Encyclopedic Reference of CANCER

Manfred Schwab (Ed.)

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Preface

Cancer, although a dreadful disease, is at the same time a fascinating biological phenotype. Around 1980, cancer was first attributed to malfunctioning genes and, subsequently, cancer research has become a major area of scientific research supporting the foundations of modern biology to a great extent. To unravel the human genome sequence was one of those extraordinary tasks, which has largely been fuelled by cancer research, and many of the fascinating insights into the genetic circuits that regulate developmental processes have also emerged from research on cancer. Diverse biological disciplines such as cytogenetics, virology, cell biology, classical and molecular genetics, epidemiology, biochemistry, together with the clinical sciences, have closed ranks in their search of how cancer develops and to find remedies to stop the abnormal growth that is characteristic of cancerous cells. In the attempt to establish how, why and when cancer occurs, a plethora of genetic pathways and regulatory circuits have been discovered that are necessary to maintain general cellular functions such as proliferation, differentiation and migration. Alterations of this fine-tuned network of cascades and interactions, due to endogenous failure or to exogenous challenges by environmental factors, may disable any member of such regulatory pathways. This could, for example, induce the death of the affected cell, may mark it for cancerous development or may immediately provide it with a growth advantage within a particular tissue.

Recent developments have seen the merger of basic and clinical science. Of the former, particularly genetics has provided instrumental and analytical tools with which to assess the role of environmental factors in cancer, to refine and enable diagnosis prior to the development of symptoms and to evaluate the prognosis of patients. Hopefully, even better strategies for causal therapy will become available in the future. Merging the basic and clinical science disciplines towards the common goal of fighting cancer, calls for a comprehensive reference source to serve both as a tool to close the language gap between clinical and basic science investigators and as an information platform for the student and the informed layperson alike. Obviously this was an extremely ambitious goal, and the immense progress in the field cannot always be portrayed in line with the latest developments. The aim of the Encyclopedia is to provide the reader with an entrance point to a particular topic. It should be of value to both basic and clinical scientists working in the field of cancer research. Additionally both students and lecturers in the life sciences should benefit highly from this database. I therefore hope that this Encyclopedia will become an essential complement to existing science resources.

The attempts to identify the mechanisms underlying cancer development and progression have produced a wealth of facts, and no single individual is capable of addressing the immense breadth of the field with undisputed authority. Hence, the 'Encyclopedic Reference of Cancer' is the work of many authors, all of whom are experts in their fields and reputable members of the international scientific community. Each author contributed a large number of keyword definitions and in-depth essays and in so doing it was possible to cover the broad field of cancer-related topics within a single publication. Obviously this approach entails a form of presentation, in which the author has the freedom to set priorities and to promote an individual point of view. This is most obvious when it comes to nomenclature, particularly that of genes and proteins. Although the editorial intention was to apply the nomenclature of the Human Genome Organisation (HUGO), the more vigorous execution of this attempt has been left to future endeavours.

In the early phase of planning the Encyclopedia, exploratory contacts to potential authors produced an overwhelmingly positive response. The subsequent contact with almost 300 contributory authors was a marvellous experience, and I am extremely grateful for their excellent and constructive cooperation. An important element in the preparation of the Encyclopedia has been the competent secretarial assistance of Hiltrud Wilbertz of the Springer-Verlag and of Ingrid Cederlund and Cornelia Kirchner of the DKFZ. With great attention to detail they helped to keep track of the technical aspects in the preparation of the manuscript. It was a pleasure to work with the Springer crew, including Dr. Rolf Lange as the Editorial Director (Medicine) and Dr. Thomas Mager, Senior Editor for Encyclopedias and Dictionaries. In particular I wish to thank Dr. Walter Reuss, who untiringly has mastered all aspects and problems associated with the management of the numerous manuscripts that were received from authors of the international scientific community. It has been satisfying and at times comforting to see how he made illustration files come alive. Thanks also to Dr. Claudia Lange who, being herself a knowledgeable cell biologist, has worked as the scientific editor. Her commitment and interest have substantially improved this Encyclopedia.

As a final word, I would like to stress that although substantial efforts have been made to compose factually correct and well understandable presentations, there may be places where a definition is incomplete or a phrase in an essay is flawed. All contributors to this Encyclopedia will be extremely happy to receive possible corrections, or revisions, in order for them to be included in any future editions of the 'Encyclopedic Reference of Cancer'.

Heidelberg, September 2001 Manfred Schwab

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A

AACR

Definition

AACR stands for American Association for Cancer Research. The AACR is a scientific society of over 15,000 laboratory and clinical cancer researchers (http://www.aacr.org/).

AAV

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Definition

Adeno-associated viruses (AAV) are small DNA-containing viruses that belong to the family of Parvoviridae. So far six → serotypes of adeno-associated viruses (AAV-1 to AAV-6) have been isolated from humans and primates. According to current knowledge, none of these serotypes are pathogenic in humans. AAV type 2 (AAV-2) has been studied for over 30 years and is thus the best characterized AAV serotype. All AAV serotypes are currently being developed and evaluated as gene transfer → vectors for the human → gene therapy of various inherited or acquired diseases, including different types of cancer.

Characteristics

As with all other members of the → Parvovirus family, AAV are non-enveloped, icosahedral capsids of about 18-24 nm in diameter. These capsids carry linear, single-stranded DNA genomes of approximately 4.6-4.8 kilobases. The genomes of all known AAV serotypes have been cloned and sequenced. With the exception of AAV-5, which is distinct from the other serotypes at both the nucleotide and amino acid level, the AAV genomes display a high level of sequence homology. Consequently, the genomic structure is also very similar among the various AAV serotypes.

As an example, the organization of the 4681 nucleotide AAV-2 genome is described (Fig. 1). The AAV-2 genome consists of two large open reading frames (orf), one at the left end encoding the nonstructural proteins (replication, → rep orf), and one at the right end encoding the structural proteins (capsid, cap orf). In addition, a single intron sequence is found in the middle of the genome, where the rep and cap orfs overlap. The AAV-2 *rep* gene actually encodes four closely related proteins (Rep proteins) with partially overlapping amino acid sequences. On the basis of their molecular weights, these proteins were designated Rep78, Rep68, Rep52 and Rep40. Unspliced and spliced transcripts originating from a promoter located at map unit 5 (p5) are translated into the two large Rep proteins, Rep78 and Rep68. Rep52 and Rep40 are expressed from identically spliced mRNAs that initiate from a second promoter, p19. The third AAV-2 promoter, p40, controls transcription of the *cap* gene. Translation of differentially spliced cap mRNAs results in expression of the three proteins that form the AAV-2 capsid, VP1, VP2 and VP3. The two ends of the viral genome are constituted by short (AAV-2: 145 nucleotides) inverted terminal repeats (→ ITR). These contain palindromic sequences which are able to fold into T-shaped stem loop structures. The ITRs

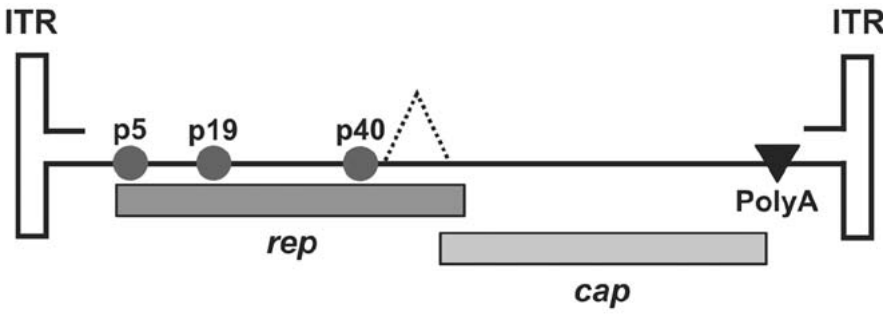
are necessary and sufficient for replication and encapsidation of the viral genome during a productive infection of cells. Moreover, they are important for integration and rescue of the AAV DNA into or from the infected cell's genome, respectively.

Cellular Characteristics (Viral Life Cycles)

The AAV serotypes belong to the Parvovirus genus Dependovirus. This classification indicates their requirement for a second helpervirus to undergo a productive infection of target cells. Thus, only if AAV-2 infects a cell that is also infected by one of these helperviruses, is the AAV-2 genome replicated and packaged into viral capsids to yield new AAV-2 particles. The typical helpervirus for AAV-2 is human → Adenovirus type 2 or 5, but also Herpes simplex virus, Vaccinia virus and Cytomegalovirus can provide full helper functions.

Analyses at the molecular level have shown that Adenovirus exerts its helper functions by inducing a strong expression of AAV-2 proteins. This occurs primarily via direct transactivation of the AAV-2 promoters. Additional helper functions mediated by adenoviral proteins are found at post-transcriptional levels. These include facilitation of the cytoplasmic transport of AAV-2 mRNAs, and later in the infection, stabilization of replicated AAV-2 genomic DNA. However, AAV-2 is not fully dependent on helperviral proteins. In fact, once the AAV-2 Rep proteins are expressed in the infected cell, they subsequently regulate and coordinate further gene expression from the AAV promoters. They also play important roles in AAV DNA replication, as well as encapsidation of the viral genomes into empty new capsids formed by AAV-2 VP proteins. In order to mediate these multiple functions, the Rep proteins can bind to the AAV-2 ITRs and to sequences located in the AAV-2 promoters. They also interact with various cellular proteins, e.g. the TATA-box binding protein (TBP), as well as with each other and the AAV-2 VP proteins. The final step in a productive AAV-2 infection is the helpervirus-mediated lysis of the infected cell. This results in cell death and release of both new AAV-2 and helpervirus particles.

In contrast to this productive (or lytic) phase, AAV-2 enters a state of latency in the absence of helpervirus. Under these conditions, the AAV-2 DNA rather than replicating itself, is integrated into the target cell genome, where it persists in a stable manner as a so-called provirus. Interestingly, the integration process does not occur randomly, as is the case for → retroviruses and other integrating viruses, but is targeted to a specific region on the long arm of human chromosome 19 (19q13.3-ter). Although the large Rep proteins are only weakly expressed in the absence of a helpervirus, they mediate this site-specific integration through binding to the AAV-2 ITRs, as well as to homologous sequences located in chromosome 19. If, however, a latently AAV-in-



AAV. Fig. 1 – Structure of the AAV-2 genome. The 4681 nucleotide single-stranded genome is depicted as a solid line; by convention, the genome is drawn in the 3′-5′-orientation. Shown are the locations of the rep and cap orfs and the single intron (caret), as well as the position of the three promoters (p5, p19, p40) and the PolyA signal, which is used for polyadenylation of all AAV-2 transcripts. Both ends of the genome are constituted by inverted terminal repeat (ITR) sequences, which assume hairpin structures.

fected cell is later challenged by a helpervirus, AAV-2 gene expression is induced and the AAV-2 genome is rescued from its integrated state. From this point on, a typical productive AAV-2 infection takes place. Thus, the helpervirus can act as an efficient switch between the two different phases, productive and latent, which characterize the AAV-2 life cycle.

Clinical Relevance

Are AAV pathogenic in humans?

According to the bulk data available, all AAV serotypes are considered to be nonpathogenic in humans. In fact, although about 80% of adults in the United States of America were found to be seropositive for AAV-2, no human disease has ever been causally linked to an infection with this virus. This is even more remarkable considering the fact that AAV-2 can infect a large variety of cells from all kinds of organs and tissues. In contrast, only a few cell lines have so far been identified as being resistent to AAV-2 infection. However, although not having pathological consequences for the cell, a latent AAV-2 infection can induce subtle phenotypic changes. Examples of this are an increased ability to respond to stress factors or a perturbation of the cell cycle. Most probably, these various effects are mediated by the large Rep proteins of AAV-2.

Is there a connection between AAV infection and cancer?

Under experimental conditions, AAV-2 infected cells exhibit an increased resistance to oncogene- or tumorvirus-induced transformation. It was also shown that AAV-2 infection can inhibit the proliferation of cells derived from human cancers, e.g. → melanoma, in culture. These observations strongly suggest that AAV-2 is not only nonpathogenic, but even possesses oncosuppressive properties. Moreover, certain human cancer cell lines become more sensitive to gamma irradiation and chemotherapeutic drugs upon experimental infection with AAV-2, as compared to non-infected controls. From a clinical point of view, these findings are of particular interest, since a major limitation of chemotherapeutic cancer treatments has been the increased resistance of transformed cells towards the drugs used. The observations of AAV-2-mediated cell sensitization therefore suggest that the virus could be used to support or improve cancer → chemotherapy.

What are recombinant AAV vectors?

A broad clinical relevance of AAV-2 and the related serotypes arises from the feasibilty to generate recombinant AAV (rAAV). These are derivatives of the viruses that no longer carry the viral *rep* and *cap* genes, but instead, contain genes of potential therapeutic interest. Consequently, when target cells are infected with such recombinant viruses, it is expected that the foreign genes are delivered to and eventually expressed in the cell, leading to the intended therapeutic effect. Recombinant AAV therefore serve as gene transfer vectors, and as such, are very promising tools for human gene therapy.

What are the special properties of AAV-2 vectors?

As opposed to vectors derived from other human viruses, which are currently being developed and tested in preclinical studies, rAAV are characterized by a unique set of advantageous properties. Firstly, the parental virus is considered to be nonpathogenic, which gives AAV-derived vectors a high grade of biosafety. Secondly, common rAAV vectors retain no viral genes and thus were found to cause only mild host immune responses, which makes vector readministration possible. Thirdly, AAV-2 vectors share with the wild type virus the ability to infect a large variety of cells, e.g. from muscle, lung, retina, central nervous system or liver. However, the efficiency of gene transfer to a target cell may vary with its mitotic state, and is influenced by a number of factors. Fourthly, vectors derived from wild type AAV-2 retain the potential for site-specific and stable integration of their genome into human chromosome 19. However, since this

process requires the large Rep proteins, which are not expressed from common AAV-2 vectors, genome integration occurs less efficiently relative to the wildtype virus and is probably no longer site-specific. On the other hand, rAAV genomes can form stable concatamers or interlocked circles and thus persist in an episomal state. This provides the appealing potential of rAAV vectors to mediate long-term foreign gene expression in infected cells. Indeed, such permanent gene expression has already been observed for up to 18 months after rAAV administration in immunocompetent animals.

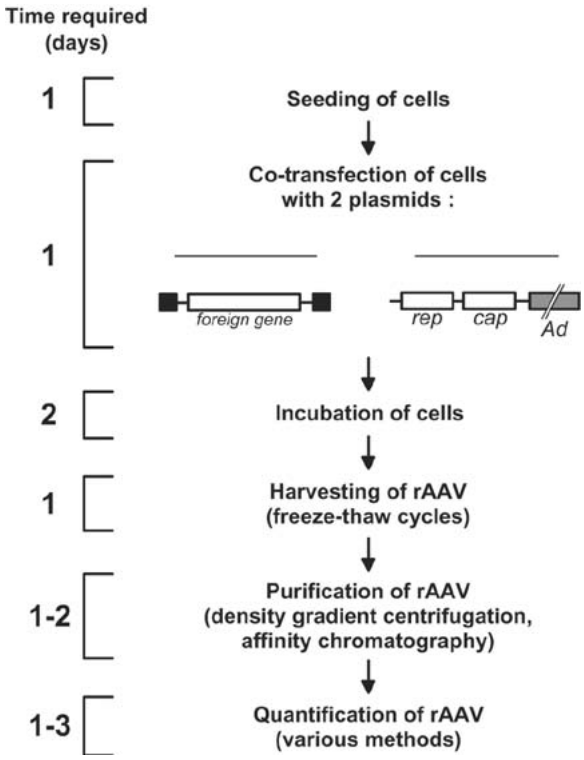
Are recombinant AAV 'perfect' vectors for human gene therapy?

Although AAV-2 vectors may appear to be ideal candidates for human gene therapy, there are a few inherent drawbacks of this particular vector system. Firstly, due to the small diameter of the viral capsid, the length of foreign DNA that can be packaged into rAAV particles is limited to a maximum of approximately 4.9 kilobases. This, on the other hand, is enough for a large variety of genes of therapeutic interest, e.g. the human factor VIII or the erythropoietin gene. Secondly, the high serumprevalence of antibodies against AAV-2 in the population may limit the success of rAAV-mediated gene transfer in humans. This is actually one of the main reasons why a growing number of research groups are currently focusing their attention on the further development of the other AAV serotypes as vectors. If successful, the ideal gene therapy would involve initial determination of the patient's immune status, and subsequent choice of the appropriate AAV serotype (against which the lowest concentration of antibodies were present) before engineering the recombinant vector. In addition, with respect to the transduction (i.e., gene delivery) efficiency to a given cell type, it is becoming clear that the various AAV serotypes exhibit different properties. Thus, if a target cell type should prove to be resistant to infection with common AAV-2 vectors, it is quite possible that one of the other serotypes could form the basis for a suitable vector. A third obstacle in the use of AAV-derived vectors is the lack of efficient and simple methods to generate, purify and quantify the recombinant viruses. Although rAAV vector production was initiated in the mid 1980's, when the first groups reported recombinant viruses carrying reporter genes, it took until the end of the last millennium before AAV-2 vector technology underwent significant improvements. As a result, the new and streamlined protocols (Fig. 2) now allow the production of large amounts of highly purified AAV-2 vector particles in a relatively short time span. This has already led to an increase in the number of phase I clinical trials that aim to evaluate rAAV-mediated gene transfer in human patients.

How could AAV vectors be used for cancer treatment?

Results from a large number of preclinical studies have already demonstrated that AAV-derived vectors could indeed prove useful for the treatment of a variety of human diseases, such as cystic fibrosis, hemophilia, or neurological disorders. With particular respect to the treatment of cancer, several strategies involving AAV vectors have already been proposed and, in part, tested:

- *Purging (removal) of tumor cells from autologous transplants.* Autologous grafts, e.g. peripheral blood progenitor grafts, can be contaminated with tumor cells that give rise to relapse after graft transplantation. There is recent evidence that following infection of such contaminated grafts with recombinant AAV-2, the contaminating tumor cells are preferentially infected. It has thus been proposed, but not yet proven, that AAV-based vectors could prove useful for the purging of such tumor cells from transplants.
- *Chemoprotection of cells.* For the treatment of a variety of advanced tumors, high-dose chemotherapy in combination with other therapies appears promising. However, this approach is also associated with severe myelosuppresssive effects, e.g. infection or hemorrhaging. One approach to this prob-



AAV. Fig. 2 – Streamlined protocol for rAAV production. Cells in culture are transfected with two plasmids. The vector plasmid contains the foreign gene that is to be packaged into the AAV-2 particles, flanked by the AAV-2 ITRs. The helper plasmid carries the AAV-2 rep and cap genes to supply the Rep and VP proteins, respectively. In addition, it contains all adenoviral (Ad) genes which encode proteins with helper function for AAV-2, but it does not yield adenovirus after transfection.
Helpervirus infection is thus superfluous, and contamination of the resulting AAV-2 vectors with adenovirus is eliminated. Following a two-day incubation of the transfected cells, the rAAV particles are harvested, purified and quantified.

lem could be to confer myeloprotection to the patients. This could be achieved, for example, by AAV-2 vector-mediated transduction of the human multidrug resistance gene (→ MDR1) to hematopoietic progenitors. Such MDR1-expressing rAAV vectors have already been generated and tested *in vitro*, but their application *in vivo* has not yet been fully explored.

- *Induction of antitumor immune responses.* A failure of the immune system to recognize or react to cancer antigens is generally considered to contribute to tumor manifestation and progression. Gene transfer protocols have thus been developed which aim to potentiate the patient's antitumor responses. Using AAV-2 vectors for gene delivery, various tumor cells have been transduced *in vivo* with genes encoding cytokines, e.g. interleukins 2 and 4, or costimulatory immune molecules, such as B7. In animal models, this resulted in augmented localized gene expression and subsequently led to enhanced tumor immunogenicity. At best, a significant growth retardation of the tumors was observed.
- *Selective inhibition of gene expression via transdominant molecules.* Following the discovery that overexpression of particular oncogenes can contribute to tumor development, transdominant molecules have been developed to selectively inhibit the expression of these genes. The most commonly used transdominants are RNA-based antisense [→ antisense nucleic acid] or → ribozyme molecules. Molecular targets include cellular oncogenes, such as → bcl-2 or → myc, as well as viral transforming genes, e.g. E6/E7 from human papillomaviruses. There is evidence that rAAV can function as an efficient vehicle for the transduction of such oncogene-targeting transdominants into tumor cells. AAV-2 vectors are therefore good candidates for further development of this particular type of cancer therapy.
- *Transduction of suicide or tumor suppressor genes.* Another approach for cancer gene therapy is to sensitize tumor cells to antiviral drugs, such as ganciclovir, by transducing them with the thymidine-kinase (tk) gene from Herpes simplex virus. Treatment of the genetically modified cells with ganciclovir results in the inhibition of DNA synthesis and eventually cell death. A further anticancer strategy is the transfer of the tumor-suppressor gene → p53, which is mutated in a large proportion of human cancers, into tumor cells. It is expected that this would result in growth suppression and induction of apoptosis. AAV-2 vectors have already been successfully used as delivery vehicles for both genes (*tk* and *p53*) in animal mod-

els. So far, the recombinant AAV have shown a high antitumor efficacy, which supports the further development of this approach.

- *Delivery of antiangiogenic factors.* The efficacy of → angiogenesis inhibitors (e.g., endostatin or angiostatin) to undermine tumor neovascularization and cancer progression has been demonstrated in many animal models. However, this cancer therapy requires that the inhibitors are chronically administered as recombinant proteins, which is usually associated with severe problems. Therefore, AAV-2 vectors with their unique ability to mediate sustained gene expression might prove particularly useful for this type of tumor therapy. In fact, recombinant AAV delivering antiangiogenic factors are currently being developed and tested.
- *Development of tumor vaccines.* The latest antitumor approach involving AAV-2 vectors focuses on the development of vaccines, in particular targeting cervical tumors induced by → human papillomaviruses (HPV). It has recently been proposed that such vaccines consist partly of the E6/E7 and/or the L1 structural gene of the high-risk papillomavirus types 16 or 18, perhaps fused to an additional gene to provide a protein adjuvant. There is hope that following rAAV-mediated delivery of the chimeric gene into target cells, the potential vaccine will be expressed to stimulate a host immune response. This should eventually result in protection against HPV-induced tumor formation.

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ABC Transporter

Definition

ABC transporters are a superfamily of prokaryotic and eukaryotic proteins. They are usually involved in membrane transport and share a homologous nucleotide-binding domain, ABC (ATP-binding cassette). In addition to the ABC domain, ABC transporters contain or interact with hydrophobic domains containing multiple transmembrane segments. Examples of mammalian ABC transporters include → P-glycoprotein, MRP (→ multidrug-resistance protein), cystic fibrosis transmembrane conductance regulator (CFTR) and the transporter associated with antigen processing (TAP).

ABL

Definition

The ABL gene encodes a nuclear tyrosine kinase that is involved in chromosomal translocations in → CML.

Accelerated Senescence

Definition

Accelerated senescence, the process of rapid terminal growth arrest, is accompanied by phenotypic features of cell → senescence (enlarged and flattened morphology, increased granularity, expression of specific biochemical and enzymatic markers such as senescence-associated β-galactosidase activity). It can be induced in normal cells by DNA damage or introduction of mutant → RAS and is also induced in tumor cells by different anticancer drugs or ionizing radiation.

Achaete-scute

Definition

This → bHLH protein family comprises the *Drosophila melanogaster* proteins Achaete and Scute, the mammalian homolog Mash-2, the human homolog Hash-2 and the Fugu homolog Fash-2. Members of the Achaete-scute family are involved in the determination of the neuronal precursors in the peripheral nervous system and the central nervous system. Efficient DNA binding requires dimerization with another bHLH protein.

Acidosis

Definition

Tissue acidosis is present whenever the pH is below 7.0 (or the H^+-concentration is higher than 10^{-7} mol/L).

Acini

Definition

The arrangements of secretory epithelial cells with a compound gland are called acini (plural; singular: acinus; adjective: acinar).

Acquired Mutation

Definition

A change in the DNA sequence that develops throughout the lifetime of an organism is an acquired mutation. Although mistakes occur in DNA all the time, especially during cell division, a cell has normally the ability to fix them. In case this DNA repair mechanisms fails, mutations can be passed on to subsequent cell generations.

Acrocentric Chromosome

Definition

An acrocentric chromosome has the centromere located closer to one end than the other.

Acromegaly

Definition

Acromegaly is a chronic debilitating disease associated with the overgrowth of bony and soft tissues most commonly due to a growth hormone (GH)-producing pituitary adenoma.

ACTH

Definition

ACTH stands for adrenocorticotrophic hormone. The correct nomenclature according to the Human Gene Nomenclature (HGNC) Database is → POMC.

Activating Mutation

Definition

A substitution of one amino acid residue by another that confers a new or higher activity upon the protein is called activating mutation.

Acute Cellular Rejection

Definition

The process of severe damage or destruction of engrafted organs, cells and tissues is called acute cellular rejection. It occurs usually within days to months after transplantation and is caused by T-cell mediated immune responses; → graft acceptance and rejection.

Acute Lymphoblastic Leukemia

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Synonyms

Acute lymphoblastic leukemia (ALL), also known as acute lymphocytic leukemia and acute lymphoid leukemia, is a rapidly growing leukemia of lymphocytes.

Definition

Acute lymphoblastic leukemia (ALL) is a malignant disease of immature T or B lymphocytes, or lymphoblasts. It arises from one or more genetic abnormality in a single lymphoblast, leading to altered blast cell proliferation, survival and maturation, and eventually to the lethal accumulation of leukemic cells. Although cases can be subclassified further according to the stage of T- or B-cell maturation, these distinctions are not therapeutically useful, except for the recognition of a mature B-cell, B-cell precursor or T-cell stage.

Characteristics

ALL accounts for about 12% of all childhood and adult leukemias diagnosed in developed countries, and for 60% of those diagnosed in persons younger than 20 years. It is the most common cancer in children (25% of all cases) and has a peak incidence in patients between the ages of 2 and 5 years, with a second, smaller peak in the elderly.

The factors predisposing children and adults to ALL remain largely unknown. Fewer than 5% of cases are associated with inherited genetic syndromes defined by chromosomal instability and defective DNA repair. Ionizing radiation and mutagenic chemicals have been implicated in some cases of ALL, but their contributions appear negligible. Nonetheless, evidence collected over the past two decades has revealed that ALL is essentially a disease of acquired genetic abnormalities. Specific genetic abnormalities are found in the leukemic cells of 60% to 75% of patients with ALL to date. These include chromosomal translocations leading to the formation of transforming fusion genes or the dysregulation of proto-oncogene expression, as well as chromosomal gains or losses resulting in hyperdiploidy [→ hyperdiploid] or hypodiploidy [→ hypodiploid], respectively. In all likelihood, with the improved genetic techniques, specific abnormalities will be identified in all cases.

Although most leukemias begin in the bone marrow and spread to other parts of the body, some may arise in an → extramedullary site, such as the thymus or intestine, and subsequently invade the bone marrow. The presenting features of ALL generally reflect the degree of bone marrow failure and the extent of extramedullary spread. Common signs and symptoms are:

- Fever.
- Fatigue and lethargy.
- Dyspnea, angina, and dizziness (older patients mainly).
- Limp, bone pain, or refusal to walk (young children).
- Pallor and bleeding in the skin or mouth cavity.
- Enlarged liver, spleen, and lymph nodes (more pronounced in children).
- Anemia, low neutrophil count, and low platelet count.
- Metabolic abnormalities (e.g., high serum uric acid and phosphorus levels).

The diagnosis of ALL is based on a morphologic examination of bone marrow cells (Figs. 1, 2 and 3), as well as the cytochemical properties and immunophenotype of cells from the same sample. Karyotyping and molecular genetic analysis by RT-PCR (reverse transcriptase-polymerase chain reaction) are now routinely performed by many centers to identify subtypes of ALL with prognostic significance, for example:

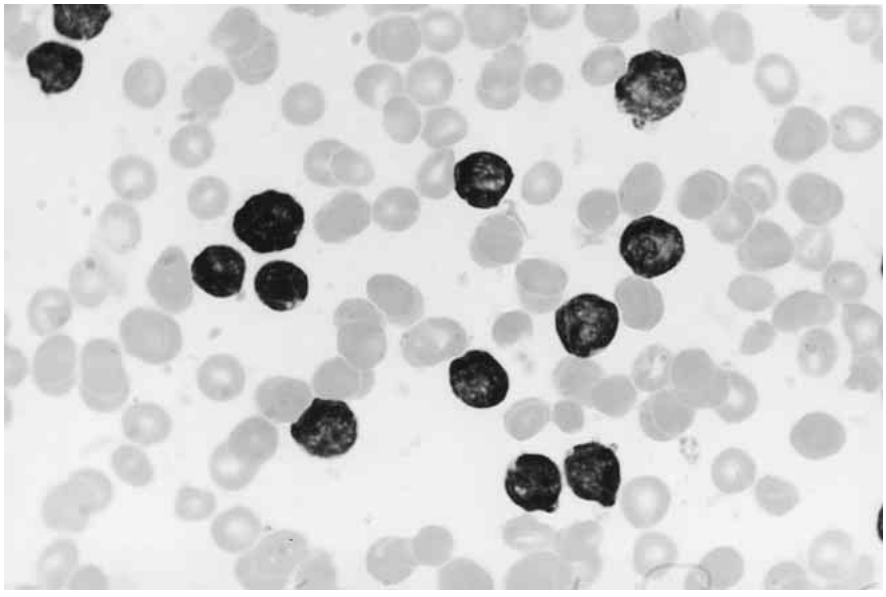
- BCR-ABL fusion gene due to the t(9;22), or → Philadelphia chromosome - 25% of adult

cases and 3-4% of childhood cases (dismal prognosis).
- *TEL-AML1* fusion gene due to a cryptic t(12;21) - 25% of childhood cases (favorable prognosis).
- Hyperdiploidy (more than 50 chromosomes per cell) - 25% of childhood cases (favorable prognosis).
- Hypodiploidy (fewer than 45 chromosomes per cell) - 1% of childhood cases and 4% of adult cases (unfavorable prognosis).

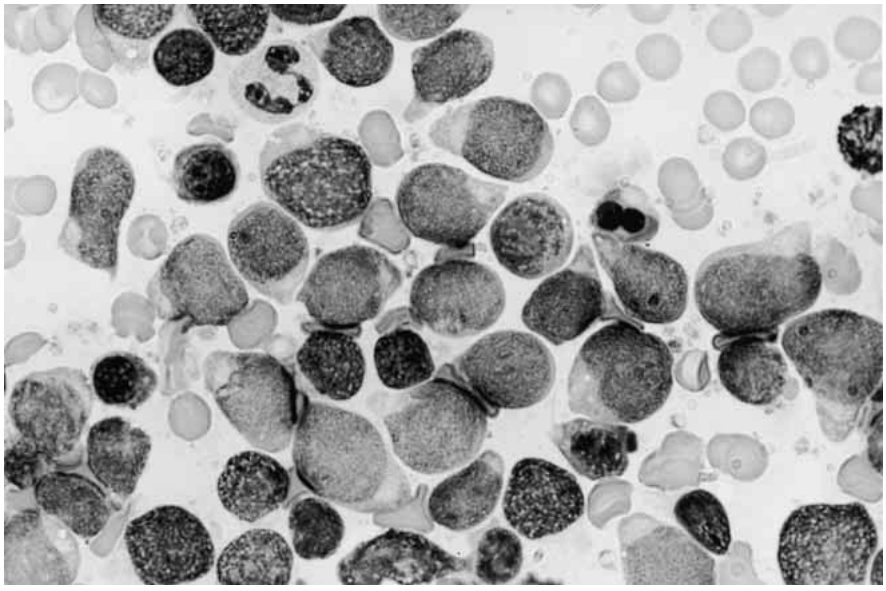
Contemporary treatment modified according to the risk of relapse can cure 80% of children and up to 40% of adults with ALL. Cases are generally classified as standard or high risk in adults and as low, standard and high risk in children. Factors used to determine the relapse hazard include the presenting leukocyte count, age at diagnosis, gender, immunophenotype, → karyotype, molecular genetic abnormalities, initial response to therapy, and the amount of 'minimal residual leukemia' after therapy to induce a complete → remission.

Multidrug remission induction regimens almost always include a glucocorticoid (prednisone, prednisolone, or dexamethasone), vincristine and at least a third agent (L-asparaginase or anthracycline), administered for 4 to 6 weeks. Some treatments rely on additional agents to increase the level of cell kill, thereby reducing the likelihood of the development of drug resistance and subsequent relapse. However, several studies suggest that intensive remission induction therapy may not be necessary, providing that patients receive post-induction intensification therapy. Remission induction rates now range from 97% to 99% in children and from 70% to 90% in adults. Complete remission is traditionally defined as restoration of normal blood cell formation with a blast cell fraction of less than 5% by light microscopic examination of the bone marrow. With this definition, many patients in complete remission may harbor as many as 1×10^{10} leukemic cells in their body. To improve treatment outcome, most protocols specify an intensification (or consolidation) phase in which several effective antileukemic drugs are administered in high doses soon after the patients attain a complete remission.

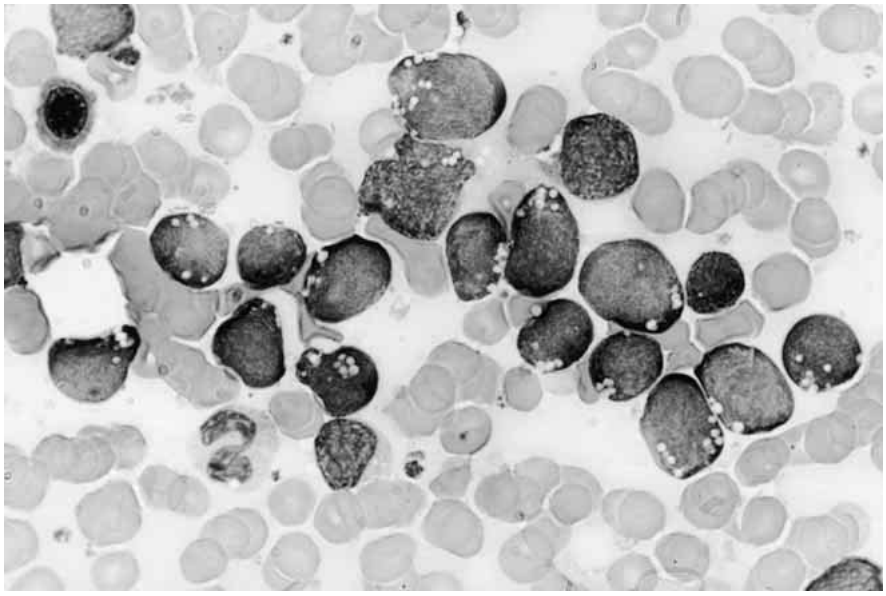
Regardless of the intensity of induction/consolidation therapy, all children require 2 to 3 years of continuation treatment, usually methotrexate and mercaptopurine, with pulses of vincristine and dexamethasone for low-risk cases, and multiagent intensive chemotherapy for standard-risk cases. The need for continua-



Acute Lymphoblastic Leukemia. Fig. 1 – Small regular blasts with scanty cytoplasm, homogeneous nuclear chromatin and inconspicuous nucleoli.



Acute Lymphoblastic Leukemia. Fig. 2 – Admixture of large blasts with moderate amounts of cytoplasm and smaller blasts. Such cases may be mistaken for acute myeloid leukemia, emphasizing the importance of immunophenotyping to corroborate the differential diagnosis.



Acute Lymphoblastic Leukemia. Fig. 3 – B-cell ALL blasts characterized by intensely basophilic cytoplasm, regular cellular features, prominent nucleoli and cytoplasmic vacuolation.

tion therapy in adults is less clear, although in most cases it is discontinued after 2 to $2^1/_2$ years of complete remission. The central nervous system can be a → sanctuary site for leukemic cells, requiring intensive, intrathecally administered chemotherapy that begins early during the remission induction phase, extending through the consolidation phase and into the continuation phase. Once considered standard treatment for central nervous system leukemia, cranial irradiation is now reserved for approximately 10% of patients who are at high risk

for relapse. For selected high-risk cases, such as patients with Philadelphia chromosome-positive ALL, and those who require extended therapy to attain initial complete remission, transplantation is currently the treatment of choice. Finally, the optimal clinical management of patients with ALL requires careful attention to methods for the prevention or treatment of metabolic and infectious complications, which may otherwise be fatal.

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Acute Lymphocytic Leukemia

Definition

→ Acute lymphoblastic leukemia.

Acute Lymphoid Leukemia

Definition

→ Acute lymphoblastic leukemia.

Acute Promyelocytic Leukemia (APL)

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Definition

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML) characterized by the expansion of leukemic cells blocked at the promyelocytic stage of the myelopoiesis. According to the French-American-British (FAB) classification of acute leukemia, APL corresponds to the M3 and M3-Variant subtypes, and accounts for 10 – 15% of adult AML patients. APL is distinct from other subtypes of leukemia because of its invariable association with reciprocal chromosomal translocations involving the retinoic acid receptor α (RARα) gene on chromosome 17 (Table) and by its sensitivity to the differentiating action of all-trans retinoic acid (ATRA).

Characteristics

Clinical and laboratorial presentation

The symptoms of APL are similar to those of other subtypes of AML such as weight loss, fatigue, weakness, pallor, fever and bleeding. These symptoms manifest acutely and are accompanied by petechiae, bruising, oral bleeding or epistaxis as well as symptoms and signs related to specific bacterial infections. Patients with APL are particularly susceptible to disseminated intravascular coagulation (DIC) and extensive bleeding is common at onset. The most common sites of clinically overt extramedullary leukemic infiltration include superficial lymphonodes, the liver and spleen. The leukocyte counts are usually lower than those observed in other AML subtypes and the differential counts reveal a variable percentage of blasts in the majority of patients. In most cases, anemia and thrombocytopenia are present at diag-

Acute Promyelocytic Leukemia (APL). Table – Molecular Genetics of Acute Promyelocytic Leukemia.

translocation	fusion proteins		response to RA
t(15;17)	PML-RARα	RARα-PML	good
t(11;17)	PLZF-RARα	RARα-PLZF	poor
t(5;17)	NPM-RARα	RARα-NPM	good
t(11;17)	NuMA-RARα	RARα-NuMA?	good
t(17;17)	STAT5b-RARα	RARα-STAT5b?	poor?

nosis. Abnormal promyelocytes constitute more than 30% of marrow nucleated cells and are morphologically characterized by the presence of distinctive large cytoplasmic granules, frequent multiple Auer rods and a folded nucleus. The M3-variant is a rarer atypical hypogranular variant in which the promyelocytes contain a large number of small granules that may be difficult to distinguish by light microscopy. However, both in the classical and variant M3 subtypes the cells are strongly positive for myeloperoxidase staining.

DIC occurs in 75% of M3 patients accompanied by secondary fibrinolysis, which is thought to be due to the release of procoagulants from the azurophilic granules in the leukemic promyelocytes. Thus, laboratory evidence of DIC (prolonged prothrombine time and partial thromboplastin time, decreased fibrinogen and increased fibrin degradation products) should be investigated in all APL patients. It must be emphasized that DIC is often precipitated by induction chemotherapy, presumably by the release of large amounts of procoagulant, when disruption of promyelocytes is maximal.

Molecular characterization

APL has been well characterized at the molecular level and has become one of the most compelling examples of aberrant transcriptional regulation in cancer pathogenesis. Due to reciprocal translocations, the retinoic acid receptor α (RARα) gene on chromosome 17 is fused to one of five distinct partner genes (for brevity, hereafter referred as X genes; Table 1). In the vast majority of cases RARα fuses to the *PML* gene (promyelocytic leukemia gene, originally named myl) on chromosome 15. In a few cases RARα fuses to the promyelocytic leukemia zinc finger (PLZF) gene, to the nucleophosmin (NPM) gene, to the nuclear mitotic apparatus (NuMA) gene, or to the signal transducer and activator of transcription 5B (STAT 5B) gene located on chromosome 11, 5, 11, or 17, respectively. The various translocations result in the generation of X-RARα and RARα-X fusion genes and the co-expression of their chimeric products in the leukemic blasts. The characterization of the genetic events of APL, and the availability of techniques such as FISH and RT-PCR, render it possible to confirm the diagnosis at the molecular level and to monitor minimal residual disease.

RARα is a member of the superfamily of nuclear receptors, which acts as a retinoic acid (RA) -dependent transcriptional activator in its heterodimeric form with retinoid-X-receptors (RXR). In the absence of RA, RAR/RXR heterodimers can repress transcription through histone deacetylation by recruiting nuclear receptor co-repressors (SMRT), Sin3A or Sin3B, which in turn, form complexes with histone deacetylases (HDAC) resulting in nucleosome assembly and transcription repression. RA causes the disassociation of the co-repressor complex and the recruitment of transcriptional co-activators to the RAR/RXR complex. This is thought to result in terminal differentiation and growth arrest of various types of cells, including normal myeloid hemopoietic cells. The X-RARα fusion proteins function as aberrant transcriptional repressors, at least in part, through their ability to form repressive complexes with co-repressors such as NCoR and HDACs. PLZF-RARα can also form, via its

PLZF moiety, co-repressor complexes that are less sensitive to RA than the PML-RARα co-repressor complexes, thus justifying the poorer response to RA-treatment observed in these patients (see below). The X-RARα oncoproteins retain most of the functional domains of their parental proteins and can heterodimerize with X proteins, thus potentially acting as double-dominant-negative oncogenic products on both X and RAR/RXR pathways.

Therapeutics

The exquisite sensitivity of APL blasts to the differentiating action of RA makes APL a paradigm for therapeutic approaches utilizing differentiating agents. This therapeutic approach conceptually differs from the treatments involving drug and/or irradiation therapies, because instead of eradicating the neoplastic cells by killing them, it reprograms these cells to differentiate normally. The application of the all-trans retinoic acid (ATRA) in APL patient management has reduced the early death from DIC-related complications and improved the prognosis dramatically. However treatment with ATRA alone in APL patients induces disease remission transiently and relapse is inevitable if remission is not consolidated with chemotherapy. In addition, in the majority of cases relapse is accompanied by RA resistance. Unlike t(15;17)/PML-RARα APL, t(11;17)/PLZF-RARα leukemias show a distinctly worse prognosis with poor response to chemotherapy and little or no response to treatment with RA, thus defining a new APL syndrome. Furthermore, up to 50% of patients treated with ATRA alone develop an 'ATRA syndrome' characterized by a rapid rise in circulating polymorphonuclear leucocytes and associated with weight gain, fever, occasional renal failure and cardiopulmonary failure, which may be life threatening in some patients. The combination of ATRA and chemotherapy in the induction and consolidation treatment phases has been proven to be a better strategy to prevent 'ATRA syndrome' and achieve long-term disease-free survival.

Arsenic trioxide (As_2O_3), a chemical used in Chinese medicine, is also extremely effective in the treatment of APL. About 90% of APL patients treated with As_2O_3 alone achieve complete remission, especially in relapsed patients who are resistant to RA and/or conventional chemotherapy. RA triggers blast differentiation while As_2O_3 induces both apoptosis and partial differentiation of the leukemic blasts. Utilizing PML-RARα and PLZF-RARα transgenic mouse models of APL, it has been demonstrated that the association of RA and As_2O_3 is effective in the former but not in the latter.

Considering the importance of transcriptional repression in APL pathogenesis, the utilization of histone deacetylase inhibitors (HDACIs) such as suberanilohydroxamic acid (SAHA) or sodium phenylbutyrate (SPB) in combination with RA may represent a promising experimental therapeutic approach. Preclinical studies in transgenic mouse models of APL suggest that in fact HDACIs work as growth inhibitors and inducers of apoptosis, and that these effects are potentiated by RA.

Modeling APL in mice

The transgenic approach in mice has been used successfully in modeling APL with various APL fusion genes. *In vivo*, transgenic mice (TM) harboring X-RARα oncoproteins develop leukemia after a long latency suggesting that the fusion proteins are necessary, but not sufficient to cause full-blown APL. In the PML-RARα TM model, mice develop a form of leukemia that closely resembles human APL, presenting blasts with promyelocytic features that are sensitive to the differentiating action of RA. On the contrary, the leukemia developed by the PLZF-RARα TM lacks the distinctive differentiation block at the promyelocytic stage and morphologically resembles more the chronic myeloid leukemia ('CML-like' leukemia). This analysis demonstrated that the X-RARα fusion protein also plays a critical role in determining response to ATRA in APL, since leukemia in PML-RARα but not the PLZF-RARα TM is responsive to ATRA treatment.

Modeling APL in TM contributed to the understanding of the role of the reciprocal RARα-X fusion proteins. RARα-PML and RARα-PLZF TM do not develop overt leukemia. However, the co-expression of RARα-PML with PML-

RARα increases the penetrance and the onset of leukemia development in double mutants. Strikingly, in the PLZF-RARα TM model, the co-expression of RARα-PLZF with PLZF-RARα metamorphoses the 'CML-like' leukemia in PLZF-RARα TM to a leukemia with classical APL features. In addition, RARα-PLZF renders the leukemic blasts more unresponsive to the differentiating activity of RA. At the transcriptional level, RARα-PLZF acts as an aberrant transcription factor that can interfere with the repressive ability of PLZF. Therefore, both RARα-X and X- RARα fusion products in combination play a crucial role in determining the distinctive phenotypic characteristics of the disease. Modeling of APL *in vivo* in the mouse is thus allowing a better comprehension of the molecular mechanisms underlying the pathogenesis of APL as well as the development of novel therapeutic strategies.

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Acute Vascular Rejection

Definition

Acute vascular rejection is the severe damage or destruction of engrafted organs, usually from days to several months after transplantation. It is probably caused by several humoral factors including antibodies and complement that may result in unwanted activation of donor endothelium; → graft acceptance and rejection.

Adaptive Response

Definition

The process of adaption, which allows survival under adverse conditions, is called adaptive response.

Adducts

Definition

Adducts are reaction products of chemicals with cellular macromolecules (DNA, RNA, protein) that contain one or more covalent bonds between the two components; → adducts to DNA.

Adducts to DNA

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Synonyms

- macromolecule-bound carcinogens

Definition

DNA-bound carcinogen adducts reflect the amount of a chemical that covalently interacts with nucleic acid bases at the target site (biologically active dose) or in → surrogate tissues. DNA adducts are mechanistically more relevant to carcinogenesis than internal doses of a mutagen, since they take into account individual differences in metabolism and DNA repair capacity (Fig. 1). The rationale of measuring DNA adducts as relevant dosimeters of biological effects and predictors of cancer risk is de-

rived from extensive experimental and human data supporting their role in the → initiation and possibly the progression of cancer. Several hundred DNA adducts, many with miscoding properties, are known to be produced by approximately 20 classes of carcinogens and through endogenous processes including oxidized DNA bases. These lesions provide powerful tools for studying disease pathogenesis, etiology and for verifying preventive measures in human cancer or other chronic degenerative diseases.

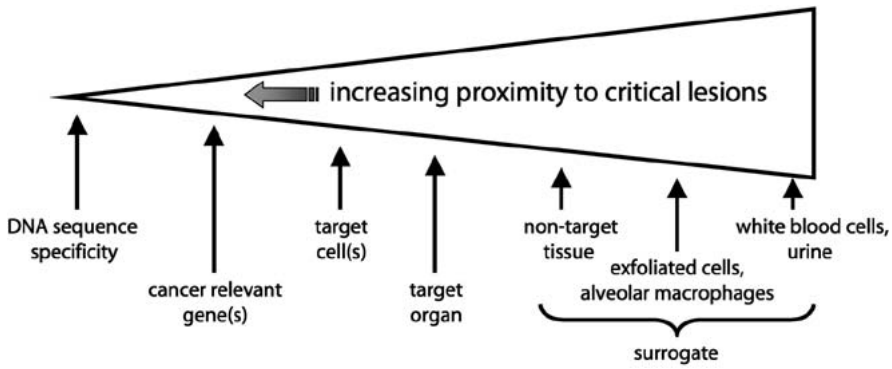
Characteristics

What is the rationale for using DNA adducts as biomarkers for exposure and adverse effects?

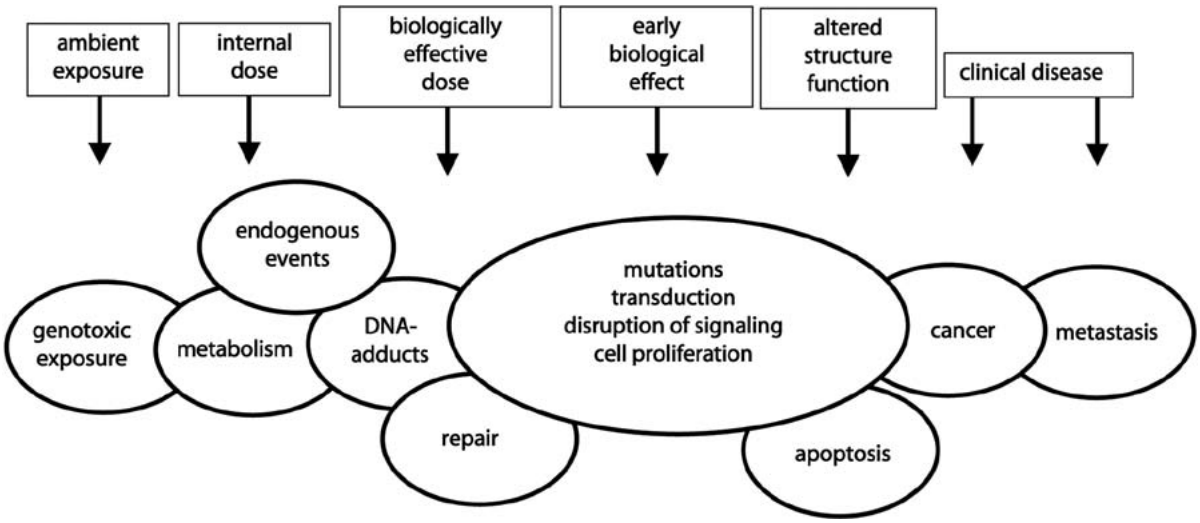
Evidence for the biological significance of DNA adducts in carcinogenesis is supported by the following findings:

- over 80% of identified (or suspected) chemicals (often after metabolic activation) act as carcinogens in humans and react with nucleic acids and proteins to form macromolecular products (adducts);
- carcinogen-DNA adducts represent the initiating events leading to mutations in → oncogenes and → tumor suppressor genes, and to malignant cell → transformation;
- humans with inherited defects in DNA repair have an elevated risk of developing cancer;
- the carcinogenic potency of a large number of chemicals is proportional to their ability to bind to rodent liver DNA.

Biological effect markers are defined as indicators of irreversible damage that result from toxic interactions at the target site; consequential adverse effects should be pathologically linked to cancer development. As DNA adducts do not often cause completely irreversible lesions, because the DNA undergoes repair (which may not be complete, though), they are not in a strict sense biological effect markers. Therefore, DNA adducts do not strictly belong in the category of biological effect markers. However, carcinogen dosage is linked to cancer outcome, and permanent mutations can be caused by DNA adducts. DNA adducts, as markers of the biologically effective dose, must therefore be associated with cancer risk (discussed by Vineis and Perera, 2000). This has been shown for several carcinogens and their adducts when critical toxico-kinetic parameters are taken into account. Examples are the steady state concentration of the adduct, the concentration of the critical adduct compared to others of lesser biological relevance, the organ, cell and gene selectivity of the adduct as well as the adduct half-life after carcinogen exposure has stopped (Fig. 2).



Adducts. Fig. 1 – Paradigm for the multistage process of carcinogenesis with DNA adducts as initiating lesions. They are used mostly as biomarkers for the biologically effective dose both of exogenous carcinogens and of DNA-reactive agents produced by endogenous processes.



Adducts. Fig. 2 – Measurement of carcinogen-DNA adducts in target tissue and cells or in surrogates. The predictive value of DNA adducts for disease risk increases with the proximity of measurements to critical lesions. Accordingly, from right to left, the specificity of this marker increases while the sensitivity for predicting disease outcome decreases.

What are the advantages and disadvantages of DNA adducts compared to other biomarkers?

For biomonitoring, both DNA and protein adducts can be used for exposure assessment as long as the response in target organs *versus* surrogate tissue is shown proportionally. The latter has to be determined individually for each carcinogen. The advantage of certain protein adduct measurements is that they often reflect cumulative past exposure (of several months), while the majority of DNA adducts are rapidly repaired or lost within several days after cessation has ceased. However, a small proportion of DNA adducts can survive in subpopulations of non-dividing cells and/or those with slow repair for several months or even years.

Since somatic genetic or cytogenetic effect markers are neither chemical specific nor exposure specific, only macromolecular adducts allow the identification of the structure and the determination of genotoxic exposure sources. Also, cytogenetic markers are more easily affected by lifestyle and environmental components (→ confounders) that often act as uncontrolled (or uncontrollable) variables in molecular epidemiology studies. In addition, at equal levels of carcinogen exposure, DNA adducts are a measure of the host's metabolic-activating capability of carcinogens and can be used to determine the effect of genetic polymorphism in carcinogen-metabolizing enzymes on DNA damage and cancer susceptibility by a given class of carcinogens.

Cellular regulation (defense): repair of DNA adducts

DNA repair [→ repair of DNA] systems such as nucleotide → excision repair, O^6-alkylguanine-DNA methyltransferase and → mismatch repair, which have been highly conserved during evolution, operate in human cells to remove adducted and oxidatively damaged DNA bases. Mutated genes, involved in nucleotide excision repair cause *Xeroderma pigmentosum* (*XP*) and a high-rate occurence of skin cancers. Also, *XP* repair gene deficient animals are highly susceptible to ultraviolet light- and hydrocarbon-induced carcinogenesis. A defective mismatch repair system is thought to cause hereditary nonpolyposis colorectal cancer (= → HNPCC). In this case patients inherit mutated mismatch repair genes through the germ line.

Genetic defects in these DNA repair functions or the inhibition of repair proteins may have dramatic consequences when DNA ad-

ducts, DNA mismatches and DNA loops are not repaired prior to cell replication and/or when damaged cells are not eliminated by → apoptosis. Thus, the characterization of germ line and somatic mutations (caused by exposure to carcinogens) in DNA-repair genes could identify high-risk subjects who, especially in the case of bi-allelic mutation, may suffer from functional defect of repair proteins that could lead to cancer.

Adduct measurement in disease epidemiology

Cross-sectional and longitudinal studies in disease epidemiology assess the relationship between carcinogen exposures and → biomarker (adduct) levels. Moreover, adduct measurements allow the detection, quantification and structural elucidation of specific DNA damage in humans. Recent findings from such studies include the detection of background exposures manifested in 'unexposed' control populations and a significant interindividual variation in adduct levels in persons with comparable exposure; the latter being in part due to genetic variation in carcinogen metabolism and DNA-repair processes. Dramatic effects of occupational and environmental exposures on adduct levels have been observed, and significant correlations have been seen between DNA adducts and adverse effects, e.g. mutations in oncogenes and tumor suppressor genes. For example, large scale studies on geographical variations of hepatocellular carcinoma and exposure to → aflatoxins have used aflatoxin-bound albumin adducts, urinary aflatoxin B_1-N7-guanine adducts and mutational hotspots in the → *TP53* gene as biomarkers. The results revealed more than an additive interaction between the chemical hepatocarcinogen and hepatitis-B virus [→ hepatitis viruses] (→ HBV) infection.

Case control studies in disease epidemiology allow the evaluation of the role of markers as cancer risk factors and the exploration of underlying mechanisms. Again, such studies cannot establish causality between biomarker response and cancer. This is especially the case when the latency period (between exposure and cancer) is long. In such cases, adduct measurements are of lesser relevance to the risk of cancer, unless exposure has been continuous. An optimal study design that establishes causality, is a nested case-control study that uses questionnaire data and biological sample collection prior to disease manifestation. Once the diagnosis of cancer has been made, cases are matched to appropriate controls and their stored samples are analyzed. The predictive value in terms of specificity and sensitivity of a certain marker, e.g. DNA adducts, in biological samples collected prior to clinical disease can thus be determined.

Association of DNA adducts with cancer risk

Not all types of DNA adducts are associated with the same cancer risk. Using alkylating agents such as aflatoxins and aromatic amines (that induced 50% tumor incidence; TD50) animal experiments were carried out to compare DNA adduct concentration. A 40- to 100-fold difference in the ability of DNA adducts to induce the same tumor incidence in target tissues was detected. These findings make it difficult to predict the tumor induction potential of unknown DNA adducts. In the past, most of the available assays for DNA adduct determination provided information on the total amount of adducts in bulk genomic DNA. However, new methods are capable of pinpointing critical targets in the DNA (Fig. 2), but because of the multistage and complex nature of human carcinogenesis, carcinogen-macromolecular adducts *per se* have so far not been a precise and quantitative predictor of individual cancer risk. Therefore, risk estimation is, at least presently, limited to a group level.

Background DNA-adduct levels: sources, variations and cancer risk prediction

By using ultrasensitive analytical methods (e.g. ^{32}P-→ postlabeling) DNA adducts have been detected in unexposed humans and untreated animals. Lipid peroxidation products, often produced in response to oxidative stress, have been associated with a variety of pathological conditions including cancer. As a consequence of physiological processes, lipid peroxidation products are formed in human tissues and can re-

act with DNA to yield background levels of a variety of adducts. These background adducts have been shown to generally increase with age both, in humans and in laboratory animals. Moreover, significant variations between human individuals were found, particularly when they were affected by risk factors that induced oxidative stress (including chronic inflammatory processes and infections) nutritional imbalances, and metal storage disorders. In addition to DNA-reactive lipid peroxidation products, oxidized DNA bases have been recognized as common lesions that occur more frequently in cells whose antioxidant defense mechansims are impaired. The biological relevance of both types of DNA lesions is supported by the fact that they are miscoding lesions and that they are recognized by specific DNA-repair enzymes. Exogenous carcinogens can also induce oxidative stress. Secondary oxidative DNA base damage can be caused in addition to agent-specific stress and agent-specific DNA adducts. It is assumed that both types of lesions play a role in the initiation and promotion of the multistage carcinogenesis process. Some open questions are:

- What is the significance of endogenously formed adducts in human cancer, particularly in relation to spontaneous tumors?
- Has the proportion of cancers that result from environmental agents been overestimated compared to those arising from endogenous processes?
- Can one protect humans against endogenously derived DNA damage and degenerative diseases by administration of chemopreventive agents, using DNA-adduct measurement to verify their efficacy?

Contributions of DNA-adduct measurements to disease etiology and pathogenesis

Adduct measurement allows new insights into disease etiology since

- adduct analysis permits the identification of hitherto unknown DNA-reactive chemicals and of carcinogenic components in complex exposures, thus increasing the power to establish causal relationships in molecular epidemiology;
- highly exposed individuals can be more readily identified; exposure to carcinogenic risk factors can be minimized or even avoided;
- subgroups in the population (pharmacogenetic variants) that are, due to genetic polymorphism of → xenobiotic-metabolizing enzymes, more suceptible to the action of environmental carcinogens, are identifiable by the combination of genotype determination and DNA-adduct measurement;
- repeated applications of dosimetry methods for macromolecular adducts can evaluate the effectiveness of intervention studies, either by the reduction of carcinogen exposure or through chemopreventive stragies;
- incorporation of DNA-adduct measurements (and of other critical endpoints involved in carcinogenesis) will reduce a) the enormous uncertainties currently associated with high-to-low dose and species-to-species extrapolation and b) yield information on inter-individual risk assessment procedures;
- the role of specific carcinogen exposures may be retrospectively implicated in the etiology of cancers by analyzing mutational spectra in tumors that arise from exogenous and endogenous mutagens after their reaction with DNA. Some carcinogens, i.e. their DNA adducts, leave specific mutational fingerprints in critical genes involved in human carcinogenesis, that are still found in tumors decades after the period of exposure. For example, specific mutational spectra, detected in the tumor suppressor gene *TP53*, were associated with distinct past carcinogen exposure to tobacco [→ tobacco carcinogenesis] smoke, aflatoxin B1, vinyl chloride and UV light. Thus, this approach might be useful to trace etiological agents that were causing tumors decades ago;
- adducts and derived mutations allow the study of pathogenesis and preventive approaches of degenerative diseases other than cancer, such as atherosclerosis or Alzheimer and Parkinson disease.

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Adenocarcinoma

Definition

Adenocarcinoma is a type of cancer arising from glandular epithelial cells, i.e., cells that line the walls of various organs.

Adenoma-carcinoma Sequence

Definition

Adenoma-carcinoma sequence is a sequence of mutations leading from benign adenomatous polyps in the colon to colon cancer; → multistep development.

Adenomatous Polyps

Definition

A polyp in general is defined as any overgrowth of gastrointestinal epithelium that protrudes into the lumen. Polyps are classified as hyperplastic or neoplastic, benign or malignant, and in various histopathologic subtypes. Adenomas (neoplastic polyps) are sessile or pedunculated and represent the most common localized neoplastic tumor of the colon-rectum; colorectal cancer [→ colon cancer].

Adenoviral Vector

Definition

An adenoviral vector is a gene transfer vehicle based on → adenovirus, used to deliver genes into host cells.

- A first-generation adenoviral vector is characterized by the deletion of the E1A and E1B genes that are the first genes to be expressed from a wild type adenovirus.
- A second-generation adenoviral vector is characterized by the deletion of the E1A and E1B genes plus the E2 and/or E4 gene.
- A high-capacity adenoviral vector is the most advanced adenoviral vector; the only viral elements that are retained on the vector genome are the adenoviral ends that contain the origin of replication and the packaging signal.

Adenovirus

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Definition

Adenoviruses were originally isolated as etiologic agents for upper respiratory infections. Their name is derived from the initial observation that primary cell explants from human adenoids were found to degenerate secondary to the infection by an, at the time, unknown virus. The adenovirus particle is composed of an outer icosahedric capsid with an inner linear double-stranded DNA genome of approxi-

mately 36 kilobases (kb) size. There are seven capsid proteins, among them hexon, penton base and fiber, which are the major constituents of the adenoviral capsid. Internalisation of the viral particle during infection requires the interaction of the fiber and the penton base with surface proteins of the cell. At least five virally-encoded proteins are associated with the viral DNA. Adenoviruses have been identified in many species and more than 50 different serotypes have been isolated from humans alone. The individual serotypes are distinguished by different parameters such as immunological properties, tumorigenicity and DNA sequence. Some serotypes may cause more serious infectious diseases such as epidemic keratoconjunctivitis, gastroenteritis or hemorrhagic cystitis.

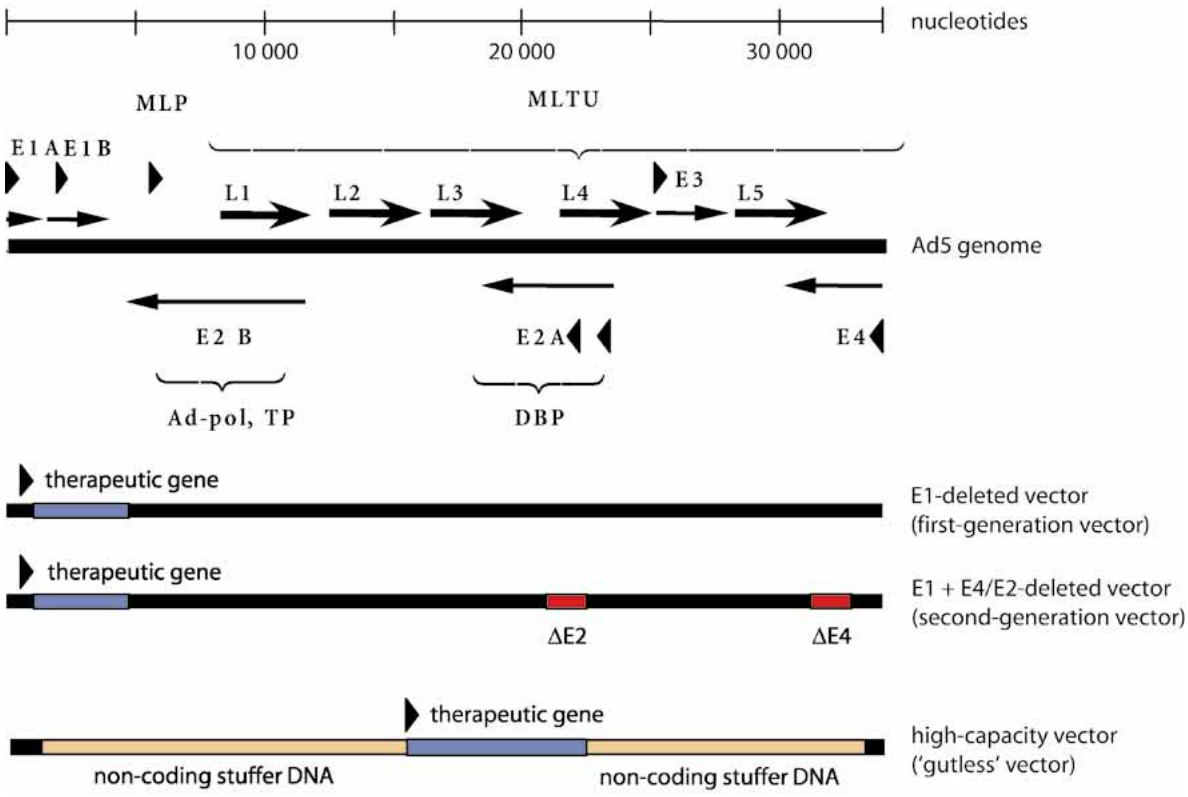
Adenovirus is being used as a gene carrier for →gene therapy. Most adenoviral vectors (see below) are derived from the serotypes 2 and 5 (Ad2, Ad5) which are frequent causes for mild colds. During childhood most individuals will become immunized against different adenoviral serotypes by natural infection. Ad2 and Ad5 are not oncogenic in humans and have a good safety record based on vaccination studies that have been performed in military recruits two to three decades ago. As detailed below, during natural infection of permissive cells the adenoviral DNA is transcribed, replicated and packaged into capsids within the nuclei of infected cells. Similar to other DNA viruses, two main phases can be distinguished during infection:

- an early phase that is characterized by the expression of the →early virus genes E1, E2, E3 and E4 and
- a late phase after onset of viral replication in which the viral structural proteins are produced.

Characteristics

Infection and viral transcription

A productive infectious cycle takes approximately two to three days and under optimal conditions about 1,000 to 10,000 infectious particles are produced in every infected cell. The infection begins with the attachment of the virus particle to the cell surface via interaction of the tip of the capsid fiber protein with the membrane protein CAR (Coxsackie-Adenovirus receptor). As it is apparent from the name, CAR is also used by some coxsackie viruses for adhesion to the cell surface and has been recently identified as a transmembrane protein of unknown function. The adenoviral particle is internalized by receptor-mediated endocytosis into clathrin-coated pits requiring a secondary interaction of the penton base with an αv-integrin. Following endocytosis the viral particle is sequentially disassembled, initally losing the fiber proteins, later most of the other viral structural proteins. Finally, the viral DNA is released as a DNA-protein complex through nuclear pores into the nucleus of the host cell. Shortly thereafter, transcriptional activation of the early genes E1A and E1B initiates a complex transcriptional program designed to first replicate the viral DNA and later to generate new infectious viral particles. The activation of early and late transcription units follows a relatively well understood transcriptional pattern. The gene products of the E1A and E1B genes are involved in the activation of both viral and cellular genes. Under certain conditions, in particular if infection of a cell does not result in a productive but rather abortive infection (abortive infection = the infectious cycle is blocked at an early stage following infection of the host cell), cellular transformation may be a consequence. The E2A and E2B gene products are involved in the replication of the viral genome and include the viral DNA polymerase (Ad-Pol), the terminal protein (TP) and the DNA-binding protein (DBP). The E3 and E4 gene products have diverse functions leading to transcriptional activation of other promoters, preferential export of viral RNAs out of the nucleus of infected cells and suppression of host defences. With the replication of the viral genome approximately six hours after infection, late phase transcription units are activated. Most of the late phase porteins are capsid proteins or proteins that are involved in the organisation and packaging of the viral genome inside the viral capsid. The most active promoter



Adenovirus. Fig. – Organisation of the adenovirus genome and the different adenoviral vector types employed for gene transfer. Promoters are indicated by arrowheads, transcribed genes by arrows. The genes that are transcribed early during infection are the E1A, E1B, E2, E3 and E4 genes. The main gene products, generated late during infection, are transcribed from the Major Late Promoter (MLP), which directs a very long RNA message (MLTU = major late transcription unit). Different RNA species (L1-L5) that code for structural proteins are generated by alternative splicing and differential polyadenylation (for clarity not all adenoviral genes and gene products are indicated). First-generation adenoviral vectors are characterized by deletion of the E1 genes, second-generation adenoviral vectors by the additional deletion of the E2 and/or E4 genes. High-capacity adenoviral vectors have most of the viral genome removed and retain only the non-coding viral ends. In high-capacity adenoviral vectors, stuffer DNA is included in the vector genome for stability reasons.

at this stage is the major late promoter (MLP) that directs the transcription of a large primary RNA transcript that covers more than two thirds of the viral genome. From this transcript five families (L1-L5) of structural proteins are generated by differential splicing and polyadenylation. During the course of an infection the metabolism of infected cells is redirected to support a predominant production and assembly of viral proteins.

Adenoviral functions and oncogenesis

Adenoviruses have played important roles as experimental tools in the discoveries of several fundamental principles in molecular biology, including RNA splicing and oncogenic transformation of cells. The induction of malignant tumors by injection of adenovirus type 12 in newborn hamsters was the first direct demonstration of a human virus causing malignant cellular transformation. This observation greatly stimulated the interest in using viruses as experimental systems in the study of the pathogenesis of cancer. While there is no epidemiological evidence for an involvement of adenoviruses in the pathogenesis of human cancers, several serotypes have been shown to cause tumors in rodents. Some serotypes, such as Ad12 or Ad18 are highly oncogenic

in animals, others, for example Ad4 or Ad5 have a low oncogenic potential. Based on several complementing observations cellular transformation is mediated by the viral E1A and E1B genes: In most virus-transformed cells the viral E1 genes are consistently found integrated into the cellular genome where they are expressed. Transfection of cells with the E1A and E1B genes is necessary and sufficent for cell transformation, and viruses with mutations in the E1 genes are defective for transformation. Several RNAs are transcribed from the E1A genes, the main species in Ad5 being the 12S and the13S RNAs coding for E1A proteins of 243 and 289 amino acids. To a large extent, the E1A proteins exert their transforming activity by interaction with cellular proteins that are involved in cell cycle regulation such as the tumor suppressor pRB. While E1A alone is capable of immortalizing cells, cooperation with E1B functions is required to achieve a full transformation phenotype. Two main proteins are produced from the E1B gene by alternative splicing. The 21 kD E1B protein that has been shown to inhibit apoptosis, and the 55 kD E1B protein that interacts with the tumor supressor protein p53. The expression of additional viral functions may contribute to E1 mediated tumorigenesis. For example, a 19 kD protein expressed from the E3 region can decrease MHC class I levels in transformed cells, and certain functions expressed from the E4 region can cooperate with the transforming activity of the E1B 55 kD protein.

Gene therapy: First- and second-generation adenoviral vectors

First-generation adenoviral vectors do not replicate in human cells under normal conditions because the E1A and E1B genes are deleted from the vector genome. These vectors are produced in complementing cell lines that express the E1A and E1B genes. First-generation vectors have been used for gene transfer in cultured cells, animals and even clinical trials in humans to express a large number of genes in different cell types and tissues. So far the results of experiments performed in animals and clinical studies in humans have been relatively disappointing. Several significant disadvantages of first-generation adenoviral vectors have been acknowledged:

- Because first-generation vectors still contain a nearly complete set of viral genes, toxicity and antiviral immune responses are frequently observed resulting in the clearance of transduced cells. Consequently, gene expression is only transient. Contributing factors for short-term gene expression include immune responses directed to the transgenic proteins expressed from the vector, if the organism is not tolerant to that protein.
- The upper DNA packaging limit for adenoviruses is about 38 kb. Because most viral genes are retained on the vector only about 7 to 8 kb of non-viral DNA can be incorporated into such vectors. However, in many conditions the therapeutic cDNAs are either large, additional elements have to be included to achieve regulated gene expression, or multiple genes need to be expressed to obtain a therapeutic effect. Thus, it is clear that the size constraints in first-generation adenoviral vectors may be a limiting factor for many potential applications. In order to further decrease expression of late viral proteins, adenoviral vectors with inactivation of the E2 and/or E4 functions in addition to the deletion of the E1 region have been generated. These vectors are produced in cell lines that complement both E1 and E2 and/or E4 functions. Currently it is controversial whether these second-generation adenoviral vectors have any significant advantages over first-generation vectors and lead to a longer duration of gene expression.

'Gutless' adenoviral vectors

Recently, in an attempt to address several of the problems observed with first-generation adenoviral vectors a novel adenoviral vector has been developed that will be useful for the functional analysis of genes *in vivo* and clinical studies. This vector has been variably called 'high-capacity (HC)' adenoviral vector, 'gutless', 'gutted' or 'helper-dependent (HD)' adenoviral

vector. Because all viral genes are deleted from this vector the capacity for the uptake of foreign DNA is more than 30 kb. The current production system involves the use of an adenoviral helper virus and takes advantage of the Cre-loxP recombination system. In this production scheme a first-generation adenoviral vector carries two loxP-recognition sequences that flank the adenoviral packaging signal. The vector is produced in E1-complementing cells that express the Cre-recombinase of bacteriophage P1. After infection of these cells both by helper virus and vector the packaging signal of the helper virus is excised. Therefore, vector and only little helper virus is packaged. From several *in vivo* experiments performed in different animal species it is apparent that these new vectors have clear advantages compared to earlier versions of adenoviral vectors and are considerably improved in safety and expression profiles. Their increased capacity for foreign DNA allows gene transfer of several expression cassettes, large promoters and some genes in their natural genomic context, a significant advantage over first and second-generation adenoviral vectors.

Replication-competent adenoviral vectors for cancer gene therapy

While the above mentioned adenoviral vectors have been widely used in preclinical and, with the exception of 'gutless' adenoviral vectors, also in clinical studies to express a wide variety of transgenes including cytokines, p53 and thymidine kinase (TK), it would be desirable to achieve gene transfer into all or most neoplastic cells within a tumor. This is clearly not possible with current vector technology. Recently, a new concept has been proposed that is based on the use of an adenovirus that is both replication competent and tumor-restricted in its growth. This virus is based on an Ad5 mutant virus that has an inactivating deletion within the E1B gene and does not express the E1B 55 kD protein. Initially, it was thought that replication of the virus was dependent on the p53 status of the host cell and that the virus was able to grow only in cells deficient for function p53 expression. However, more recent results indicate that the growth of this virus is independent of the p53 status cells and may depend on other cell cycle related factors. Currently, clinical studies are under way to have this new concept tested in different cancer types.

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Adenylyl Cyclase

Definition

Adenylyl or adenylate cyclase is the critical enzyme in the formation of cyclic adenosine monophosphate (cAMP). Various isoforms exist, they represent a ubiquitous group of enzymes that catalyse the formation of the second messenger cAMP from ATP. The substrate of the reaction is ATP into which a 3′-5′ link via phosphodiester bonds is introduced to form cAMP. The various mammalian isoforms are differentially regulated through → G-protein α-subunits and βγ-complexes as well as by Ca^{2+} or → protein kinase C.

ADH

Definition

→ Aldehyde dehydrogenase.

Adherens Junctions

Definition

Adherens junctions are intercellular junctional structures, most prominent in epithelial cells. In the adherens junction, the cell-cell adhesion is mediated by Ca^{2+}-dependent → cell adhesion molecules, the → cadherins; the cytoplasmic tail of these cadherins is indirectly linked to the actin → cytoskeleton.

Adhesion

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Definition

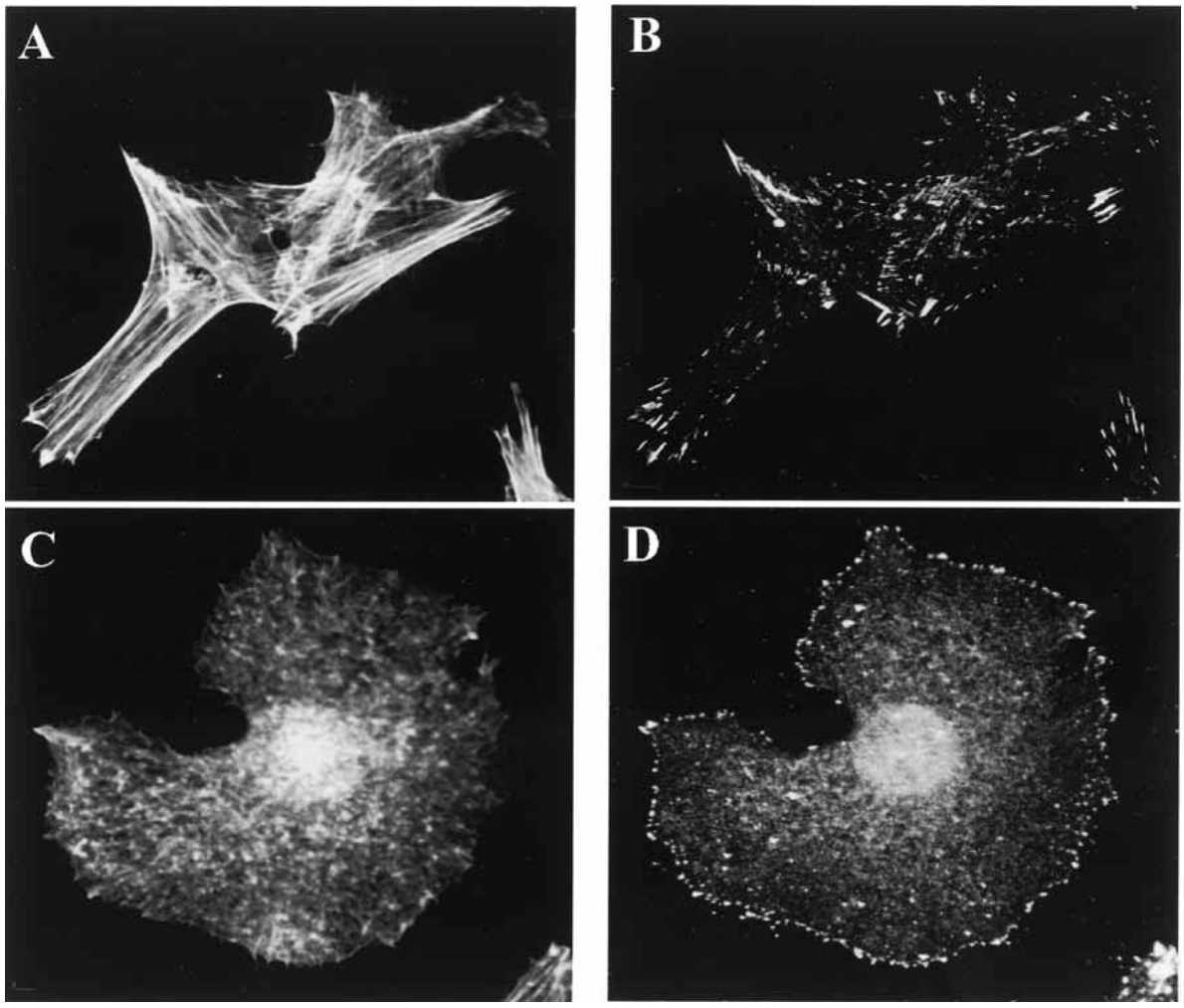
Cell adhesion is a dynamic process that results from specific interactions between cell surface molecules and their appropriate ligands. Adhesion can be found between adjacent cells (cell-cell adhesion) as well as between cells and the → extracellular matrix (ECM) (cell-matrix adhesion). Besides keeping a multicellular organism together, cell adhesion is also a source of specific signals to adherent cells; their phenotype can thus be regulated by their adhesive interactions. In fact, most of the cell adhesion receptors were found to be involved in → signal transduction. By interacting with growth factor receptors they are able to modulate their signaling efficiency. Therefore, gene expression, cytoskeletal dynamics and growth regulation all depend, at least partially, on cell adhesive interactions.

Characteristics

Cell adhesion receptors

Cell adhesion molecules were grouped into distinct classes according to structural and/or functional homologies. The following receptors have been directly implicated in the malignant phenotype of tumor cells.

- Integrins represent a family cell surface → glycoproteins that depend on divalent cations and are important in cell-ECM and cell-cell adhesion. The non-covalent association of an alpha and a beta subunit results in heterodimers that span the plasma membrane, enabling contacts with elements of the → cytoskeleton and signal transducing intermediates.
- The immunoglobulin superfamily of adhesion receptors is mainly involved in cell-cell adhesion. Named after a 90-100 amino acid domain that is also present in Ig molecules, these kind of receptors can be expressed either as plasma membrane-spanning molecules. However, some of them are alternatively spliced and are anchored to the cell membrane by covalent linkage to phosphatidylinositol.
- → Selectins represent a class of structurally related monomeric cell surface glycoproteins that bind specific carbohydrate ligands via their → lectin-like domains. Since the ligands are expressed in a specific way by vascular endothelial cells, selectins are important in lymphocyte trafficking and homing of malignant tumor cells.
- Cell surface → proteoglycans consist of → glycosaminoglycans (GAG) attached to core proteins through an O-glycosidic linkage. They can mediate cell-cell and cell-ECM adhesion.
- → CD44 comprises a large family of proteins generated from one gene by alternative splicing. Variants of CD44 (CD44v) differ from the standard form (CD44s) by their imlementation of ten variant exons in various combinations. Some variants have been causally related to the metastatic spread of some tumor cells. Among the ligands for CD44 are hyaluronic acid (HA), fibronectin and collagen, and chondroitin sulfate-modified proteins.
- → Cadherins are surface glycoproteins involved in cell-cell interactions. They are involved in the formation of adherens-type functions between cells. Through their cytoplasmic tail they interact with catenins, which are important for the signal transducing ability of cadherins.



Adhesion. Fig. 1 – Cell adhesion in normal (A, B) and cancer (C, D) cells. Normal mesenchymal cells show regular actin stress fibers (A: stained with phalloidin) and focal contacts (B: stained with anti-vinculin antibodies). In contrast, cancer cells (a highly motile melanoma cell is shown) often present with a completely disorganized actin cytoskeleton (C) and few focal contacts (D). Vinculin is typically arranged in patches at the periphery of the cell (D).
(Confocal micrograph courtesy of Dr. Jörg Hagmann, FMI, Basel).

- → Connexins are gap junction-forming proteins that oligomerize into specialized intercellular channels, connecting apposing plasma membranes. They allow the exchange of low molecular weight metabolites such as second messengers that are important in signal transduction.

Adhesion and cancer

The selective adhesion of one cell to another or to the surrounding ECM, is of paramount importance during embryonic development as well as for the maintenance of normal adult tissue structure and function. Severe perturbations of these interactions can be, at the same time, cause and consequence of malignant transformation and also play a fundamental role during malignant progression and metastatic dissemination (Fig. 1).

- Adhesion to the ECM through integrin receptors is important for anchorage dependent cell growth and cell survival. Normal cells that are detached from the ECM are locked in the G1 phase of the cell cycle

Adhesion. Table – Adhesion Receptors

family	main members	type of adhesion
integrins	characterized by the different α- and β-subunits	cell-ECM cell-cell
IgG superfamily	ICAM-1, V-CAM, N-CAM, CD2 (LFA2), LFA3, CD4, CD8, MHC (class I and II)	cell-ECM cell-cell
cadherins	E, P, L	cell-cell (adherens junction)
selectins	E, P, N	cell-cell
connexins	26 (tumor suppressor) 32 (liver) 43 (glial cells)	cell-cell (gap junctions)
cell surface proteoglycans	syndecan, glypican	cell-ECM cell-cell
CD44	CD44s, CD44v	cell-ECM cell-cell

(by loss of activity of the cyclinE/cdk2 complex) and undergo apoptosis (anoikis). Transformed cells, in which integrin signaling is altered, acquire the ability to grow in suspension and do not succumb to anoikis.

- Adhesion to neighboring cells, mediated by cell-cell adhesion molecules (e.g. N-CAM and C-CAM) and by gap-junctions, inhibits growth of normal cells (what is commonly known as 'contact growth inhibition'). Loss of these contacts due to the disrupted function of the relative adhesion molecules may result in uncontrolled proliferation.
- The differentiated state of mature cells (their 'identity') is also maintained through specific adhesion to the ECM and adjacent cells: a loss of identity is thus a likely consequence if specific contacts are lost, finally resulting in the ambiguous phenotype of many tumor cells.

Certain genes that code for cell adhesion molecules may therefore be considered as → tumor suppressor genes or even → metastasis suppressor genes since their loss or a functional mutation can strongly contribute to the acquisition of the malignant phenotype.

Adhesion in metastasis

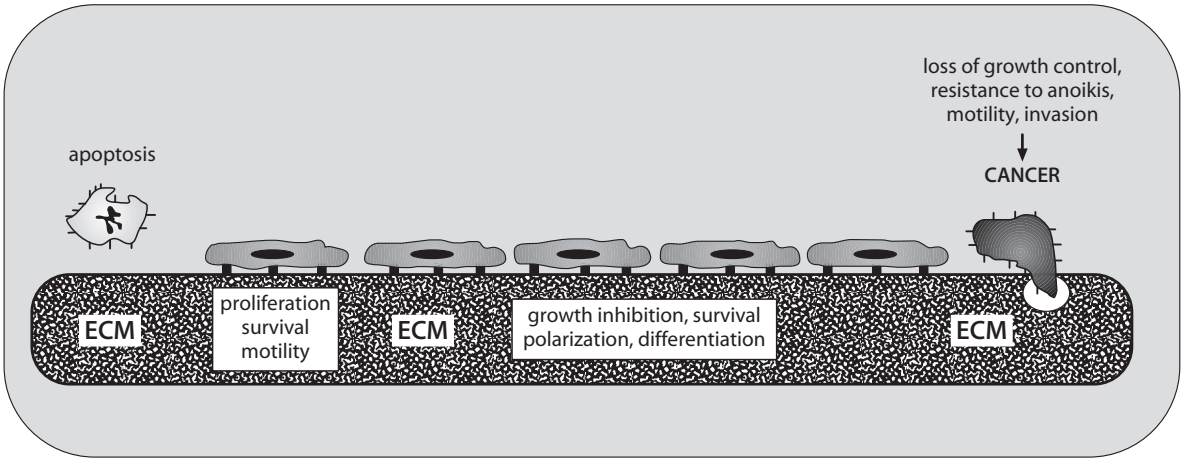
Adhesive interactions play a very critical role in the process of metastatic tumor dissemination, and the abnormal adhesiveness that is generally displayed by tumor cells appears to contribute to their metastatic behavior. Both positive and negative regulation of cell adhesion are required in the metastatic process, since metastatic cells must break away from the primary tumor, travel in the circulation where they can interact with blood cells and then adhere to cellular and extracellular matrix elements at specific secondary sites.

Adhesion within the tumor mass

The majority of normal adult cells are restricted by compartment boundaries that are usually conserved during the early stages of development of a tumor. Therefore, the detachment of malignant cells from the primary tumor is an essential step for the initiation of the metastatic cascade. During → tumor progression changes on the cell surface that lead to a weakening of the cellular constraints, contribute to the release of such mutant cells from the primary tumor mass. Indeed, it was found that tu-

mor cells separate more easily from solid tumors than normal cells from corresponding tissues.

- Cadherin expression has been shown to influence intercellular cohesion in direct correlation with invasive behavior. An increased cadherin expression in tumor lines, generally causes a tighter association of tumor cells. *In vitro* experiments have shown that cells which do not express cadherins or in which cadherins are functionally inhibited are more invasive than cells with normal cadherin activity. In cases where E-cadherin was involved, re-introduction of a wild type copy of the gene could revert the invasive phenotype. The loss of cadherin activity however, is not sufficient to make cells invasive. → Invasion also requires other cellular activities, such as → motility and protease production. *In vivo*, tumors expressing low levels of cadherins tend to be less differentiated and to exhibit higher invasive potential, although they are not necessarily more metastatic. In human cancer, a reduction in cadherin activity correlates with the infiltrative ability of tumor cells, a correlation that in many tumors is also retained in distant metastasis.
- A different type of cellular constraint is provided by gap junction communication. Gap junctions play an essential role in the integrated regulation of growth, differentiation and function of tissues and organs. The disruption of gap junction communication can cause irreversible damage to the integrity of the tissue and finally contribute to tumor promotion and malignant progression by favoring local cell isolation. There is experimental evidence that a loss of intercellular junction communication affects the metastatic potential of cell lines. Normal cells use gap junctions to control the growth of tumor cells. Once gap junctional communication is lost, the signalling mechanism responsible for the exertion of such growth control is also lost. Both quantitative and qualitative changes in gap junction protein (connexins) expression were found to be associated with tumor progression during



Adhesion. Fig. 2 – Cell adhesion and maintenance of a normal differentiated phenotype:

- Detachment of a normal cell from the extracellular matrix (ECM) would normally lead to apoptosis.
- Normal cells that keep contact with the ECM are protected from apoptosis and may migrate and grow. Normal cells tend to be organized as sheets onto the ECM, which contributes to their polarization and differentiation.
- Extensive intercellular contacts among cells adhered onto the ECM lead to contact-mediated growth inhibition.
- Tumor cells do not undergo apoptosis when detached from the ECM and may grow, migrate and invade into the matrix, to enter the circulation and give rise to distant metastases.

multistage skin carcinogenesis in the mouse model system as well as with tumorigenesis in a rat bladder tumor cell line.

Malignant tumor cells in the blood stream: Adhesion to blood cells and platelets

Blood-borne tumor cells undergo various homotypic and heterotypic interactions, the effect of which will also influence their metastatic behavior. Some of these interactions may be detrimental to circulating tumor cells such as tumor cell recognition by natural killer (NK) cells, or by tumor infiltrating lymphocytes (TIL). Others may provide, to a certain extent, a protective effect and/or contribute to metastatic spreading, such as interactions with platelets or, in certain cases, with leucocytes.

- *De novo* expression of the cell adhesion molecule ICAM-1 by melanomas might lead to heterotypic adhesion between melanoma cells and leukocytes bearing the relative receptor (LFA-1). Such interaction might thus enhance tumor cell adhesion to migratory and invasive leukocytes, thereby contributing to further dissemination of malignant tumor cells. In this regard, it has been suggested that site specificity of cancer metastasis might be, at least partially, a consequence of the formation of 'multicellular metastatic units' (MSU) consisting of tumor cells, platelets and leukocytes. A subset of leukocytes within the 'MSU' would be responsible for site-specific endothelium recognition, adhesion and stable attachment, thus serving as 'carrier cells' targeting the metastatic 'spheroids' to specific sites of secondary tumor foci formation.
- Several lines of evidence have provided strong support for the concept that tumor cell-platelet interaction significantly contributes to haematogenous metastasis. Two categories of molecules can trigger tumor cell induced platelet aggregation (TCIPA) and activation; soluble mediators and adhesion molecules. The latter are likely to be responsible for the initial contact between tumor cells and platelet cells, and might later stabilize the interaction. P-selectin and αIIbβ3 integrin on the platelet surface may bind Le^x carbohydrate determinants and fibrin on the surface of tumor cells, thus triggering platelet activation. Sialylation appears to be a general requirement for TCIPA, and → sialoglycoconjugates present on both tumor cells and platelets have been involved in tumor cell-platelet interactions. Mechanistically, platelets may contribute to metastasis by stabilizing tumor cell arrest in the vasculature, shielding tumor cells from physical damage, providing additional adhesion mechanisms to endothelial cells and subendothelial matrix, and serving as a potential source of growth factors. If tumor cell interaction with host platelets occurs while tumor cells are circulating, an organ-specific colonization ability of blood-borne tumor cells may be inflenced. In fact, the resulting embolus will be more easily arrested in the vasculature of the first organ downstream from the primary tumor site. If this organ represents a favorable milieu for tumor growth, then interaction with platelets will enhance tumor metastasis at that site; if this is not the case, it may prevent tumor cells from reaching their preferred organ and thus cause a reduction of the metastatic potential. It seems, however, that in most cases platelets are involved only after tumor cells have arrested, and platelet activation may then stabilize the initial tumor cell arrest in the microvasculature.

Adhesion in the target organ

Circulating tumor cells, either as single cells or most likely as homotypic and/or heterotypic aggregates that have escaped killing by the host immune system and lysis by mechanical shear forces associated with passage in the blood stream, need now to arrest in the microvasculature and extravasate into the organ parenchyma. In fact, the survival time of tumor cells entering the circulation is very short, usually less than 60 minutes. Therefore those cells that can rapidly arrest and are able to get out the blood stream might have a selective advantage in giving rise to metastatic colonies.

Specific adhesion in the target organ has been proposed as a critical determinant of organ specific metastasis, and experimental data indicates that malignant tumor cells preferentially adhere to organ-specific adhesion molecules. Tumor cells, for instance, adhered more efficiently to disaggregated cells or to histologic sections prepared from their preferred site of metastasis than from other organs. These type of assays, however, do not accurately mimic the physiological situation *in vivo*, where the first contact of circulating tumor cells happens with the luminal surface of the vascular endothelium, and, after endothelial retraction, with the subendothelial → basement membrane.

Adhesion to endothelial cells (EC)

The arrest of tumor cells in the capillary bed of secondary organs and their subsequent extravasation occur through interactions with the local microvascular endothelium and the subendothelial matrix.

- Biochemical heterogeneity of EC is related to both the heterogeneous microenvironment within tissues and the size of the vessel. Heterogeneity is seen in the differential expression of plasma membrane glycoproteins, cytoskeletal proteins and surface receptors in microvascular endothelium of different organs. Such heterogeneity of endothelium underscores the importance of using organ-specific capillary endothelium in studying the role of organ-specific tumor cell adhesion in metastasis.
- The specificity of the adhesive interactions that depends on the heterogeneity of microvascular EC and tumor cells, may favor, in a selective way, the initial adhesive events in preferred metastatic sites. As a consequence it may also facilitate metastatic dissemination to those organs, in a way that is similar to extravasation of lymphocytes from high endothelial venules of lymphoid tissues. In fact, lymphocyte 'homing' represents the paradigm for organ-specific cell adhesion and it has been shown to follow specific interactions between surface 'homing' receptors on lymphocytes with vascular 'addressins' expressed on the high endothelial venule surface. In a similar way, tumor cells express various combinations of cell surface molecules that may serve as ligands for EC surface receptors, which are typically induced upon stimulation by mediators of inflammation. A local inflammatory response might thus facilitate circulating tumor cells adhesion and arrest. The relevance of this type of interaction in directing tumor metastasis has been recently demonstrated *in vivo* using strains of transgenic mice that constitutively express cell surface E-selectin either in all tissues or in the liver alone. Metastatic tumor cells that do not express the ligand, colonized mostly the lung. However, following the induction of ligand expression, tumor cell colonization was redirected to the liver with tremendous efficiency.

Adhesion to extracellular matrix components

Mammalian organisms are composed by a series of tissue compartments separated from one another by two types of extracellular matrix (ECM): basement membranes and interstitial stroma. ECM consists of three general classes of macromolecules, including collagens, proteoglycans and non-collagenous glycoproteins (such as fibronectin, laminin, entactin and tenascin among others), which are expressed in a tissue-specific fashion.

Malignant cells arrested in the microcirculation sometimes do not migrate further into the organ parenchyma but grow locally in an expansive fashion until they rupture the vessel wall. In most cases however, the contact between tumor cells and the endothelium results in EC retraction with exposure of the underlying basement membrane, followed by invasion of tumor cells in the tissue.

The presence of specific adhesion receptors on the membrane of metastatic cells, and the peculiar composition of the extracellular matrix at a given site will influence tumor cell retention, motility and invasion, and growth at target organs.

- Electron microscopy observation on the formation of pulmonary metastasis has shown

that tumor cells often adhere to regions of exposed basal lamina. The exposed subendothelial matrix is usually a better adhesive substrate for tumor cells than the endothelial cell surface.

- In order to move through the ECM, tumor cells must make firm contacts with matrix molecules, be able to break these adhesive contacts as they move on and respond to chemotactic molecules that direct their movement. Interactions with the ECM may fulfill all these scopes, through the signaling effect of several cytokines (growth factors, motility factors, enzymes and enzyme inhibitors) that are stored bound to ECM molecules, and released upon interaction with tumor cells. Moreover, ECM macromolecules themselves may also function as motility attractants, and have been shown to stimulate both → chemotaxis and → haptotaxis. Haptotactic migration over insoluble matrix components may occur predominantly during the initial stages of metastatic invasion, while at later stages partially degraded matrix proteins, derived from proteolytic processing of the matrix, could be the major determinant of directed motility.
- Finally, it has to be considered that some ECM components may actually impede cell adhesion and thus might influence directional tumor cell motility by promoting the localized detachment of the trailing edge of migrating cells. ECM-associated chondroitin sulfate proteoglycans such as decorin, or the glycoprotein tenascin, have been suggested to modulate tumor cell adhesion and motility in this way.

Adhesion and drug resistance

The malignant phenotype of tumor cells depends, at least partially, on the weakening of cell-matrix and cell-cell interactions that occurs during tumor progression. However, late stage tumors maintain some level of intercellular adhesion, or even tend to reactivate certain adhesion mechanisms, indicating that modulation of cell adhesion is a dynamic process. Given the beneficial effect of cell adhesion on apoptosis resistance, an increased level of adhesion may facilitate survival of tumor emboli, and there is evidence that it can help tumor cells to evade the cytotoxic effects of anticancer therapy.

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Adhesion Molecules

Definition

Adhesion molecules are cell membrane molecules that either mediate adhesion among cells or attach cells to elements of the cellular matrix. Most adhesion molecules are also involved in signal transduction. There are five major families of adhesion molecules:

- cadherins
- integrins
- selectins
- members of the Ig (immunoglobulin) superfamily
- CD44.

Adjuvant Therapy

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Definition

Adjuvant therapy is an auxiliary therapy (e.g. ovarian ablation) administered concomitant with another therapy (e.g. surgery or radiation)in the treatment of primary breast cancer.

Characteristics

As defined in Webster's Deluxe Unabridged Dictionary adjuvant is:

1. 'an assistant';
2. 'in medicine, a substance added to a drug to aid in the operation of the principal ingredient' (e.g. Freund's adjuvant in immunotherapeutic research).

In clinical cancer research and treatment any therapy that in some way helps another modality is considered an 'adjuvant'. Most of the time cancer therapy is a multidisciplinary endeavor involving specialists from many treatment modalities.These specialists include (but are not limited to) medical oncologists, surgical oncologists and radiation oncologists. As more specific and targeted drug treatments become available, specialists in biologic and immunotherapeutic approaches will need to join the multidisciplinary team.

Cancer treatment for many tumor types involves some combination of surgery, cytotoxic chemotherapy and radiation therapy. In some hormonally-sensitive tumor types such as breast and prostatic cancer, hormonal manipulations may also be utilized. At present 'adjuvant' therapies usually imply systemic therapies such as chemotherapy or hormonal therapies, with surgery and/ or radiation therapy as the primary treatments. It is possible that as systemic therapies become more effective they may be the primary treatments, with surgery and radiation therapy then being used as 'adjuvants'.

It has been well established in animal tumor systems, that forms of chemotherapy unable to cure established metastatic cancer could lead to a cure in animals initially rendered disease-free by surgery. These seminal studies have been translated into the clinical setting. The concept of adjuvant therapy is based on the premise that even relatively early cancers may have already disseminated to distant sites by the time of diagnosis. With current diagnostic technologies it is seldom possible to detect systemic metastasis that are less than 1 cm in size. Since this would contain approximately one billion tumor cells, it is likely that many tumors may have already disseminated microscopically via the bloodstream leaving tumor foci of millions of tumor cells undetectable by current diagnostic imaging techniques. This premise underlies current theories attempting to explain why only 70% of women with early stage breast cancer (tumors between 1 and 2 cms with negative axillary lymph nodes) are cured by standard surgical techniques. Much research is being done in an effort to identify those 30% of women destined to relapse and ultimately die of metastatic breast cancer, so that aggressive chemotherapeutic 'adjuvant' therapies can be directed toward that high risk subset, and thus sparing the cured 70% the toxic side effects of chemotherapy. Various attempts at prognostication including (but not limited to) the analysis of bone marrow cells for micrometastasis using immunocytochemistry or, more recently, polymerase chain reaction technologies still require validation in large-scale clinical trials.

As examples, reasonably well accepted adjuvant therapies include; the use of chemotherapy, hormonal therapy or both after primary surgery ± radiation therapy for stages I and II → breast cancer; radiation therapy to the breast for women undergoing lumpectomy instead of mastectomy for early stage breast cancer; chemotherapy for primary colon cancer that has spread to regional lymphnodes; and radiation therapy after surgery for women with locally advanced cancers of the uterine cervix. In some cancers, such as small cell carcinoma of the lung, chemotherapy is generally consid-

ered to be the primary form of therapy thus making surgery and/or radiation therapy, when used, the 'adjuvants'. Similarly in certain forms of leukemia where chemotherapy is considered the standard form of treatment, the use of whole brain radiation to prevent central nervous system relapse has been used as another example of 'adjuvant' therapy.

In summary, as of the year 2000 the term adjuvant therapy in clinical cancer therapy is usually applied to the use of systemic cytotoxic chemotherapy and or hormonal therapy (for hormonally sensitive tumors). However, in reality the term adjuvant can be applied to any treatment modality applied (generally with curative intent) after whatever initial treatment modality is considered to be the standard primary therapeutic intervention.

Adoptive Immunotherapy

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Definition

Adoptive immunotherapy is the transfer of either donor or recipient immune system cells that may have been cultured *ex vivo* for therapeutic benefit. Cell populations that have been adoptively transferred in clinical studies include donor leukocyte infusions after allogeneic bone marrow transplant, lymphokine activated killer (LAK) cells, tumor infiltrating lymphocytes (TILs) and antigen specific cytotoxic T cells (CTLs). The two main applications of this approach have been in patients with cancer and viral infections.

Characteristics

Unmanipulated T cells from an allogeneic donor

When allogeneic hemopoietic bone marrow transplantation is undertaken for malignant disease, cure is achieved not only by the high dose chemotherapy and radiotherapy administered as conditioning but also by the → graft-versus-leukemia (GVL) effect. The presence of GVL was originally suggested by the higher relapse rates in recipients of syngeneic or T cell depleted transplants and lower relapse rates in patients who developed graft versus host disease. Adoptive immunotherapy with donor lymphocyte infusion provides a means of augmenting the GVL effect after allogeneic bone marrow transplantation to eliminate residual disease or treat relapse. In patients with chronic myeloid leukemia → CML who relapse post transplant with chronic phase disease approximately 70% achieved complete cytogenetic remission after infusion of donor lymphocytes. For other hematological malignancies relapsing after transplant, administration of donor lymphocytes has resulted in a much lower response rate with only 29% of patients with acute myeloid leukemia (AML) and 5% with acute lymphoblastic leukemia (ALL) responding. The response rate may be increased if cytokines such as → Interleukin-2 (IL2) are given to the patients in conjunction with adoptive immunotherapy. One of the risks of this approach is that the unmanipulated donor cells will also contain alloreactive T cells that can induce → graft versus host disease (GVHD). The reported incidence of GVHD with the administration of donor lymphocytes varies between 55 and 90%, with a treatment-related mortality of approximately 20%. One solution is to transduce donor T cells with a 'suicide gene' so they may be destroyed if GVHD occurs. The vector most commonly used encodes the Herpes simplex thymidine kinase (Tk) gene. Unlike mammalian Tk, viral Tk can phosphorylate nucleotide analogues such as ganciclovir rendering host cells sensitive to the cytotoxic effects.

Lymphokine activated killer (LAK) cells

Lymphokine-activated killer (LAK) cells can be generated by culturing peripheral blood lymphocytes with interleukin 2 (IL2) *ex vivo*. LAK cells nonspecifically lyse autologous and allogeneic tumor cells *in vitro* and were one of the first adoptive immunotherapy approaches used to treat advanced cancer. While

some benefit was seen in patients with renal cell cancer and melanoma, it was not greater than responses seen with IL2 alone.

Tumor infiltrating lymphocytes (TIL Cells)

Tumor infiltrating lymphocytes (TILs) are lymphocytes that have been harvested from tumor sites and expanded *ex vivo* with IL2. They have more specific anti-tumor activity *ex vivo* and in murine models than LAK cells. In clinical studies some responses have been observed, particularly in patients with renal cancer or melanoma, and in one study correlated with homing to tumor sites.

Antigen specific cytotoxic T lymphocytes

Another approach to selectively isolate cells with specific antitumor acidity is to generate antigen-specific cytotoxic T lymphocytes (CTLs) *ex vivo*. CTL recognize processed intracellular proteins presented as short peptide fragments (together with MHC molecules) on the cell surface. Hence, internal proteins unique to the malignant clone may act as tumor-specific antigens for CTL. A prerequisite for generating CTLs is identification of a target antigen and availability of a suitable antigen presenting cell. Because tumor-specific antigens have not been identified for most tumors, initial studies have focused on treatment of viral infections where antigens are better defined. Cytotoxic T cell lines are generated by co-culturing T cells with antigen presenting cells expressing or pulsed with the target antigen and then expanding by restimulation with antigen and addition of IL2. Antigen specific CTLs were initially used clinically to prevent or treat viral infections after bone marrow transplantation. Reactivation of CMV infection in immunocompromised patients results in significant morbidity and mortality. CMV-specific CTLs have been generated using peripheral blood lymphocytes from bone marrow donors stimulated with autologous CMV-infected fibroblasts. These cells have shown effectiveness in the prevention of CMV reactivation in a small group of patients.

In solid organ transplant and certain allogeneic bone marrow transplant recipients, who are receiving immunosuppressive therapy or who lack an efficient CTL response, → Epstein-Barr Virus latent infection can reactivate and induce EBV-associated lymphoproliferative disease (EBV-LPD). The malignant B cells express latent cycle virus-encoded antigens (i.e. EBNA 1, 2, 3A, 3B, 3C, LMP1, 2a, 2b), most of which are targets for virus-specific immune activity. The immunogenicity of these latent cycle virus-encoded antigens has been exploited to generate donor-derived EBV specific CTLs by culturing donor T cells with donor-derived EBV-infected lymphoblastoid cell lines. Patients receiving infusions of donor derived EBV-specific CTL as prophylaxis post-BMT, did not develop EBV-lymphoproliferative disease (LPD) in contrast with a 12% incidence in a control group who did not receive prophylaxis. EBV-specific CTLs were also successfully used to treat 2 patients with EBV-LPD who achieved complete resolution of their disease. In contrast to the experience with unmanipulated donor T cells, none of the patients developed acute GVHD.

This approach has also been extended to other viruses such as HIV and other EBV-associated malignancies such as → Hodgkin disease. Autologous CTL clones specific for the HIV protein gag were administered to patients with HIV. The infused CTLs accumulated adjacent to HIV-infected cells in lymph nodes and transiently reduced the levels of circulating productively-infected $CD4^+$ T cells. With advances in modern tumor immunology additional tumor specific antigens are being identified that may be candidate targets for CTL approaches.

Augmenting function of adoptively transferred immune cells

One way of enhancing the tumor-specific activity of T cells or CTL is to increase the levels of cytotoxic cytokines [e.g., tumor necrosis factor (TNF)] they produce at local tumor sites. Tumor cell recognition may be improved by the introduction into T cells of antigen-specific T cell receptors that confer novel recognition properties. For example, many tumors express surface antigens, which may be recognized in

their native form by specific antibodies. CTL expressing a chimeric gene encoding a single chain antibody variable region specific for tumor antigen linked to the intracellular gamma or zeta chain of the immunoglobulin receptor of the T cell receptor may recognize and kill the tumor cell via the chimeric receptor. So far there has been no evidence of therapeutic benefit in early studies of solid tumors.

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Adrenal Medulla

Definition

The adrenal glands, which lie in the abdomen adjacent to the kidneys, have two distinct components:

- the outer cortex, which secretes steroid hormones,
- the central medulla, which secretes catecholamines such as adrenaline and noradrenaline.

The medulla is of neural ectodermal origin.

AFB1

Definition

→ Aflatoxin B_1.

Aflatoxin B_1

Definition

Aflatoxin B_1 is a potent hepatocarcinogen produced by the mold *Aspergillus flavus;* → aflatoxins.

Aflatoxins

Definition

Aflatoxins are toxic fungal metabolites, some of which are hepatocarcinogenic, produced by some strains of *Aspergillus flavus*; → adducts to DNA; → detoxification.

AFP

Definition

→ α-Fetoprotein.

AKAPS

Definition

AKAP is the acronym for A-kinase anchoring protein. AKAPs are a family of functionally related proteins that bind the regulatory (R) subunit of cAMP-dependent protein kinase A (PKA) with high affinity. They target the kinase to specific subcellular organelles. This type of subcellular localization is a mechanism of compartmentalization of PKA. The compartmentalization of signal transduction enzymes into signaling complexes is an important mechanism to ensure the specificity of intracellular events. In part, the preferential phosphorylation of specific proteins by PKA is thus mediated by the anchoring of PKA to AKAPs that are positioned in close proximity to target proteins. A whole family of AKAPs exist but for

most of them only little is known about their exact function.

AKR

Definition

→ Aldo-keto reductase.

AKT

Definition

Akt is the mammalian homologue of retroviral oncogene v-akt encoding a serine/threonine protein kinase, also termed protein kinase B (PKB). It is a serine/threonine protein kinase, the cellular homolog of the viral transforming oncogene v-Akt. The c-Akt protein contains a pleckstrin homology domain and a catalytic kinase domain. c-Akt is activated by translocation to the cell membrane mediated by its lipid-binding pleckstrin homology domain and enzymatic activation by specific phosphorylation events. These phosphorylations are mediated by protein kinases that are themselves activated by phosphatidylinositol-3′-kinase. → Scatter factor-induced activation of c-Akt results in a cell survival signal that renders cells resistant to → apoptosis.

AKT Signal Transduction Pathway in Oncogenesis

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Definition

Akt, also called protein kinase B, represents a serine/threonine protein kinase subfamily. To date, three isoforms of this family have been cloned, AKT1/PKBα, AKT2/PKBβ, and AKT3/PKBγ. AKT1 is the cellular homolog of the transforming oncogene of the AKT8 retrovirus. At the amino acid level the overall homology of these three isoforms is >85%.

Characteristics

The genes, encoding the three isoforms of AKT are located on different human chromosomes (AKT1 on 14q32, AKT2 on 19q13.1-13.2 and AKT3 on1q44). AKT proteins share a very similar structure, they all contain an N-terminal pleckstrin-homology (PH) domain, a central kinase domain and a serine/threonine-rich C-terminal region. At the amino acid level the PH domain and C-terminal region of these three isoforms are more diverse (homology 73%~84%) than the kinase domain (90%~95%), suggesting that PH and C-terminal regions may represent functional difference between AKT1, AKT2 and AKT3. All three AKT isoforms localize to the cytoplasm but can translocate to nucleus in the cells-overexpressing exogenous AKTs following growth factor stimulation.

Expression and oncogenic activity

Although AKT1, AKT2 and AKT3 display high sequence homology, there are clear differences between these three isoforms in terms of biological and physiological functions:

- AKT1 expression is relatively uniform in various normal organs whereas high levels of AKT2 and AKT3 mRNA are detected in skeletal muscle, heart, placenta and brain,
- overexpression of wild type AKT2, but not AKT1, transforms NIH 3T3 cells,
- active forms of AKT1 and AKT2 are highly oncogenic.

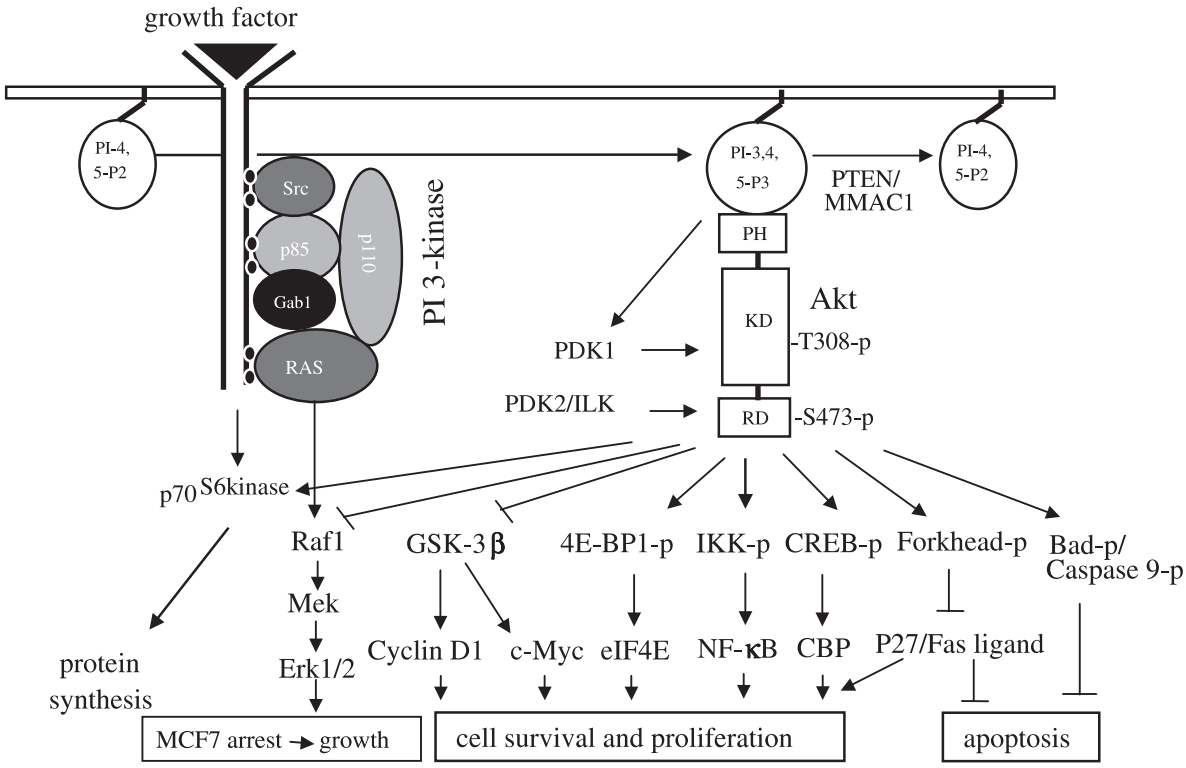
Alteration of Akt in human malignancy

Altered expression levels of AKT2 have been frequently detected in human cancer. Amplification of AKT2 was been observed in 15% of human ovarian carcinomas and 20% of human

pancreatic cancers. Alterations of AKT2 have been predominantly observed in late stage and high-grade tumors, suggesting that AKT2 plays an important role in tumor progression. In contrast to AKT2, AKT1 has been reported to be amplified in only a single human gastric carcinoma. Since it is located at chromosome band 14q32, proximal to the IGH locus, AKT1 had been proposed as a candidate gene targeted by 14q32 chromosome rearrangements in human T-cell malignancies, prolymphocytic leukemias, and mixed lineage childhood leukemia. Overexpression of AKT3 at the mRNA level has been shown in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer cell lines.

Signal transduction

Akt signaling has extensively been studied in the last 5 years. A variety of stimuli can activate Akt in a PI 3-kinase dependent manner. These include growth factors, protein phosphatase inhibitors, and cellular stress. Activation of Akt by growth factors depends on the integrity of the PH domain, which binds to PI 3-kinase product PI-3,4,5-P3, and phosphorylation of Thr-308 and Ser-473 by PDK1 and PDK2/ILK,



AKT Signal Transduction Pathway in Oncogenesis. Fig. – Proposed pathways of Akt signaling. Activation of Akt through the PI 3-kinase pathway involves recruitment of Akt to the cell membrane by means of PH domain binding to product of PI 3-kinase: $PI(3,4,5)P_3$. This promotes a conformational change in Akt which results in phosphorylation of Thr308 and Ser473 by PDK1 and PDK2, respectively. Activation of PDK1 and PDK2/ILK is also mediated by PI 3-kinase. Upon its release from the membrane, Akt becomes available to phosphorylate a) GSK-3β, leading to its inactivation; b) 4E-BP1, resulting in disinhibition of translation initiation factor; c) pro-apoptotic proteins BAD, caspase-9, and transcription factor FKHRL1 resulting in reduced binding of BAD to Bcl-X_L, inhibition of caspase-9 protease activity and Fas ligand/p27 transcription; d) IKKα leading to the degradation of IKBα and activation of NF-κB; e) Raf1 to overcome constitutively active MAPK-induced cell-cycle arrest in MCF7 cells. Another possible downstream target is the ribosomal protein $p70^{S6K}$. (KD = kinase domain, RD = regulatory domain, PH = pleckstrin homology domain). In addition, activated Ras, Src and Gab1 have been shown to induce the PI 3-kinase/Akt signaling pathway.

respectively (Fig.). In addition, growth factor-induced Akt activation is also mediated by Ras, Src, and Gab1, which bind and activate PI 3-kinase. Akt activity is indirectly regulated by the product of the PTEN tumor suppressor gene that is mutated in a number of human malignancies. PTEN encodes a dual-specificity protein and lipid phosphatase that reduces intracellular levels of PI-3,4,5-P3 in cells by converting PI-3,4,5-P3 to PI-4,5-P2 and, thus, inhibits the PI 3-kinase/Akt signaling pathway. In numerous cell types it has been shown that apoptosis, induced by growth factor withdrawal and loss of cell adhesion, can be suppressed by Akt. Possible mechanisms by which Akt promotes cell survival include phosphorylation of the proapoptotic proteins BAD, caspase-9, Forkhead transcription factors, and IκB kinase α/β (IKKα/β). This results in reduced binding of BAD to Bcl-XL, the inhibition of caspase-9 protease activity and Fas ligand and p27 transcription, and activation of NF-κB cascade. Moreover, glycogen synthase kinase (GSK)-3β is also phosphorylated and inactivated by Akt (Fig.). Akt inhibits Raf-MEK-ERK pathway through phosphorylation of Raf-1 in myotubes and MCF7 cells to overcome constitutively activated MAPK-induced cell cycle arrest. All the Akt phosphorylation proteins, except CREB, contain Akt phosphorylation consensus sequence RXRXXS/T (R is arginine; S/T is serine/threonine). In addition, Akt is capable of inducing Bcl2, Bcl-xl, c-myc, and cyclin D1 expression.

Akt pathway as a target for cancer intervention

Numerous studies showed that inhibition of PI 3-kinase activity by PI 3-kinase inhibitors (LY29004 and wortmannin) leads to cell growth arrest and programmed cell death. Dominant-negative p85, a regulatory subunit of PI 3-kinase, significantly diminishes Ras-transformation.

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ALA

Definition

→ 5-Aminolevulinic acid.

Aldehyde Dehydrogenase

Definition

Aldehyde dehydrogenases are a family of enzymes that catalyze the oxidation of aldehydes to carboxylic acids, using NAD^+ as cofactor.

Aldo-keto Reductase

Definition

Aldo-keto reductases are a superfamily of enzymes that catalyze the NADPH-dependent reduction of numerous carbonyl-containing compounds; substrates include, not only xenobiotics, but also monosaccharides, prostaglandins and steroid hormones. Some isoenzymes may also catalyze the oxidation of alcohols to aldehydes and ketones; → detoxication.

ALL

Definition

→ Acute lymphoblastic leukemia.

Alleles

Definition

Genes in the nucleus of the cell are present in pairs on the two homologous chromosomes, with one copy inherited from each parent. Many genes exist in a number of variant forms, known as alleles. A dominant allele prevails over a recessive allele. A recessive allele becomes apparent if its counterpart allele on the other chromosome becomes inactivated or lost.

Allelic Loss

Definition

Allelic loss is the deletion of one of the two alleles from the two homologous chromosomes. If two alleles can be distinguished by → RFLP or by other molecular genetic means (e.g. → microsatellite analysis), allelic loss can be identified as → loss-of-heterozygosity (→ LOH).

Allograft

Definition

Allograft is the engraftment of organs, cells or tissues between individuals of the same species.

α-Fetoprotein

Definition

α-Fetoprotein is a 70 kD glycoprotein synthesized in early embryonal development by cells of the yolk sac, liver and gastrointestinal tract.

α-Interferon

Definition

α-Interferon is a cellular cytokine that is produced by macrophages and exerts immunomodulatory, antiviral and antiproliferative functions. Recombinant forms of α-interferon are generally used for the treatment of viral → hepatitis.

ALPS

Definition

→ Autoimmune lymphoproliferative syndrome.

Alternative Lengthening of Telomeres

Definition

Alternative lengthening of telomeres (ALT) is a telomere maintenance mechanism that does not involve telomerase, which probably involves recombination. It is found in a minority of cancers and immortalized cell lines. A minority of immortalized cell lines and cancers have no detectable → telomerase activity and maintain their → telomeres by an alternative mechanism. Although the details are not yet known, it is likely to be a recombinational mechanism in which one telomere uses another telomere (or itself *via* looping back) as a template for synthesis of new telomeric DNA. Cells that maintain their telomeres by ALT characteristically have very heterogeneous telomere lengths, ranging from undetectable to extremely long.

Alternative Splicing

Definition

Alternative splicing is the excision and combination of the coding regions (exons) of the primary transcript of a gene in an alternative manner, i.e. not by normal RNA splicing; exons, which are often silent (alternative usage), are included into the mature RNA. The regulation of this process is not fully understood. Alternative splicing is a widespread gene regulation mechanism able to generate diversity in a reversible way and without requiring the expression of new genes. Some eukaryotic genes, such as the gene encoding for tenascin, produce multiple isoforms by alternative mRNA splicing. In general the exon changes caused by alternative mRNA splicing do not produce radically different proteins, rather they produce a set of similar proteins called protein isoforms.

Alu Elements

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Definition

The most abundant class of dispersed repeat elements in the human genome, and one member of the family of short interspersed repeat elements (SINEs). An estimated one million copies comprise about 10% of DNA in human cells.

Characteristics

Structure

Alu elements are 280 basepairs (bp) in length, and consist of two similar monomers that have homology to, and were originally derived from, the 7SL RNA gene. [Cytoplasmic 7SL RNA is one of the components of the signal recognition particle (SRP) that functions in protein translocation across the endoplasmic reticulum.] Individual *Alu* elements (Fig.) are flanked by direct repeats, end in a 3′ A-rich tract, and the left monomer contains an internal RNA polymerase III promotor which directs transcription initiation to the first residue of the element. *Alu* are retrotransposable elements, and several subfamilies, mobilised from different 'source' genes at different evolutionary times, can be recognised on the basis of their sequence divergence and diagnostic bases. Although recent investigations suggest that *Alu* expression increases in cells stressed by chemical agents or viral infection, most human *Alu* repeats are silent in somatic cells, with only the minor, evolutionarily younger, subgroups actively transcribed.

Alu Elements. Fig. – Genomic structure of *Alu* elements.

Function

The function of *Alu* elements is presently unknown but subject to intense debate and speculation. Proposed roles include modulation of chromosome structure and packaging of DNA around → nucleosomes, regulation of gene transcription possibly through *Alu*-specific protein binding domains, and initiation or switch sites for DNA replication in cells. *Alu* elements are also differentially methylated in male germ cells compared with female, suggesting a role in genomic → imprinting.

Role in Human Cancer Gene Mutations

Alu-mediated gene mutation underlies several important constitutional diseases, including familial breast and colon cancer. Different mechanisms for these mutations include recombination between homologous or nonhomologous regions of *Alu* elements at different locations within a gene, or on the same or different chromosomes, expansion of 3′ polynucleotide tracts to form fragile sites, or disruption of coding regions of functional genes by transpositional insertion of actively transcribed younger *Alu* elements. Instability of 3′ polynucleotide tracts may also indicate a DNA → mismatch repair deficiency. Although likely candidates to mediate somatic cell gene mutations leading to gene rearrangements with neoplastic potential, the reality and underlying mechanisms of involvement of *Alu* elements in these diseases has only recently begun to be appreciated.

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Alu Family

Definition

The *Alu* family are a set of dispersed, related sequences in the human genome each ~300 bp long. The individual members have *Alu* cleavage sites at each end.

Amino Acid Single Letter Code

Definition

There exists a three-letter abbreviation and a one-letter symbol for all of the 21 amino acids.

Amino Acid Single Letter Code. Table

Amino acid	Three-letter abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

5-Aminolevulinic Acid

Definition

5-Aminolevulinic acid is the precursor of protoporphyrin IX in the heme synthetic pathway. It is used as a photodiagnostic agent; → fluorescence diagnostics.

Aminopeptidase

Definition

An aminopeptidase is an enzyme that cleaves one or two (dipeptidase) amino acids at a time from the amino terminus of proteins and peptides.

AML

Definition

→ Acute myelocytic leukemia.

AML-1

Definition

Acute-myeloid-leukemia-1, also known as Runx-1, CBFA1 and PEBP2A2 is a DNA binding transcriptional regulator.

AML-1/ETO/CBFβ/TEL in Chromosomal Translocations

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Definition

The inv(16) is perhaps the most frequent translocation in acute myeloid leukemia (AML) accounting for up to 15% of all cases with discernable chromosomal abnormalities. This pericentric inversion leads to the fusion of the majority of CBFβ coding sequences to the coiled-coiled domains of a smooth muscle myosin heavy chain gene, *MYH11*. The second most frequent transolcation in acute myeloid leuke-

mia (up to 12% of the patients) is the t(8;21). The t(8;21) fuses the first 177 amino acids of AML-1 to nearly all of ETO. Finally, the t(12;21) is associated with acute B-cell leukemia (ALL) in up to 25% of the pediatric cases. It fuses the amino terminus of the TEL transcriptional repressor to the amino terminus of AML-1.

Characteristics

The AML-1/CBFβ transcription factor complex is frequently disrupted by chromosomal translocations in acute leukemia. AML-1 binds DNA in concert with CBFβ, which does not bind DNA alone, but increases the ability of AML-1 to bind DNA. Transcriptional studies demonstrated that AML-1 binds a DNA motif (T-G-T or C-G-G-T) that is required for the tissue-specific expression of a number of genes including some T-cell receptors, cytokines, and growth factor receptors. AML-1 can bind to co-activators, but its central role is as a promoter or enhancer 'organizing' factor. Thus, AML-1 synergistically activates transcription with a wide variety of transcription factors including → Myb, → AP-1, C/EBP, ETS [→ ETS transcription factors], and TCF family proteins. As well, AML-1 can repress transcription by interacting with the mSin3 and Groucho co-repressors.

By contrast, the translocation fusion proteins inhibit the expression of AML-1-dependent target genes. The t(8;21) fuses the amino terminal AML-1-DNA binding domain to nearly all of ETO. AML-1/ETO interacts with the mSin3 and nuclear hormone co-repressors that recruit histone deacetylases to repress transcription. It appears that the normal role of ETO is as a component of a co-repressor complex because ETO is found exclusively in high molecular weight complexes that co-sediment with histone deacetylases. Although ETO cannot bind DNA, it can interact with DNA binding proteins, such as PLZF. Thus, the t(8;21) fuses a co-repressor to the DNA binding domain of AML-1 to repress the transcription of AML-1-regulated genes.

The t(12;21) removes the TEL DNA binding domain, but retains the ability to regulate transcription via the AML-1 DNA binding domain. Structure-function analysis indicated that the t(12;21) fuses a repression domain from TEL to AML-1 to create a potent transcriptional repressor. The recognition that AML-1 binds co-repressors led to the observation that TEL also binds co-repressors. The first 336 amino acids of TEL that is fused to AML-1 by the t(12;21) contains domains that bind both mSin3A and the nuclear hormone co-repressors. Thus, the fusion protein contains at least two mSin3 interaction domains that creates a stable, perhaps unregulated association between TEL/AML-1 and the co-repressor. Because mSin3 and the nuclear hormone co-repressors recruit histone deacetylases to repress transcription, it is presumed that histone deacetylase inhibitors will inactivate the fusion protein. If so, the t(12;21) would be analogous to the t(8;21) in its mechanism of action.

The inv(16) fusion protein is perhaps the most intriguing of these chimeric proteins. By fusing the first 165 amino acids of the 182 amino acid CBFβ protein to a smooth muscle myosin heavy chain, the translocation creates a transcriptional repressor protein. Initial work suggested that the fusion protein dimerizes and when expressed at high levels it may be able to form rod-like structures. Because the fusion protein retains the AML-1-interaction domain, it may partially block AML-1-dependent transcription by preventing AML-1 from reaching the nucleus or by preventing it from binding DNA. However, more recent work suggests that the myosin heavy chain portion of the inv(16) fusion protein contains a transcriptional repression domain. In AML-1-dependent repression assays, the fusion protein cooperated with AML-1 to repress transcription. Thus, the inv(16) may create a co-repressor specific for AML-1. Because AML-1 uses the mSin3 co-repressor, it is possible that the inv(16), like the t(8;21) and t(12;21), may be sensitive to inhibitors of histone deacetylases.

Clinical relevance

The inv(16) or t(8;21) are good prognostic indicators, and up to 60% of patients with these translocations respond well to conventional chemotherapy. The t(12;21) is an excellent

prognostic indicator with up to 90% of the patients responding to chemotherapy. The response to chemotherapy may be due to fortuitous repression of the multi-drug resistance-1 gene by these chromosomal translocation fusion proteins.

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Amplification

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Definition

Amplification is the selective increase in DNA copy number either intracellularly, as a local genomic change, or experimentally, by polymerase chain reaction [→ PCR] (PCR). Increase in the level of mRNA or protein should not be referred to as amplification.

Characteristics

Intracellular amplification results in a selective increase in gene copy number with the consequence of elevated gene expression. Gene amplification has been seen in three different settings

- scheduled amplification as part of a developmental gene expression program, e.g. chorion genes in ovaries of the fruitfly *Drosophila melanogaster* or actin genes during myogenesis in the chicken
- unscheduled amplification during acquisition of cellular → drug resistance. For example; amplification of the gene encoding dihydrofolate reductase (DHFR) can result in up to 1000 gene copies per cell with the consequence of cellular resistance against methotrexate.
- unscheduled amplification of cellular genes involved in growth control (→ oncogenes) during tumor → progression. Amplification of oncogenes can result in up to several hundred gene copies and enhanced gene expression. Usually large DNA stretches (from 100 Kb up to several Mb) are amplified, and therefore → syntenic genes in addition to the particular oncogene can be co-amplified due to their close linkage to the oncogene. Alternatively, different → non-syntenic oncogenes can amplify independently in the same cell.

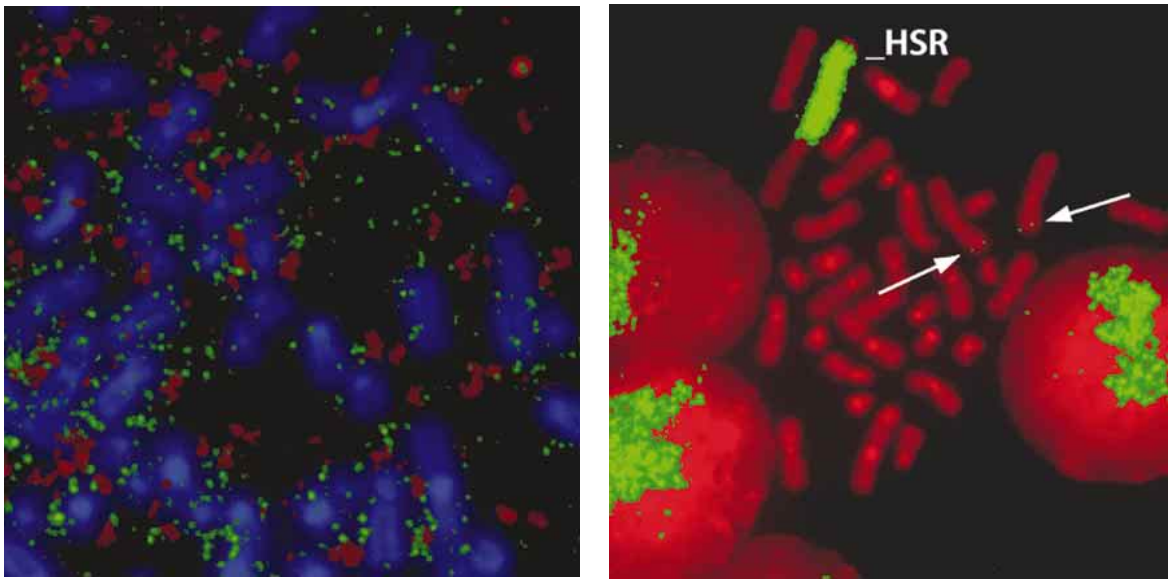
Amplified DNA can be visualized cytogenetically as a → homogeneously staining region within chromosomes (→ HSR), as → double minutes (→ DM) or as → C-bandless chromosomes (→ CM).

Cellular & Molecular Regulation

Amplification can follow different pathways, the 'onion skin model' and 'breakage fusion-bridge' (BFB) cycles both fit experimental observations. Little is known about genomic or environmental elements involved in amplification. Unscheduled amplification presumably is a sporadic event that can become stabilized under selective pressures, i.e. cytostatic drugs or if cells acquire a growth advantage within a certain tissue architecture.

Clinical Relevance

Resistance against cytostatic drugs poses a big problem in cancer therapy. Amplified oncogenes contribute to → tumor progression, many different oncogenes have been found amplified



Amplification. Fig. – Cytogenetics of *MYCN* amplification in neuroblastoma cells. Chromosomal fluorescence *in situ* hybridization. *MYCN* amplification appears in human neuroblastoma cells as two alternative cytogenetic manifestations:

1. **Double minutes (DMs)** (left), this tumor cell has in addition to amplified → *MYCN* (red) amplification of another oncogene *MDM2* (green). The two oncogenes are non-syntenic (2p24, and 12q13-14, respectively), and the amplification is the result of two independent genetic events.
2. **Homogeneously staining region (HSR)** (right), multiple copies are amplified in an HSR on chromosome 12 (with strong signal), while single copy gene is retained on the two parental chromosomes (arrows). The retention of *MYCN* at 2p24 indicates that not the original *MYCN* gene but rather a copy, presumably the result of extra-replication, has been amplified. Note also the strong signal in interphase nuclei which allows detection of amplified *MYCN* in tumor biopsies when chromosomes cannot be prepared.

(e.g. → *RAS*, → *MYC*, → *MYCN*, → *MYCL*, *HER-2* [→ HER-2/neu], → *ABL*, etc.), in some tumor types the oncogene status provides information about patient prognosis: Amplified *MYCN* indicates poor *prognosis* for stage 1-2 → neuroblastoma; and amplified *HER-2* indicates unfavourable outcome in a subgroup of → breast cancer.

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Amplified

Definition

Amplified, as related to gene → amplification, is the accumulation of multiple copies of a gene per cell.

A-MYB

Definition

A-MYB is member of the → *MYB* family of oncogenes.

Anchorage Dependence

Definition

Anchorage dependence describes the need of eukaryotic cells for a surface to attach to in order to grow in culture.

Androgen Signaling Pathway

Definition

Androgens are male specific hormones that are produced and secreted by the testes as well as by the adrenal gland; these hormones are essential for male characteristics and sexual function. With respect to the latter, androgens are necessary for the growth, differentiation, and function of the normal prostate and also drive proliferation and survival of prostate adenocarcinomas. Androgens or their derivatives bind to the androgen receptor, and the resultant complex serves as a sequence-specific transcription factor. Variations in the androgen receptor gene may correlate with prostate cancer [→ prostate cancer, basics and clinical parameters] risk, and mutations of the androgen receptor are frequently observed in late stage disease.

Anemia

Definition

Anemia is abnormally low hemoglobin concentration in the blood (females: < 120 g/l, males: < 130 g/l).

Aneuploid

Definition

Aneuploid is a chromosome constitution that differs from the usual diploid constitution, which occurs by loss or duplication of chromosomes or chromosomal segments. It results in abnormal gene imbalances, the resulting perturbation of normal gene expression along with other genomic changes and may contribute to tumorigenesis.

Aneuploidy

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Definition

Aneuploidy refers to the condition of having either less than or more than the normal diploid set of chromosomes in a cell. Such losses or gains (aneusomies) may involve segments of chromosomes or complete chromosomes leading to a major imbalance in the genetic makeup of the affected cells. Loss of one chromosome (monosomy) or both homologous chromosomes (nullisomy) as well as gain of one or more chromosomes (trisomy, tetrasomy etc.) have been observed in a variety of different cell types. Occasionally, gain of entire set of chromosomes (triploidy, tetrapolidy etc.) or loss of one set of chromosomes (haploidy) have also been reported. While germline inheritance of aneuploid condition often leads to developmental defects, somatic origin of similar anomalies are frequently associated with malignant phenotypes. It has been convincingly demonstrated that aneuploidy arise due to an underlying chromosomal instability in malignant cells.

Characteristics

Chromosomal instability in the form of aneuploidy, is one of the most common genetic anomalies detected in human cancer. Whether aneuploidy is a cause or consequence of cancer has long been debated. Several lines of evidence now make a compelling case for aneuploidy being a discrete mutation event that contributes to malignant transformation and progression. This evidence includes precise assay of chromosome aneuploidy in several primary tumors with *in situ* hybridization (ISH) and comparative genomic hybridization (CGH) techniques, which have revealed that specific chromosome aneusomies correlate with distinct tumor phenotypes. In addition, aneuploid tumor cell lines and *in vitro* transformed rodent cells have been reported to display an elevated rate of chromosome instability, thus indicating that aneuploidy is a dynamic chromosome mutation event associated with transformation of cells. Finally, most importantly, a number of mitotic genes regulating chromosome segregation have recently been found mutated in human cancer cells, implicating such mutations in the induction of chromosomal instability in tumors. Some of these gene mutations, possibly allowing unequal segregation of chromosomes, also cause tumorigenic transformation of cells *in vitro*. These findings provide a new direction towards understanding the molecular mechanisms responsible for induction of aneuploidy in cancer. The knowledge is expected to help design novel therapeutic strategies in the future.

Genetic alterations in cancer

Tumor cells manifest aberrations in genetic loci regulating growth, senescence and apoptosis. These observations have led to the current understanding of cancer as a genetic disease. The genetic changes identified in tumors include:

- subtle point mutations in genes at the nucleotide level,
- chromosomal translocations leading to structural rearrangements in genes,
- gain and loss of partial chromosome segments or whole chromosomes (aneuploidy) resulting in gene dosage imbalances.

Both segmental and whole chromosome imbalances leading to DNA dosage changes in cancer cells are manifestations of aneuploidy.

Aneuploidy in cancer

Aneuploidy involving one or more chromosomes has been commonly reported in human tumors. It is estimated that numerical chromosomal imbalance, referred to as aneuploidy, is the most prevalent genetic change recorded among over 20,000 solid tumors analyzed thus far. These observations were originally made using classical cytogenetic techniques late in a tumor's evolution and were difficult to correlate with cancer progression. More recent studies have reported an association of specific non-random chromosome aneuploidy with different biological properties such as loss of hormone dependence and metastatic potential.

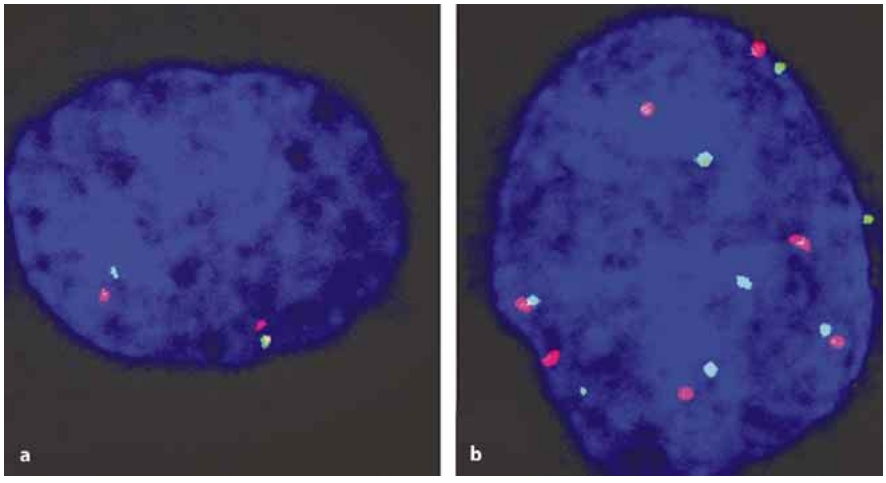
Classical cytogenetic studies of analyzing metaphase chromosomes from tumor cells had serious limitations in scope since these were applicable only to those cases in which mitotic chromosomes could be obtained. Because of low spontaneous rates of cell division in primary tumors, analyses depended on cells either derived selectively from advanced metastases or those grown *in vitro* for varying periods of time. In both instances, the metaphases analyzed represented only a subset of the tumor cell population. The two major advances in analytical cytogenetic techniques, *in-situ* hybridization (ISH) and comparative genomic hybridization (CGH), have allowed better resolution of chromosomal aberrations in freshly isolated tumor cells. ISH analyses with chromosome-specific DNA probes can be performed on interphase nuclei and allow assessment of numeric chromosomal anomalies within tumor cell populations in the contexts of whole nuclear architecture and tissue organization (Fig. 1). CGH allows genome-wide screening of chromosomal anomalies without the use of specific probes, even in the absence of knowledge of the

chromosomes involved. While both techniques have certain limitations in terms of their resolution power, they nonetheless provide a better approximation of chromosomal changes occurring among tumors of various histology, grade and stage compared with what was possible with the classical cytogenetic techniques.

DNA ploidy measurements, have also been performed with flow cytometry and cytofluorometric methods. Although these assays underestimate chromosome ploidy due to the possibility of a gain occasionally masking a loss in the same cell, several studies employing these methods have concluded that DNA aneuploidy closely associate with poor prognosis in different cancers. A few recently reported examples of aneuploidy in cancer are mentioned in the following discussion that deals with DNA ploidy measurements as well. Most of these observations are correlative without a direct proof of specific involvement of genes on the respective chromosomes. Identification of putative oncogenes and tumor suppressor genes on gained and lost chromosomes in aneuploid tumors, however, provide strong evidence in favor of chromosomes involved in aneuploidy playing a critical role in the tumorigenic process.

In renal cancer [→ renal carcinoma], either segmental or whole chromosome aneuploidy appears to be uniquely associated with specific histologic subtype. Tumors from patients with hereditary papillary renal carcinomas (HPRC) commonly show trisomy of chromosome 7, when analyzed by CGH. Germline mutations of a putative oncogene *MET* have been detected among patients with HPRC. It has been recently demonstrated that an extra copy of chromosome 7 results in nonrandom duplication of the mutant → *MET* allele in HPRC, thus implicating this trisomy in tumorigenesis. The study suggested that mutation of *MET* may render the cells more susceptible to errors in chromosome replication, and clonal expansion of cells harboring duplicated chromosome 7 reflects their proliferative advantage. In addition to chromosome 7, trisomy of chromosome 17 in papillary tumors and also of chromosome 8 in mesoblastic nephroma are commonly seen. Association of specific chromosome imbalances with benign and malignant forms of papillary renal tumors not only contributes to understanding of tumor origins and evolution but also implicate aneuploidy of the respective chromosomes in the tumorigenic transformation process.

In colorectal cancer [→ colon cancer], chromosome aneuploidy is common occurrence. Molecular allelotyping studies have suggested that the limited karyotyping data available



Aneuploidy. Fig. 1 – Fluorescence *in-situ* hybridization (FISH) analyses of normal human uroepithelial cells (A) and fresh human bladder tumor cells (B) performed with chromosome 20 short arm (labeled with red fluorescent dye) and long arm probes (labeled with green dye). Note the presence of two copies of chromosome 20 in normal uroepithelial cells in (A) but seven copies of chromosome 20 as evidence for aneuploidy in the bladder tumor cells.

from these tumors actually underestimate the true extent of these changes. Losses of heterozygosity, reflecting loss of the maternal or paternal allele in tumors, are widespread and often accompanied by a gain of the opposite allele. Thus, for example, a tumor could lose a maternal chromosome while duplicating the same paternal chromosome leaving the tumor cell with a normal karyotype and ploidy but an aberrant allelotype. It has been estimated that on an average, cancer of the colon, breast, pancreas and prostate may lose 25% of its alleles and it is not unusual for a tumor to have lost over half of all its alleles. In clinical settings, DNA ploidy measurements have revealed that DNA aneuploidy indicates high risk of developing severe premalignant changes among patients with ulcerative colitis, who are known to have an increased risk of developing colorectal cancer. DNA aneuploidy has been found to be one of the useful indicators of lymph node metastasis among patients with gastric carcinoma, and associated with poor outcome compared to diploid cases. However, CGH analyses of chromosome aneuploidy correlated gain of chromosome 20q with high tumor S phase fractions, and loss of 4q with low tumor apoptotic indices. Aneuploidy of chromosome 4 in metastatic colorectal cancer has recently been confirmed in a study that employed unbiased DNA fingerprinting by arbitrarily primed PCR to detect moderate gains and losses of specific chromosomal DNA sequences. The molecular karyotype (amplotype) generated from colorectal cancer revealed that moderate gains of sequences from chromosomes 8 and 13 occurred in most tumors suggesting that over representation of these chromosomal regions is a critical step for metastatic colorectal cancer.

Incidence of chromosome aneuploidy has also been evaluated as a marker of risk assessment and prognosis in several other cancers. Analyzing aneuploidy in non-surgically obtained squamous epithelial cells offers a promising non-invasive tool to identify individuals at high risk of developing head and neck cancer. Interphase FISH studies have revealed extensive aneuploidy in tumors from patients with head and neck squamous cell carcinomas (HNSCC) and also in clinically normal distant oral regions from the same individuals. It has been suggested that a panel of chromosome probes for FISH analyses may serve as an important tool to detect subclinical tumorigenesis and for diagnosis of residual disease. The presence of aneuploid or tetraploid populations are commonly seen in 90-95% of esophageal adenocarcinomas, and when detected with → Barrett esophagus, a premalignant condition, predicts progression of disease.

The significance of DNA and chromosome aneuploidy in other human cancers continues to be evaluated. Among papillary thyroid carcinomas, aneuploid DNA content in tumor cells has been reported to correlate with distant metastases reflecting worsened prognosis. Genome wide screening of follicular thyroid tumors by CGH, on the other hand, reveal frequent loss of chromosome 22 in widely invasive follicular carcinomas. Chromosome copy number gains in invasive neoplasm compared with foci of residual *in situ* carcinoma (DCIS) with similar histology have been proposed to indicate involvement of aneuploidy in progression of human breast cancer. ISH analyses of cervical intraepithelial neoplasia have suggested that chromosomes 1, 7 and X aneusomy are likely to be associated with progression toward cervical carcinoma.

Prognostic value of numerical chromosome aberrations remains a matter of controversy in human hematopoietic neoplasia. However, several studies suggest that the presence of monosomy 7 defines a distinct subgroup of acute myeloid leukemia patients. It is relevant in this context that therapy-related myelodysplastic syndromes have been reported to display monosomy 5 and 7 karyotypes reflecting poor prognosis.

Besides clinical correlative observations, role of aneuploidy in oncogenesis has been strongly supported by *in vitro* and *in vivo* transformation experiments performed with human and rodent cells. These studies revealed that aneuploidy is induced at early stages of tranformation. Transgenic mouse models with chromosome segment-specific duplications and deletions have been generated to investigate the effect of chromosome ploidy alterations during

development. Three duplications for a portion of mouse chromosome 11 syntenic with human chromosome 17 were established in the mouse germline. Mice with duplication of 1 Mb chromosomal DNA developed corneal hyperplasia and thymic tumors. The findings document a direct role of chromosome aneusomy in tumorigenesis.

Aneuploidy as a 'driving force' and not a 'consequence' in cancer

The presence of numerical chromosomal alterations in a tumor does not mean that the change arose as a dynamic mutation due to genomic instability. While aneuploidy as a dynamic mutation due to genomic instability in tumor cells would occur at a certain measurable rate per cell generation, a consequential state of aneuploidy is expected to be acquired by similar tumors at an unpredictable random rate. In addition to genomic instability, differences in environmental factors could explain high incidence of aneuploidy and other somatic mutations in tumors compared with normal cells. These include humoral, cell substratum, and cell-cell interaction differences between tumor and normal cell environments. It could be argued that despite similar rates of spontaneous aneuploidy induction in normal and tumor cells, the latter are selected to proliferate due to altered selective pressure in the tumor cell environment while the normal cells are eliminated through activation of apoptosis. Alternatively, it could be postulated that selective expression or overexpression of anti-apoptotic proteins or inactivation of proapoptotic proteins in tumor cells may counteract default induction of apoptosis in G2/M phase cells undergoing missegregation of chromosomes. Recent demonstration of overexpression of a G2/M phase antiapoptotic protein survivin in cancer cells suggests that this protein may favor aberrant progression of aneuploid transformed cells through mitosis. This would then lead to proliferation of aneuploid cell lineages that may undergo clonal evolution.

To investigate if aneuploidy is a dynamic mutational event, different human tumor cell lines and transformed rodent cell lines have been analyzed for the rate of aneuploidy induction. When grown under controlled *in vitro* conditions, such conditions ensure that environmental factors do not influence selective proliferation of cells with chromosome instability. In one study, Lengauer and colleagues provided evidence by FISH analyses that losses or gains of multiple chromosomes occurred in excess of 10-2 per chromosome per generation in aneuploid colorectal cancer cell lines. The study further concluded that such chromosomal instability appeared to be a dominant trait. Utilizing another *in vitro* model system of Chinese hamster embryo (CHE) cells, Duesberg and colleagues have also obtained similar results. With clonal cultures of CHE cells, transformed with nongenotoxic chemicals and a mitotic inhibitor, these authors demonstrated that the majority of the transformed colonies contained more than 50% aneuploid cells, indicating that aneuploidy would have originated from the same cells that underwent transformation. All the transformed colonies tested were tumorigenic. It was further documented that the ploidy factor, representing the quotient of modal chromosome number divided by the normal diploid number, in each clone correlated directly with the degree of chromosomal instability. Thus chromosomal instability was found proportional to the degree of aneuploidy in the transformed cells, and the authors hypothesized that aneuploidy is an effective mechanism of destabilizing the genome, and changing normal cellular phenotypes.

Aneuploidy and somatic gene mutation in cancer

The idea that numerical chromosome imbalance or aneuploidy is a direct cause of cancer was proposed at the turn of the century by Theodore Boveri. However, this hypothesis was largely ignored over the last several decades in favor of the 'somatic gene mutation hypothesis'. According to this hypothesis, mutations at the nucleotide level alone can cause cancer by either activating cellular proto-oncogenes to dominant cancer-causing oncogenes and/or by inactivating growth inhibitory tumor suppressor genes. In this scheme of things,

chromosomal instability in the form of aneuploidy is a mere consequence rather than a cause of malignant transformation and progression process.

Evidence accumulating in the literature on:

- specific chromosome aneusomies recognized in primary tumors,
- incidence of aneuploidy in cells undergoing transformation and
- aneuploid tumor cells showing a high rate of chromosome instability, have led to the rejuvenation of Boveri's hypothesis.

The role of aneuploidy as a cancer causing mutation helps resolve the paradox that with known mutation rate in somatic cells (~10^{-7} per gene per cell generation), tumor cell lineages cannot accumulate enough mutant genes during a human life time. It is argued that aneuploidy significantly enhances the mutation rate to cause transformation in somatic cells. The concept is gaining credibility following the discovery that genes regulating chromosome segregation are mutated in human cancer. Some of these genes have also been shown to have transforming capability in *in vitro* assays.

Potential mitotic targets involved in aneuploidy

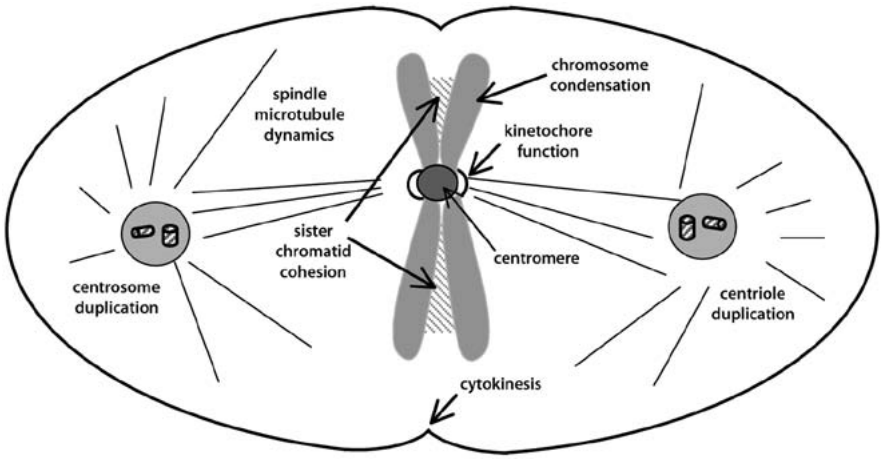
Since aneuploidy represents numerical changes in chromosomes, it is is expected that aneuploidy arises due to either failed segregation or missegregation of chromosomes during cell division. There are many mitotic processes and their regulatory proteins that could function in an anomalous manner to cause unequal segregation of chromosomes (Fig. 2).

Among the mitotic processes implicated in cancer, defects in centrosome function have been frequently suggested to be involved in a wide variety of malignant human tumors. Centrosomes play a central role in organizing the microtubule network in interphase cells and the mitotic spindle during cell division. Multipolar mitotic spindles have been observed in human cancers *in situ* and abnormalities in the form of supernumerary centrosomes, centrosomes of aberrant size and shape, as well as aberrant phosphorylation of centrosome proteins have been reported in prostate, colon, brain and breast tumors. It is conceivable that cells with abnormal centrosomes may missegregate chromosomes producing aneuploid cells. The molecular genetic mechanism(s) regulating centrosome structure/function that are aberrant in cancer cells remain to be elucidated. Recent discovery of a centrosome associated kinase STK15/BTAK (aurora 2), naturally amplified and over expressed in human cancers, has raised the interesting possibility that aberrant expression of this kinase is critically involved in abnormal centrosome function and unequal chromosome segregation in tumor cells. The presence of supernumerary centrosomes in aneuploid p53-deficient fibroblasts, and over expression of the centrosome kinase PLK1 in human non-small cell lung cancer have further validated the possibility that aberrant centrosome function is involved in aneuploidy and oncogenesis.

Untimely separation of sister chromatids has also been suspected as a cause of aneuploidy in human tumors. A vertebrate securin (v-securin) has recently been identified that inhibits sister chromatid separation and causes transformation of cells *in vitro*. It is proposed that elevated expression of v-securin may contribute to generation of malignant tumors due to chromosome gain or loss produced by errors in chromatid separation.

It is expected that for unequal chromosome segregation to be perpetuated through cell proliferation cycles giving rise to aneuploidy, checkpoint controls have to be abrogated. Inactivation of spindle checkpoint function in virally-induced leukemia has also recently been documented, with the finding that hMAD1 checkpoint protein is targeted by the Tax protein of human T cell leukemia virus type 1. Abrogation of hMAD1 function leads to multinucleation and aneuploidy.

The involvement of a DNA damage checkpoint defect in the induction of aneuploidy has also been indicated. Targeted deletion in the human → *BRCA1* gene, proposed to be involved in DNA damage checkpoint function, was shown to cause defective G2/M cell-cycle



Aneuploidy. Fig. 2 – Mitotic processes regulating equal segregation of chromosomes, which may become anomalous to cause aneuploidy. Aberrant expression of some of the proteins involved in these processes have been found oncogenic.

checkpoint function and genetic instability in mouse embryonic fibroblasts. The cells revealed multiple functional centrosomes and unequal chromosome segregation and aneuploidy. The findings raise the interesting possibility that aneuploidy, due to defective DNA damage checkpoint function, may be involved in the initiation/progression of malignancy among individuals carrying germline mutations of *BRCA1* gene.

Conclusions

Evidence from human tumor cytogenetic and molecular genetic studies provide compelling evidence in favor of aneuploidy being directly involved in the development of tumor phenotypes. Results from clinical findings support a correlation between origin of aneuploidy and tumorigenic transformation of cells. Molecular genetic analyses of tumor cells suggest that mutations in genes involved in controlling cell cycle checkpoint function and chromosome segregation play critical roles in causing chromosome instability leading to aneuploidy in cancer.

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Angiogenesis

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Synonyms

- Formation of new blood vessels

Definition

Angiogenesis is the formation of new capillary vasculature out of pre-existing blood vessels under the regulation of growth factors and in-

hibitors. It occurs in physiological (e.g. wound healing, ovulation, placental growth) and pathological (e.g. cancer, arthritis, inflammation) conditions.

Characteristics

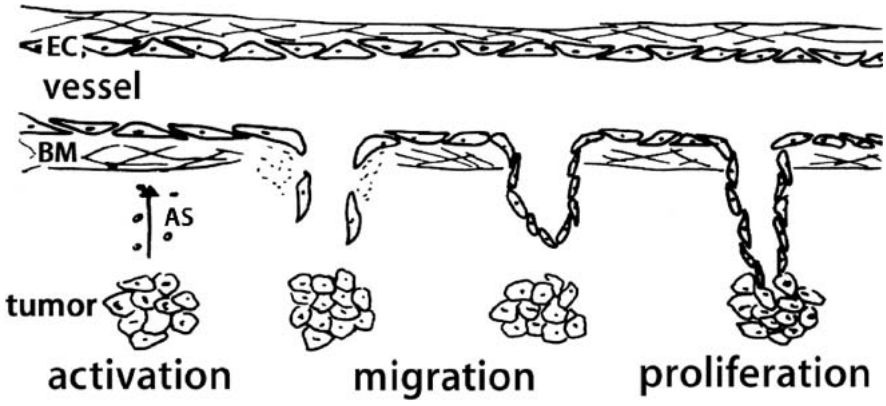
The formation of new blood vessels out of pre-existing capillaries, or angiogenesis, is a sequence of events that is of key importance in a broad array of physiologic and pathologic processes. Normal tissue growth such as in embryonic development, wound healing and the menstrual cycle is characterized by dependence on new vessel formation for the supply of oxygen and nutrients as well as removal of waste products. Also, in a large number of different and non-related diseases, formation of new vasculature is involved in abnormal physiology. Among these pathologies are diseases such as tissue damage after reperfusion of ischemic tissue or cardiac failure, where angiogenesis is low and should be enhanced to improve disease conditions. In a larger number of diseases excessive angiogenesis is part of the pathology. These diseases include cancer (both solid and hematologic tumors), cardiovascular diseases (atherosclerosis, restenosis), chronic inflammation (rheumatoid arthritis, Crohn's disease), diabetes (diabetic retinopathy), psoriasis, endometriosis and adiposity. These diseases may benefit from therapeutic inhibition of angiogenesis.

The initial recognition of angiogenesis being a therapeutically interesting process, began in the oncological arena in the early 1970s, when the hypothesis was put forward that tumors are highly vascularized and thereby vulnerable at the level of their blood supply. It was hypothesized that the process of angiogenesis might be a target for therapy. Since then, it was only after the discovery of the first compounds with specific angiostatic effects in the early 1990s, that the research field of angiogenesis rapidly expanded and provided an increasing body of evidence that inhibition of angiogenesis could attenuate tumor growth (1, 2).

The endothelial cells that line the blood vessels play a pivotal regulatory role in the execution of angiogenesis. The sequence of events in endothelial cells that follow the initiation of angiogenesis by exposure to (e.g. tumor derived) angiogenic stimulation consists of:

- synthesis of proteases that degrade the extracellular matrix,
- migration towards the stimulus,
- proliferation to increase the number of endothelial cells,
- differentiation in order to form a functional vessel (Fig.).

Negative interference in the different steps of the angiogenesis cascade enables different approaches for treatment of cancer:



Angiogenesis. Fig. – The angiogenesis cascade of endothelial cell activation, degradation of the extracellular matrix and the basal membrane, migration and proliferation. EC, endothelial cell; BM, basal membrane; AS, angiogenic stimulus (reviewed in Griffioen AW et al (1998) J Lab Clin Med 132: pp 363-368).

1. Neutralization of angiogenic factors - anti-vascular endothelial cell growth factor (VEGF) antibodies, dominant negative VEGF-receptors.
2. Inhibition of VEGF-receptors - anti-VEGF-receptor antibodies.
3. Desensitization of VEGF mediated intracellular signalling pathways - VEGF receptor tyrosine kinase inhibitor (e.g. SU5416, PTK787).
4. Inhibition of matrix metalloproteinases (marimastat, prinomastat).
5. Inhibition of endothelial cell adhesion (anti-avb3-integrin antibody Vitaxin).
6. Inhibition of endothelial cell migration (interferon-alpha).
7. Inhibition of endothelial cell proliferation (TNP-470, angiostatin, endostatin, anginex).

Clinical aspects

While many anti-angiogenic therapies for treating cancer were highly active in animal models, clinical results so far tend to be rather disappointing. This may either be a result of the fact that the most promising anti-angiogenic compounds have not been tested in the clinic yet, or that the read-out systems available for measuring clinical efficacy of anti-tumor drugs are not suitable for measuring anti-angiogenic effects. One of the advantages of anti-angiogenic therapy is believed to be the lack of induction of resistance to the therapy (3). This is explained by the fact that endothelial cells are genetically stable cells that are considered not to mutate into drug resistant variants. Although this is a beneficial feature of the anti-angiogenic approach, it is expected that inhibitors of angiogenesis will be used in future in combination with other anti-cancer modalities such as chemotherapy, irradiation and/or immunotherapy.

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Annealing

Definition

Annealing is the pairing of complementary single strands of DNA to form a double helix.

Anoikis

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Definition

Anoikis is the → apoptosis of cells that have lost contact with extracellular matrix, or that interact with matrix through an inappropriate integrin-matrix combination.

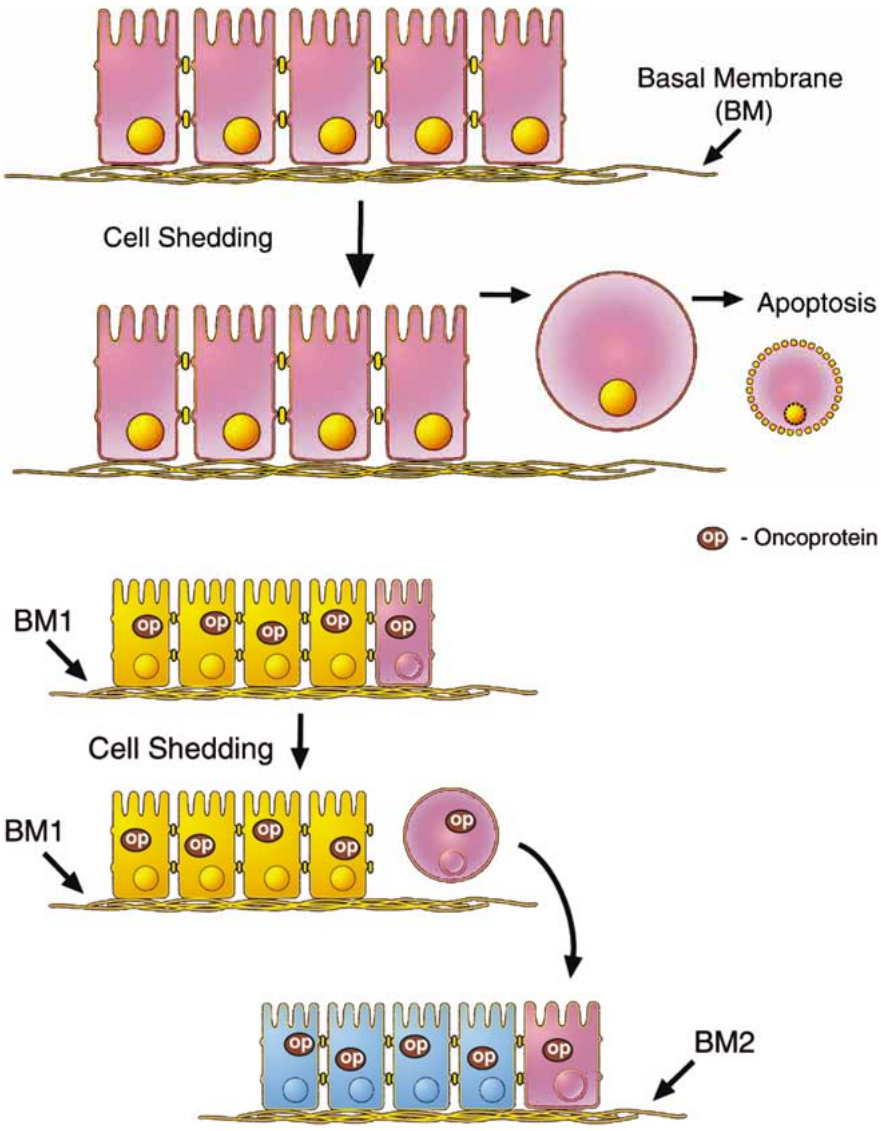
Characteristics

- Documented to occur in epithelial, endothelial, muscle cells and oligodendrocytes; also occurs in fibroblasts subjected to growth factor deprivation, possibly by different mechanisms;
- occurs through established apoptotic signaling pathways, which depend upon cell type. These include caspases of both the initiator (caspase-8) and effector (caspase-3,7) types as well as protein kinases such as MEKK-1/JNK; protection against anoikis is afforded by the activation of certain other kinases such as FAK and Akt. Bcl-2/Bcl-xL involvement depends upon cell type;
- in certain epithelial cell lines, cells must achieve complete cell-cell contact while attached to matrix, to become sensitive to anoikis;

- transformation by oncogenes or loss of tumor suppressor genes can render tumor cells resistant to anoikis, promoting anchorage-independent growth/metastasis.

Cellular and molecular aspects

- Cells undergoing anoikis-typical apoptotic characteristics: nucleosomal DNA ladder formation, cell shrinkage, caspase activation/cleavage of caspase substrates, cytochrome c release from mitochondria;
- anoikis is defined operationally, so whether a cell dies from cell-matrix dissociation or from another stimulus can only be determined experimentally, not retrospectively *in vivo*.



Anoikis. Fig. – Anoikis prevents the 'metastasis' of ephithelial cells. a) Epithelial cells interact with each other and with the basal membrane (BM1). Upon release from the tissue during normal turnover (upper panel), the shed epithelial cell undergoes anoikis. b) Transformation by oncogenes can prolong the survival time of this cell on BM1 such that it may attach to a new basal membrane (BM2), which is equivalent to metastasis (lower panel).

Clinical relevance

- Anoikis is thought to be relevant to normal tissue homeostasis of rapid turnover epithelial tissues such as in the digestive tract. It is also important for embryonic development (gastrulation) and mammary gland involution;
- sensitivity to anoikis is frequently lost in tumor cells, which probably contributes to their ability to grow independently of anchorage, and metastasize. It is also involved in muscle degeneration in muscular dystrophy;
- this sensitivity can be lost by activated oncogenes such as ras, alterations in intracellular apoptosis components, overexpression of growth factors such as EGF, HGF or IGF-related molecules, or breakdown of cadherin-catenin complexes.

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Anoxia

Definition

Anoxia is the absence of molecular oxygen in a tissue.

Ansomia

Definition

Ansomia is the lack of the sense of smell.

Anthracycline

Definition

Anthracycline is a chemical class of drugs isolated from cultures of *Streptomyces peucetius* that inhibit topoisomerase [→ topoisomerase enzymes as drug targets] II. Doxorubicin and mitoxantrone belong to this chemical class of compounds.

Antibody

Definition

An antibody is a protein (immunoglobulin) produced by B-lymphocyte cells that recognizes a particular foreign 'antigen' and triggers the immune response.

Antiestrogen

Definition

Antiestrogen is an agent that prevents the estrogenic hormone from interacting with its intracellular receptor; → estrogenic hormones and cancer.

Antigen

Definition

An antigen is any molecule whose entry into an organism provokes synthesis of an → antibody (immunoglobulin).

Antigen Processing

Definition

Antigen processing is the degradation of an antigen into peptide fragments that are displayed in association with HLA molecules on the surface of antigen-presenting cells.

Antigen Retrieval

Definition

Antigen retrieval is a process by which protein epitopes, altered during tissue fixation and processing, can be restored to a conformation that is recognized efficiently by a particular antibody. Antigen retrieval is accomplished by microwaving or pressure steaming.

Antigen-presenting Cells

Definition

Antigen presenting cells (APC) are highly specialized cells (B cells, macrophages and dendritic cells) that can process and present antigens for lymphocyte activation.

Antioxidant Defences

Definition

Antioxidant defences are intrinsic compounds (e.g. albumin, glutathione, uric acid) and enzymes (catalase, superoxide dismutase) that can convert, scavenge or inactivate free radicals (→ oxidative DNA damage). Dietary antioxidants, such as carotenoids, vitamins E and C, aid this process.

Antisense DNA Therapy

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Characteristics

It is increasingly clear that the process of tumorigenesis is intimately associated with the accumulation of specific genetic abnormalities. This recognition has led to the design of novel therapeutic strategies based on suppressing the activity of genes involved in tumorigenesis. Gene expression can be disrupted by a variety of methods targeted to the gene itself (e.g. homologous recombination), to the gene's transcriptional product (e.g. antisense strategies), or to the gene's protein product (e.g. expression of proteins with dominant-negative activity). These strategies are usually successful in tissue culture where cells subjected to gene transfer can be identified and expanded; they are, however, of limited value in anti-cancer DNA therapies where it is essential that many tumor cells carry and/or express the exogenous DNA sequences that can disrupt the function of the genes responsible for the growth advantage of neoplastic cells. Among the strategies directed to the suppression of gene expression, the most widely used (at least in preclinical models) involves the so-called 'antisense' oligodeoxynucleotides (ODNs). ODNs are short (15-20 nucleotides) single-stranded DNA sequences synthesized as exact reverse complements of the desired mRNA target's nucleotide sequence. Compared to longer DNA molecules, ODNs should exhibit more favorable cell uptake while preserving high specificity of sequence complementarity to the mRNA target. Once the ODNs form a specific DNA-mRNA duplex, translation of the message might be prevented and mRNA degradation promoted by activation of RNase H that cleaves the RNA component of the DNA-RNA duplex. The potential for highly specific targeting of mRNA transcripts of cancer genes contrasts with the mechanism(s) of action of conven-

tional anti-cancer chemotherapeutic agents, which block enzymatic pathways or randomly interact with nucleic acids irrespective of the cell phenotype. Anti-cancer chemotherapeutic agents exploit differences in biochemical or metabolic processes (e.g. growth rate) between normal and cancer cells for the preferential killing of neoplastic cells. In contrast, antisense ODNs have the potential to exploit the presence of genetically defined characteristics that distinguish neoplastic cells and are responsible for their growth advantage over normal cells. In recent years, the antisense strategy for cancer therapy has progressed from *in vitro* culture studies to investigations in animal models, and now to clinical studies. The principles underlying the *in vitro* experiments such as choice of target mRNA, oligonucleotide design, assessment of antisense effects apply also to the *in vivo* studies. We describe here the current state of progress toward gene-directed antisense-based therapies, primarily from studies in animal models and phase I clinical investigations in hematological malignancies.

Target choice and oligonucleotide design

The choice of the target mRNA selected for inhibition by antisense ODNs is dictated by the biology of a particular disease process and by the ability to predict the effects that may be achieved by inhibiting the expression of a particular cancer gene. For example, the bcr/abl [→ BCR-ABL1] transcripts of chronic myelogenous leukemia (→ CML) cells serve as an ideal target because of the role of the BCR/ABL oncoprotein in hematopoietic cell transformation and in the maintenance of the leukemic phenotype. Since bcr/abl genes are only found in leukemic cells, targeting their mRNA transcripts might also provide the advantage of a specific effect against tumor cells. Targeting bcl2 mRNA in lymphomas with the t(14;18) translocation is appropriate not only for the disease-causing effect of Bcl2 expression but also for the importance of interfering with anti-apoptotic pathways in drug response. Thus, *Bcl2* antisense ODNs, in addition to their direct effects on target cells, may also sensitize these cells to chemotherapeutic agents that promote → apoptosis. In most published studies, the sequence of the ODN targeting mRNA transcripts of a disease-causing gene is selected empirically with a preference for the mRNA transcription initiation sequence or the nucleotides surrounding the translation initiation codon. However, there are now novel approaches of oligonucleotide design based on the use of the DNA chip [→ microarray (cDNA) technology] technology and hybridization with labeled RNA to dissect accessible sites in the mRNA tertiary structure (1).

Early investigations of ODN-targeting of growth-regulatory mRNA transcripts employed natural DNA; the realization that natural ODNs are rapidly cleaved by endo-and exonucleases led to the development of nuclease-resistant ODNs by modification of the internucleotide linkages. The most common modification is the replacement of the nonbridging oxygen atoms in the phosphate group with a sulfur group. This type of modification generates the so-called phosphorothioate ODNs extensively used in preclinical studies and in phase I clinical trials. The phosphorothioate modification results in several desirable properties such as nuclease resistance, water solubility, and activation of RNase H. Nevertheless, it presents also certain disadvantages, including impaired uptake caused by the polyanionic nature of phosphorothioate ODNs, and non-sequence-dependent effects attributed to charge interactions between phosphorothioate ODNs and proteins in the extracellular environment, on the cell surface, and intracellularly. A number of strategies have been utilized to minimize the undesirable effects of the phosphorothioate ODNs while preserving their useful properties. Since these modified phosphorothioate ODNs have not been tested sufficiently *in vitro* and in *in vivo* models, we will focus on first-generation phosphorothioate ODNs with regard to delivery, subcellular trafficking, pharmacodynamics, and applications in mouse models and in humans.

Delivery, subcellular trafficking, and pharmacodynamics of ODNs

Native and phosphorothioate ODNs are polyanionic molecules that cross cell membranes inefficiently. There is evidence that ODN uptake is time- and concentration-dependent (2). Below a concentration of 1mmol/l, uptake of phosphorothioate ODNs is predominantly via a receptor-like mechanism, while fluid-phase endocytosis appears to predominate at higher concentrations (2). Several receptor-like proteins potentially involved in ODNs uptake have been identified, but evidence that they are responsible for ODNs uptake is still lacking. In culture, ODN uptake may be enhanced by a number of procedures directly or indirectly modifying the permeation properties of the ODNs. The most common methods are electroporation and streptolysin treatment which result in physical disruption or enhanced permeabilization of cell membranes. Such procedures are inpractical for *in vivo* studies, which, at present, rely on the administration of naked DNA. Inside the cells, ODNs accumulate in vacuoles, presumably endosomes and lysosomes, and slowly redistribute to the cytoplasm and nucleus where they may interact with their target mRNA molecules (3). Accordingly, strategies that promote the release of ODNs from endosomal structures may enhance the ODN's antisense effects. Pharmacokinetics and metabolism of antisense ODNs have been investigated in a variety of animal systems and also in few human trials (4, 5). In most reports, the analyses were carried out after intravenous or intraperitoneal administration. Approximately 30% of the injected dose is excreted in the urine within 24 hours and intact material is detected in most tissues up to 48 hours, and up to 7 days in liver and kidney, the organs where most ODNs accumulate. Plasma clearance is biphasic with an initial half-life of 15-25 minutes and a second half-life of 20-40 hours. Potential toxic effects of ODNs administration have been reported in rodents and in primates. Mice receiving high doses of phosphorothioate ODNs show decreased platelet counts probably related to the polyanionic charge of the ODNs. Cardiovascular toxicity, rapid pheripheral vasodilatation and death have been reported in monkeys. These effects were noted after rapid bolus administration of large doses, while slow infusion of similar doses appeared to be well tolerated.

Clinical applications of antisense ODNs in hematological malignancies

Early clinical experiences with antisense ODNs have been reported by groups targeting oncogene or apoptosis regulators. These studies were based on encouraging anti-tumor effects of systemically delivered ODNs in mice injected with leukemia or lymphoma cells of human origin (6-9). For example, the disease process induced by Philadelphia[1] leukemia cells was suppressed by the systemic delivery of antisense ODNs targeting bcr/abl or c-→ myb transcripts (6, 7). In particular, the anti-leukemia effects of the bcr/abl antisense ODNs was markedly enhanced by the combination with low-doses of cyclophosphamide (8). In the context of chronic myelogenous leukemia (CML), oligodeoxynucleotides targeting bcr/abl or c-myb mRNA have been used as marrow purging agents in the chronic as well as accelerated phase of the disease. Eight patients with CML in advanced phase were subjected to autologous bone marrow transplantation after bone marrow purging with bcr/abl antisense ODNs (10).

Infusion of the ODN-treated cells was followed by prompt engraftment and hematologic reconstitution in all patients. Evaluation of anti-leukemia effects by standard cytogenetic analysis and fluorescence *in situ* hybridization showed a complete karyotypic response in two cases and a minimal or no response in the other six. Survival of transplanted patients exceeded three years in some cases, but it is not clear that the protocol had therapeutic efficacy.

However, lack of toxicity, prompt hematopoietic reconstitution, and karyotypic response in some cases, are all encouraging observations for designing additional clinical trials. In a different study, eight CML patients were subjected to bone marrow transplantation using autologous hematopoietic progenitors (CD34+) cells that were pre-treated with antisense

ODNs targeting the mRNA transcripts of the *c-myb* gene, a key regulator of normal and leukemic hematopoiesis (11). After transplantation, seven of eight patients engrafted. Of these, four patients showed 80 to 90% normal metaphases three months post autologous bone marrow transplant, suggesting that the antisense ODNs treatment eliminated the majority of Philadelphia[1] CML cells. These patients showed hematologic improvement during the period (6 to 24 months) following the bone marrow transplant (11).

18 patients with refractory acute myelogenous leukemia were also treated by continuous infusion of c-myb antisense ODNs at dose levels ranging from 0.3 to 2.0 mg/kg/day for 7 days. There was no treatment-related toxicity, but only one patient showed a therapeutic response (11).

Studies in a mouse model of lymphoma with the t(14;18) associated with Bcl2 overexpression have demonstrated dose-dependent disease eradication in most mice treated with antisense ODNs targeting a segment of the Bcl2 open reading frame (8).

On the basis of these preclinical data, Bcl2 antisense ODNs were given via a continuous subcutaneous infusion for 2 weeks to lymphoma patients with high Bcl2 expression and resistant to conventional therapies (12). Therapeutic responses assessed by computed tomography scanning were demonstrated in six out of nine patients. The specificity of the antisense effects was validated by showing a decrease in Bcl2 levels in lymph node aspirates taken at different times after initiating the antisense ODNs therapy.

Prospects for antisense DNA therapy

Continuous advances in understanding the genetic basis of tumorigenesis are leading to the identification of an ever-increasing number of gene targets for antisense ODNs-based therapies. Most disease-causing genes identified by molecular genetics belong to the class of cell-cycle and apoptosis regulators. Accordingly, antisense ODNs may be used individually or in combination against these targets; moreover, antisense ODNs might be combined with conventional chemotherapeutic drugs to enhance apoptosis susceptibility of tumor cells. It might therefore be conceivable that various therapeutic strategies involve ODNs that target tumor-causing genes. Considering this, the success of antisense ODNs-based anti-tumor therapies is likely to depend on the development of antisense ODNs as effective therapeutic agents. Delivery of sufficient amounts of ODNs to tumor cells remains an important problem. Administration procedures that may guide ODNs to tumor cells are of great interest; in a recent *in vitro* study, neuroblastoma cells were targeted on the basis of the expression of the neuroectodermal-specific GD2 disialoganglioside by antibody-coupled neutral liposomes encapsulated with *c-myb* antisense ODNs (12). Although it is unknown if such an approach may function *in vivo*, this is an example of a potentially useful strategy. The delivery of sufficient amounts of ODNs to tumor cells does not guarantee that they will find the mRNA targets once inside the cells. Thus, methods promoting the intracellular trafficking of ODNs, to enhance the access to as many as possible mRNA target molecules, will be invaluable for efficacious ODNs-based therapies. The development of novel classes of ODNs with fewer non-specific interactions to non-target molecules will also improve the efficacy of antisense ODNs therapies.

If the goal of making effective ODN drugs is to be achieved, these and other problems need to be addressed.

While the principles underlying ODNs-based therapies remain highly attractive, the field of DNA therapeutics is now at a crossroad where rigorous validation in clinical trials is necessary.

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Antisense Nucleic Acid

Definition

Antisense nucleic acids are nucleic acids (single stranded DNA or RNA of various length) that are complementary to the mRNA of a certain gene. The antisense nucleic acid binds to the mRNA and, by mechanisms that are not completely understood, inhibits its natural function, i.e. translation into protein. Antisense nucleic acids are widely used to study the effect of genes in cultured cells. The potential of antisense nucleic acids in gene therapy, for instance to downregulate the expression of overexpressed genes, is being evaluated.

AP

Definition

1. Adaptor protein

AP1, → AP2, AP3, AP4; complexes involved in membrane protein sorting.

2. Activating protein

→ AP-1, → AP-2; complexes involved in transcriptional regulation of other genes.

AP-1

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Definition

Activating protein-1 (AP-1) is a transcription factor usually consisting of a member of the Jun family and a member of the Fos or ATF family of proto-oncogenes. AP-1 is activated in response to cytokines, growth factors and stress factors during cell differentation, tumor formation or mitogenic response.

Characteristics

Much of our present knowledge about transcription factors came from the discovery and study of the activating protein-1 (AP-1) family. AP-1 (and the transcription factor NFκB) has served to identify one of the decisive DNA binding motifs required for gene regulation by a variety of extracellular signals. These extracellular signals include growth factors, cytokines, tumor promoters, such as the phorbol ester → TPA (12-O-tetradecanoyl-phorbol-13-acetate) and carcinogens, for example UV irradiation and other DNA damaging agents. One of the members of the AP-1 family, the heterodimer Fos-Jun, was found in the mid-1980s as a protein complex containing the viral oncogene product Fos, but its function was unclear. The term AP-1 was coined for an activity that supports basal level transcription *in vitro* and to bind to a decisive control element of the human collagenase I promoter to mediate gene regulation in response to TPA. These findings served to establish the role of AP-1 in controlling both basal and inducible transcription of several genes containing AP-1 sites (5′-TGAG/CTCA-3′), also known as TPA-responsive elements (TRE). The first member of the Jun family, c-Jun, represents the cellular homologue of the transforming oncogene (v-Jun) of the chicken retrovirus ASV-17. At present, the Jun protein family consists of c-Jun, JunB and JunD. The Fos protein family consists of c-Fos, FosB, Fra-1 and Fra-2. In the last decade additional proteins, such as members of the ATF family (ATFa, ATF-2, ATF-3), have been identified that share structural homologies and form heterodimeric complexes predominantly with Jun proteins and bind to TRE-like sequences.

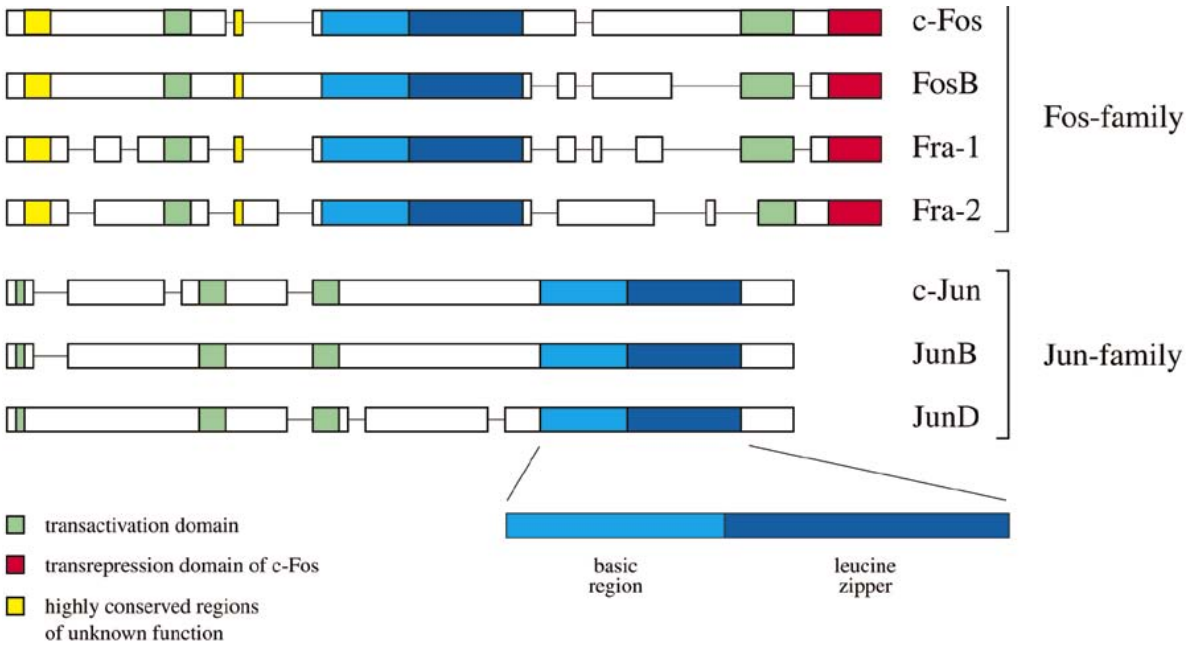
General structure of the AP-1 subunits

According to its function in controlling gene expression, the prototype of a transcription factor has to comprise at least two properties: a region of the protein that is responsible for binding to a specific DNA recognition sequence (DNA binding domain) and a second region that is required for transcriptional activation (transactivation domain) following DNA binding.

DNA binding domain

The DNA binding domain is evolutionarily conserved between the Jun, Fos and CREB/ATF proteins, thus defining the protein family of 'bZip' proteins. bZip stands for the amino acid sequences of the two independently acting sub-regions of the DNA binding domain called the 'basic domain' and the 'leucine zipper'. The basic domain is rich in basic amino acids which are responsible for contacting the DNA The leucine-zipper region is characterised by leucine heptad repeats that are part of the well known '4-3 repeats' forming a coiled-coil structure, which are responsible for dimerisation and a pre-requisite for DNA binding (Fig. 1). In addition to the leucines, other hydrophobic and charged amino acid residues within the leucine zipper region are responsible for specificity and stability of homodimer or heterodimer formation between the various Jun, Fos or CREB/ATF proteins. The Fos proteins do not form stable homodimers but heterodimerise efficiently with the Jun proteins. The Jun proteins can form homodimers, although with reduced stability compared to Jun/Fos or Jun/ATF. Jun-Jun and Jun-Fos dimers preferentially bind to the 7 bp motif 5-TGAG/CTCA-3′ whereas Jun-ATF dimers or ATF homodimers prefer to bind to a related, 8 bp consensus sequence 5′-TTACCTCA-3′. Therefore, individual AP-1 dimers are expected to regulate specific subsets of AP-1 target genes depending on the characteristics of the AP-1 site in their promoter.

In addition to the 'classical' AP-1 members (Jun, Fos, ATFs) identified on the basis of DNA sequence specificity and heterodimer formation with Jun and Fos proteins, several new bZip proteins have recently been defined. These include Maf and the Maf-related proteins Nrl, Smads and Jun-dimerising partners (JDPs). The function of these proteins in AP-1-regulated processes remains to be determined. Binding of AP-1 to DNA may also support binding of other transcription factors to adjacent or overlapping binding sites (composite elements) to allow the formation of quaternary complexes. The interaction of NFAT and Ets proteins with DNA on the IL-2 and collagenase promoters, respectively, may serve as paradigms for this type of protein/protein interaction.



AP-1. Fig. 1 – Structural organisation of Fos and Jun proteins.

Transactivation domain

In contrast to the well-defined DNA binding domain the structural properties of the domains in the AP-1 proteins mediating transcriptional activation of target genes (transactivation domain, TAD) are poorly understood. Investigations were carried out by constructing fusion proteins, in which TAD is transferred to heterologous DNA binding domains, such as the yeast transcription factor GAL4 or the mammalian transcription factor GHF. By employing such chimeric proteins (which, in contrast to the wild type proteins do not depend on a dimerisation partner) critical amino acids in the TADs were identified. Moreover, it became clear that the various Jun, Fos and ATF proteins greatly differ in their transactivation potential. For example, c-Fos, FosB and c-Jun are strong transactivators, whereas JunB, JunD, Fra-1 and Fra-2 exhibit only weak transactivation potential. Under specific circumstances they may even act as repressors of AP-1 activity by competitive binding to AP-1 sites, or by forming inactive heterodimers with c-Fos, FosB or c-Jun. Most importantly, transactivation studies using fusion proteins led to the identification of protein kinases, which bind to and phosphorylate AP-1 proteins in the TAD in response to extracellular signals, thereby controlling expression of AP-1 target genes.

Transcriptional and post-translational control of AP-1 activity

The *jun* and *fos* genes are members of a class of cellular genes, termed 'early response' or 'immediate-early' genes that are characterised by a rapid and transient activation of transcription in response to changes of environmental conditions such as growth factors, cytokines, tumor promoters, carcinogens and expression of certain oncogenes. Since this type of regulation of promoter activity is also observed in the absence of ongoing protein synthesis, it is generally accepted that pre-existing factors, whose activity gets altered by changes in post-translational modification (described in detail in the subsequent section), are responsible for the regulation of promoter activity.

Transcriptional activation

Most of our current knowledge on transcriptional activation of immediate-early genes is

derived from studies on deletion and point mutations of the *c-fos* and *c-jun* promoters, combined with *in vitro* and *in vivo* footprinting analyses. The serum-response element (SRE) is required for responding to the majority of extracellular stimuli including growth factors and phorbol esters. This element is bound by a ternary complex containing the transcription factor p67-SRF and p62-TCF (which stands for a class of related proteins described as Elk, SAP). Changes in the phosphorylation pattern of SRF and, predominantly, TCF regulate *c-fos* promoter activity by these stimuli. Other elements include the c-AMP response element (CRE) and the Sis-inducible enhancer (SIE), which is recognised by the STAT group of transcription factors. These factors are at the receiving end of the Jak/Stat signalling pathway initiated by specific classes of cytokines. In contrast, the element responsible for negative autoregulation of the *c-fos* promoter has not yet been identified conclusively.

Analysis of deletion mutants of the *c-jun* promoter identified two AP-1-like binding sites (Jun1, Jun2), which are recognised by Jun/ATF heterodimers or ATF homodimers and that are involved in transcriptional regulation in response to the majority of extracellular stimuli affecting *c-jun* transcription. In response to G-protein coupled receptors (e.g. the muscarinic acetylcholine receptor), EGF and other growth factors, the AP-1 sites and an additional element in the *c-jun* promoter, recognised by MEF2 proteins, cooperate in transcriptional control of the *c-jun* gene. Similar to the factors binding to the *c-fos* promoter, the activity of factors binding to the *c-jun* promoter is regulated by their phosphorylation status.

Regulation of activity

The most critical members of the class of protein kinases regulating the activity of AP-1 in response to extracellular stimuli are mitogen-activated protein kinases (MAPKs). Depending on the type of stimuli these proline-directed kinases can be dissected into three subgroups, the extracellular signal-regulated kinases (Erk-1, Erk-2), the Jun-N-terminal kinases (JNK-1, JNK-2, JNK-3) and p38 MAP kinases (p38α, p38β, p38γ). The extracellular signal-regulated kinases are activated by growth factors and phorbol esters, but only weakly activated by cytokines and cellular stress-inducing stimuli (UV irradiation, chemical carcinogens). In contrast Jun-N-terminal kinases, also known as stress-activated kinases (SAPK), and the structurally related class of p38 MAP kinases are strongly activated by cytokines and environmental stress, but are poorly activated by growth factors and phorbol esters.

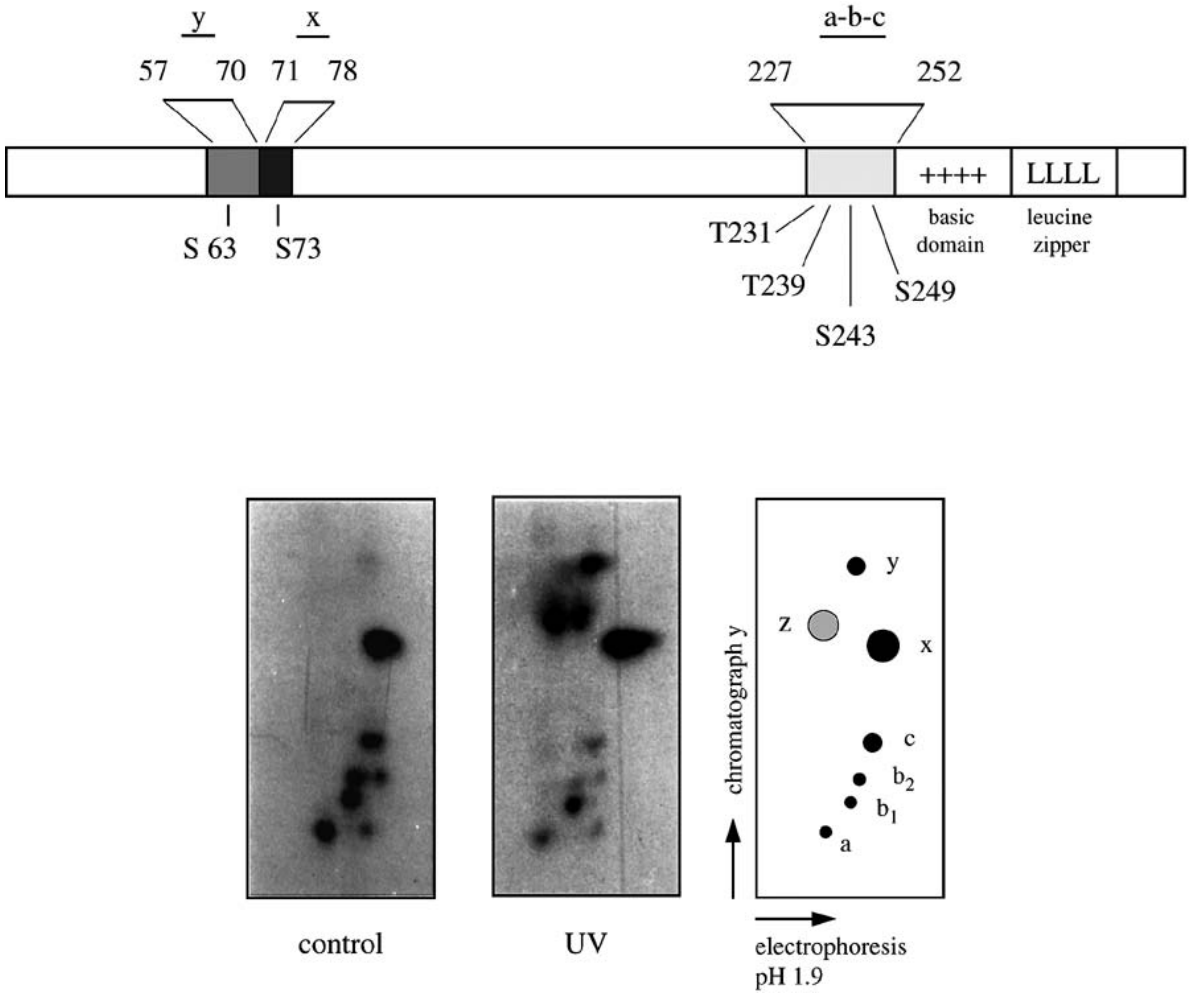
These kinases themselves are under strict control of upstream kinases and phosphatases, which are part of individual signalling pathways initiated by specific classes of extracellular and intracellular stimuli (growth factors, DNA damaging agents, oncoproteins). This network, which exhibits a high degree of evolutionary conservation between yeast, drosophila and mammals is, however, too complex to be discussed in detail in this review (for indepth information on this subject see [4, 6]).

Erk-1 and Erk-2 carry out mitogen-stimulated phosphorylation of Elk/SAP proteins. The sites phosphorylated by Erks reside in the TAD of TCF proteins and have a positive regulatory role in transactivation. The JNK/SAPKs were originally identified by their ability to specifically phosphorylate c-Jun at two positive regulatory sites (ser-63, ser-73) residing within the transactivation domain (Fig. 2). Hyperphosphorylation of both sites, which was originally identified by 2D-phospho-amino acid-peptide mapping (peptides x, y; Fig. 2), is observed in response to stress stimuli and oncoproteins and is responsible for transcriptional activation of c-Jun target genes. The JNKs can also phosphorylate and stimulate the transcriptional activity of ATF-2 and, to a lower extent TCF proteins. The same positive sites on ATF2 also serve as phospho-acceptor sites for p38, while ser-63 and ser-73 of c-Jun are not affected by p38. Most likely, hyperphosphorylation of Jun, ATF, and TCF proteins results in a conformational change of the TAD allowing more efficient interaction with co-factors such as CBP, which facilitate and stabilise the connection with the RNA polymerase II/ initiation complex to enhance transcription of target genes. Interestingly, in addition to en-

hanced transactivation, hyperphosphorylation of the TAD of c-Jun also regulates the stability of c-Jun by reducing ubiquitin-dependent degradation of c-Jun. Similar phosphorylation-dependent changes in the half-life of c-Fos have been observed.

In unstimulated cells, the DNA binding domain of c-Jun becomes phosphorylated at multiple sites (Fig. 2) by GSK-3 and/or casein kinase II (CK-II) resulting in reduced DNA binding. In response to extracellular stimuli such as UV irradiation, phosphorylation is reduced leading to enhanced DNA binding. The mechanism (reduced activity of the kinase or enhanced activity of a phosphatase) has not yet been defined conclusively.

In addition to phosphorylation other mechanisms have been identified which regulate AP-1 activity. These include redox-dependent DNA binding and regulation of nuclear localisation. Moreover, positive and negative interference between AP-1 and other cellular pro-



AP-1. Fig. 2 – Top: schematic diagram of the human c-Jun protein. Amino acids are numbered. Numbers on top refer to the trypsin cleavage sites that lead to the appearance of phosphopeptides following *in vivo* labelling of cells with ^{32}P-orthophosphate. The location of the tryptic peptides 'a-c' in the DNA binding domain and peptides 'x' and 'y' in the transactivation domain are indicated.
Bottom: Autoradiogram of *in vivo* labelled c-Jun protein, isolated by immunoprecipitation from untreated and UV-treated cells, digested with trypsin and separated by gel electrophoresis into two dimensions. On the right, positions of the tryptic peptides are schematically illustrated. Peptide 'z' most likely represents a peptide, containing residual phosphorylation at threonine-89 and/or threonine-91 of c-Jun.

teins (in addition to the protein kinases and coactivators described above) have been identified. The mutual interference between AP-1 and steroid hormone receptors, particularly the glucocorticoid receptor (GR), represents the most extensively analysed example for this type of crosstalk. There is evidence that the anti-inflammatory and immunosuppressive activities of glucocorticoids are mediated, at least in part, by GR-mediated repression of AP-1 activity. In addition to GR, transcription factors NFκB, MyoD, and YY1 have been found to modulate AP-1 activity. The exact mechanism of interaction between AP-1 and these proteins remains to be determined.

AP-1 in physiology and pathology

AP-1 activity is enhanced when cells are stimulated by agents that promote cell proliferation. Moreover, oncogenic versions of c-Jun and c-Fos have been isolated from retroviruses, and various membrane-associated or cytoplasmic oncogenes (Ras, Src, Raf) permanently upregulate AP-1 levels as part of their transforming capacity, suggesting that AP-1 members play an important role in cell proliferation and transformation. Supporting evidence was obtained by:

- blocking AP-1 activity either through expression of a dominant-negative c-Jun mutant,
- expression of antisense sequences,
- microinjection of Jun and Fos-specific antibodies.

Under these conditions cell-cycle progression in tissue culture cells, was disturbed and the efficiency of oncoprotein-mediated cell transformation was reduced. However, different lines of evidence suggested that the members of the Jun and Fos families play specific roles during these processes or may even antagonise each other. For example, over-expression of JunD resulted in a slower proliferation rate and an increase in the percentage of cells in G0/G1, whereas over-expression of c-Jun led to an increase in the populations of S/G2 and M phase cells. Moreover, by transient transfection analysis JunB was found to repress c-Jun-mediated transactivation and Ras-induced transformation, most likely by the preferential formation of inactive c-Jun-JunB dimers.

Genetic analysis of AP-1 function in transgenic mice revealed that over-expression of Fos and Jun proteins in mice induce tumor formation in specific organs (Table). Conversely, expression of a dominant-negative mutant of c-Jun in keratinocytes interferes with chemically induced skin carcinogenesis.

Since over-expression of a dominant-negative c-Jun mutant blocks total AP-1 activity inactivation of individual AP-1 members *in vivo* by gene targeting turned out to be the most informative approach to elucidate the specific contribution of a given AP-1 subunit to the regulation of AP-1-dependent processes. At present, knockout mice have been generated for most of the AP-1 members. These mice revealed characteristic phenotypes (Table) indicating in which tissue the subunit was particularly important or where its absence became limiting first. These differences also support the assumption of independent functions of AP-1 dimers *in vivo*. For example, c-Jun and JunB null embryos die at midgestation due to heart defects and deficiencies in the development of extraembryonal tissues such as the yolk sac and placenta respectively, indicating that no other AP-1 subunit can functionally compensate c-Jun and JunB deficiencies. These phenotypes are caused by defects in cell differentiation (e.g. trophoblasts, endothelial cells) rather than aberrant cell proliferation. Mice lacking specific members of the Fos and ATF families are viable (except Fra1), although the adults show specific defects in distinct tissues (Table), implying that only a subset of AP-1 target genes is affected in these mutant mice. Interestingly, a transgene expressing Fra-1 in *c-fos*-/- hematopoietic cells rescues the osteopetrosis of *c-fos* mutant mice by neutralising the block in osteoclast differentiation, suggesting that *Fra-1* is the critical c-Fos target gene during osteoclast differentiation. The *c-fos*-/- mice were also used to confirm the essential role of AP-1-regulated genes in skin carcinogenesis.

Despite a broad knowledge concerning genes that harbour AP-1 binding sites in their regulatory elements, only a few directly regu-

AP-1. Table – AP-1 function in transgenic mice.

AP-1 member	gain of function	loss of function
c-Jun	fibrosarcomas upon wounding (v-jun)	embryonic lethal E13 hepatic failure, heart defect
JunB	coat abnormalities, immune defects, enlarged hemato-poietic organs, reduced fertility	embryonic lethal E8.5-E10.0, placentation defect
JunD	N.D.	viable, reduced postnatal growth, age-dependent defcts in male reproductive function
c-Fos	osteosarcomas; chondrosarcomas	viable, defective bone remodelling, osteopetrosis
FosB	N.D.	viable, nurturing defect
Fra-1	increased bone density	embryonic lethal E10, placenta defect
ATF-2	N.D.	hypomorth allele: decreased postnatal viablility and growth, defects in endochondral ossification, ataxic gait, hyperactivity, decreased hearing, decreased number of Purkinje cells
		complete loss: neonatal lethality, meconium aspiration syndrome, decreased cytotrophoblasts in placenta

middle column: consequences of over-expressed Fos and Jun proteins in transgenic mice (gain of function) are listed.
right column: phenotypes of mice, containing loss-of-function mutations (introduced by gene targeting); (N.D.: not determined.)

lated AP-1 target genes have been identified, which are affected in AP-1 null mice. Among the best characterised AP-1 regulated genes are *c-jun* itself and genes involved in tissue remodelling processes. Examples are proteinases, such as MMP-9 (gelatinase B), MMP-13 (interstitial collagenase), uPA (urokinase plasminogen activator) and their inhibitors, the placental hormones placental lactogen-I and proliferin, and the osteoclast differentiation factor Rank/TRANCE.

Importantly, primary and immortalised fibroblasts could be isolated from almost all mice lacking individual AP-1 members. Analysis of these cells revealed that loss of c-Jun results in elevated transcription of p53 indicating that c-Jun acts as positive regulator of the cell cycle by suppressing p53 and, indirectly, the p53 target gene p21. Moreover, over-expression of c-Jun was found to up-regulate cyclin D levels. JunB, however, serves as a negative regulator of cell-cycle progression by induction of the cyclin-CDK inhibitor p16 and down-regulation of c-Jun and cyclin D expression. Data from fibroblasts lacking both c-Fos and FosB established a critical role of these AP-1 subunits in cyclin D expression. Moreover, fibroblasts lacking either c-Jun or c-Fos can not be transformed by oncogenes such as *ras* and *src*, providing additional evidence for a critical role of AP-1 members in the control of cell proliferation and transformation.

As described before, AP-1 activity is also greatly enhanced upon treatment of cells with genotoxic agents, implying that AP-1 target genes are involved in the cellular 'stress response', such as DNA repair, induction of survival functions, or initiation of the apoptotic

program. Indeed, analysis of fibroblasts from c-Jun and c-Fos -deficient mice provided experimental proof for this assumption. Fibroblasts lacking c-Fos are hypersensitive to UV irradiation when compared to wild type cells, which is caused by a higher rate of apoptosis rather than the inability to repair damaged DNA. In contrast to cells lacking c-Fos, the ability of c-Jun-deficient fibroblasts to undergo apoptosis is greatly reduced due to the absence of CD95 (Fas/APO)-ligand induction. Conversely, overexpression of c-Jun induced apoptosis in fibroblasts. Reduced CD95-L induction was also observed in cells from mice expressing a c-Jun mutant protein which lacks the critical JNK/SAPK phosphorylation sites in its transactivation domain (ser-63 and ser-73). Reduced apoptosis and CD95-L expression was found in PC12 cells upon over-expression of a c-Jun mutant lacking the JNK/SAPK phosphorylation sites. Similarly, a c-Jun dominant negative mutant protected sympathetic neurons against apoptosis. Reduced apoptosis in response to genotoxic agents was also observed in mice lacking members of the JNK/SAPK family of protein kinases, suggesting that c-Jun and ATF proteins are the major substrates of JNK/SAPKs to mediate the cellular stress response. However, it is important to note that AP-1 members, depending on the cell type and extracellular stimuli, may be involved in both apoptotic and anti-apoptotic responses. For example, primary liver cell cultures and erythroblasts derived from *c-jun* -/- embryos exhibit increased apoptotic rates. On the other hand, the lack of c-Fos results in the loss of light-induced apoptosis of photoreceptors in retinal degeneration.

Obviously, AP-1 members represent a crosspoint of multiple pathways that regulate cell proliferation and apoptosis; two apparently opposing phenotypes. Some of the specificity of the function of a given AP-1 subunit is presumably based on the choice of the heterodimeric partner, dictating sequence specificity and, in turn, the subset of AP-1 target genes. A shift in the equilibrium of gene expression of such distinct classes of c-Jun target genes, in conjunction with alterations in c-Jun-independent pathways, will contribute to the decision of the cell to either proliferate, to activate survival factors or to induce the genetic program of cell death in response to extracellular signals.

Despite the fact that AP-1 was identified more than a decade ago, it still retains a lot of its mystery. Further research on tissue-specific inactivation of AP-1 members and the identification of subunit-specific target genes may yield an even more complex picture of function and regulation of AP-1.

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AP-2

Definition

Activating enhancer-binding protein 2 (AP-2), also known as AP2TF and TFAP2, is a transcription factor that maps to 6p22.3-ter; a gene responsive to retinoic acid. It is a nuclear protein of 52 kD, and as a dimer binds to the DNA consensus sequence 5′-CCCCAGGC-3′. It regulates the expression of genes required for the development of tissues of ectodermal origin, activating CDKN1A (→ WAF1) and other genes such as those encoding → MCAM, → MMP2, → KIT/stem cell factor. Is involved in the progression of human → melanoma and potentially involved in anterior eye chamber develeopment disease; → AP-1.

AP2

Definition

AP2 is a heterotetrameric protein constituted of four polypeptides, called adaptins: α and β2 (~100 kD each), and σ2 (~25 kD) and μ2 (~50 kD). α and β2-adaptins both bind to clathrin, whereas μ2 binds to receptor tails; the α subunit further binds to several accessory endocytic proteins in a spatially and temporally organized fashion. AP2 is also often spelled as AP-2.

APAF-1

Definition

Apoptotic protease activating factor 1 (APAF-1), also known as → CED4, binds to caspase 9 (apaf-3) to activate caspase 3, resulting in → apoptosis. It is an ubiquitously expressed cytoplasmic protein of 1194 aa and 135 kD. The human gene maps to 12q22.

APAF-3

Definition

→ Caspase 9.

APC

Definition

1. Adenomatous polyposis coli (APC); familial colon tumor; colorectal cancer [→ colon cancer].
2. → Antigen-presenting cells.

APC Gene in Familial Adenomatous Polyposis

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Synonyms

- Familial Polyposis Coli (FPC)
- Adenomatous Polyposis Coli (APC)
- Familial Adenomatous Polyposis (FAP)
- Gardner Syndrome (GS)
- Attenuated Adenomatous Polyposis Coli (AAPC)

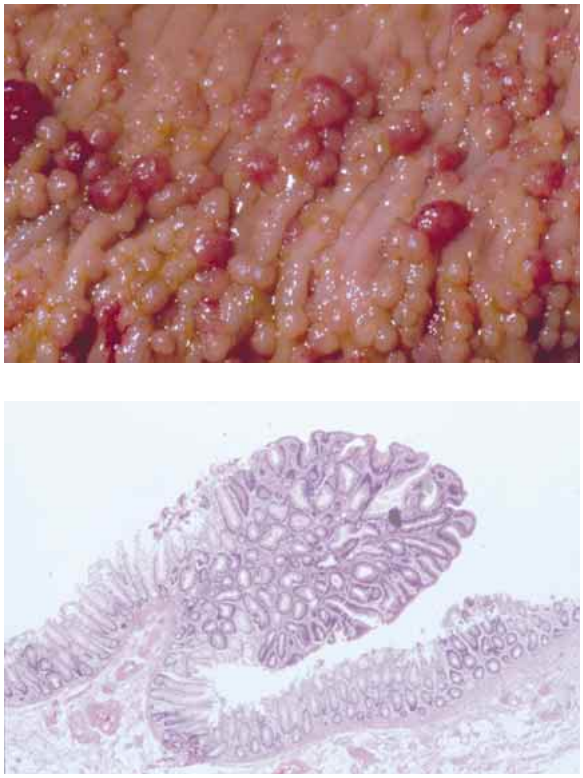
Definition

Familial adenomatous polyposis (FAP) is a dominant condition predisposing to the development of multiple colorectal adenomas (polyps) during adolescence (1, 2). Adenomatous polyps are benign tumors that can degenerate into malignant adenocarcinomas and subsequently into metastases if the affected segment of the bowel is not surgically resected (Fig. 1).

Characteristics

FAP affects on the average 1 in 10,000 individuals and, although the polyps represent the hallmark of the disease, it might be regarded as a condition of the whole body since it is often characterized by a number of extra-intestinal manifestations involving all three embryonic lineages:

- tumors of the stomach, duodenum, and of the biliary tree;
- → osteomas, → desmoids (Fig. 2), liver tumors, and dental abnormalities;
- epidermal cysts of the skin, congenital hypertrophies of the retina (CHRPE), and endocrine tumors.



APC Gene in Familial Adenomatous Polyposis. Fig. 1 – Top: Macroscopic detail of adenomatous polyps from an FAP patient;
Bottom: Section of an adenomatous polyp; HE staining. [Courtesy of Dr. Alex Kartheuser, Brussels, Belgium].

Of these tumors, the duodenal polyps and the abdominal desmoids occur respectively in 44% and 13% of the FAP patients. Next to colorectal cancer [→ colon cancer] and eventual metastases, these tumors represent the most clinically relevant complication of the disease (2).

The most benign manifestation of FAP, the → CHRPE in the eye, is found to be consistently associated in about 80% of the cases even before the appearance of the polyps in the large bowel, thus representing a very useful diagnostic biomarker.

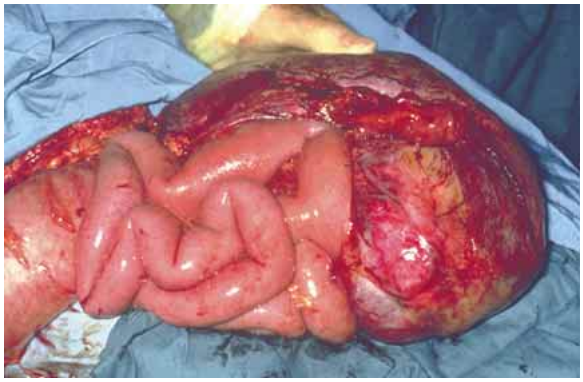
FAP is an autosomal dominant condition with very high → penetrance (close to 100%). Germline mutations in the *APC* gene are responsible for FAP. *APC* encodes for a large (312 kD) and multifunctional protein involved in several biological processes ranging from cell → adhesion, migration, and signal transduction (3, 4).

APC is nowadays considered as the gene for colorectal cancer as somatic *APC* mutations occur early in the adenoma-carcinoma sequence and are found in the vast majority (>85%) of sporadic adenomas and carcinomas. Functional studies have shown that APC plays a critical role in controlling WNT signal transduction by regulating β-catenin levels in the cytoplasm, and this feature is likely to represent APC's tumor suppressive function. Indeed, colorectal tumors with an intact *APC* contain oncogenic β-*catenin* mutations that alter phosphorylation sites which make the protein resistant against proteolytic degradation. The → WNT signal transduction pathway plays a critical role in a broad range of biological processes such as differentiation, cell polarity, and the specification of cell fate (for a schematic representation of the WNT pathway see http://www.ana.ed.ac.uk/rnusse/pathways/pathway.html). In the absence of the WNT stimulus, a multi-protein complex composed of GSK3, Axin, Conductin, and APC, earmarks β-catenin for proteolytic degradation. In the presence of the secreted Wnt glycoproteins, these interact with the frizzled receptors thereby inhibiting the formation of the above complex and β-catenin degradation. Accumulation of β-catenin in the cytoplasma results in its translocation to the nucleus where it complexes with TCF transcription factors thereby activating downstream target genes. Hence, loss of *APC* in mammalian cells or oncogenic activation of β-*catenin*, leads to constitutive signalling and cell transformation due to uncontrolled activation of downstream target genes.

A large number of disease-causing mutations in individuals affected by FAP have been characterized. The vast majority of *APC* mutations identified to date are clustered within the 5′ half of the gene (upstream of codon 1600) and are predicted to result in the truncation of the corresponding protein products.

Genotype-phenotype correlations

The identification of a large number of mutations, together with the availability of the corresponding clinical data, offers a unique opportunity to establish genotype-phenotype correla-



APC gene in Familial Adenomatous Polyposis. Fig. 2 – Mesenteric desmoid tumor [Courtesy of Dr. Diana Eccles, Southampton, UK].

tions at the *APC* gene (5). Mutations located close to the 5′ end of the *APC* gene result in a generally mild and variable FAP phenotype, the so-called attenuated adenomatous polyposis coli (AAPC), characterized by a variable and reduced polyp multiplicity and a delayed age of onset. Mutations beyond *APC* codon 1600 are rare and are also often associated with attenuated phenotypes. Consistent correlations between germline mutations at the *APC* gene and FAP extra-intestinal manifestations such as desmoid tumors, CHRPE's, and osteomas have also been reported.

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APC/β-Catenin Pathway

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Synonyms

The terms Wnt pathway and APC/β-catenin pathway have been used interchangeably. Because some Wnt proteins have β-catenin-independent effects, and since β-catenin can be affected by upstream pathways other than Wnt, the term APC/β-catenin is used here.

Definition

The APC/β-catenin pathway is a signal transduction pathway important in development and tumorigenesis. Signaling by this pathway is defined as the stabilization of β-catenin and transcriptional activation of several target genes.

Characteristics

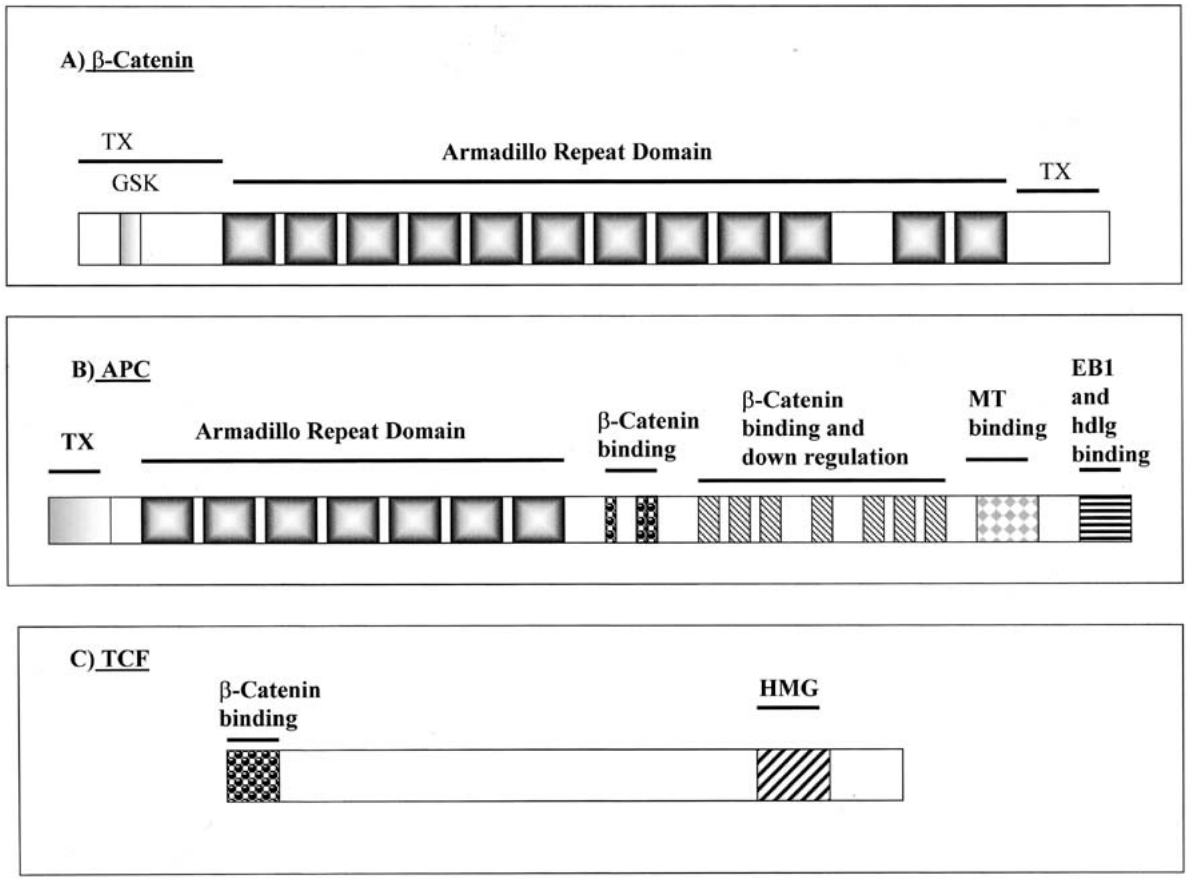
β-catenin is a multi-functional protein with roles in adhesion and signal transduction. The adhesion properties of β-catenin reflect its ability to interact with → E-cadherin at the cellular membrane, and the alteration of this important cellular function has been associated with an increased invasion potential of cancer cells. In addition to this important role, β-catenin is also found in a cytoplasmic/nuclear pool and is believed to act as a signal transduction molecule. Cytoplasmic/nuclear β-catenin (sometimes called free β-catenin) can associate with the T cell factor (TCF) family of transcription factors and activate transcription of specific genes. The TCFs provide the DNA binding domain while β-catenin contains the transcriptional activation domain (Fig. 1). Transcriptional target genes of the pathway include cMYC [→ MYC family], → Cyclin D1 and MMP-7. APC/β-catenin signaling is regulated mainly through degradation of β-catenin at the protein level (see below). This regulation is complex, and inappropriate activation of the pathway can facilitate the develop-

ment of several malignancies in humans and in animal models.

The pathway is highly conserved through evolution and the *Drosophila melanogaster* homolog of β-catenin, Armadillo, interacts with *Drosophila* TCF (Pangolin) to activate genes important for cellular fate determination during fruit fly embryonic development. Studies of the wingless pathway in *Drosophila* and *Xenopus* have been instrumental in unraveling the regulation of this pathway in normal and cancer cells.

Regulation

Normally, in differentiated cells, β-catenin protein is constantly degraded through the ubiquitin-proteasome pathway. β-catenin is earmarked for degradation through phosphorylation of specific residues at its N-terminus by GSK3β. The ubiquitin ligase β-TRCP can bind phosphorylated β-catenin, which is then polyubiquitinated (→ ubiquitination). This effectively targets β-catenin for → proteasome degradation. The phosphorylation and ubiquitination of β-catenin is regulated by a large complex of proteins that include GSK3β, Axin and APC (Fig. 2). In addition to its action on β-catenin, GSK3β phosphorylates APC and Axin but the consequences of these phosphorylation events are unclear. It appears that Axin increases the ability of GSK3β to phosphorylate β-catenin, since GSK3β phosphorylation of β-catenin is inefficient *in vitro*. APC has been sug-

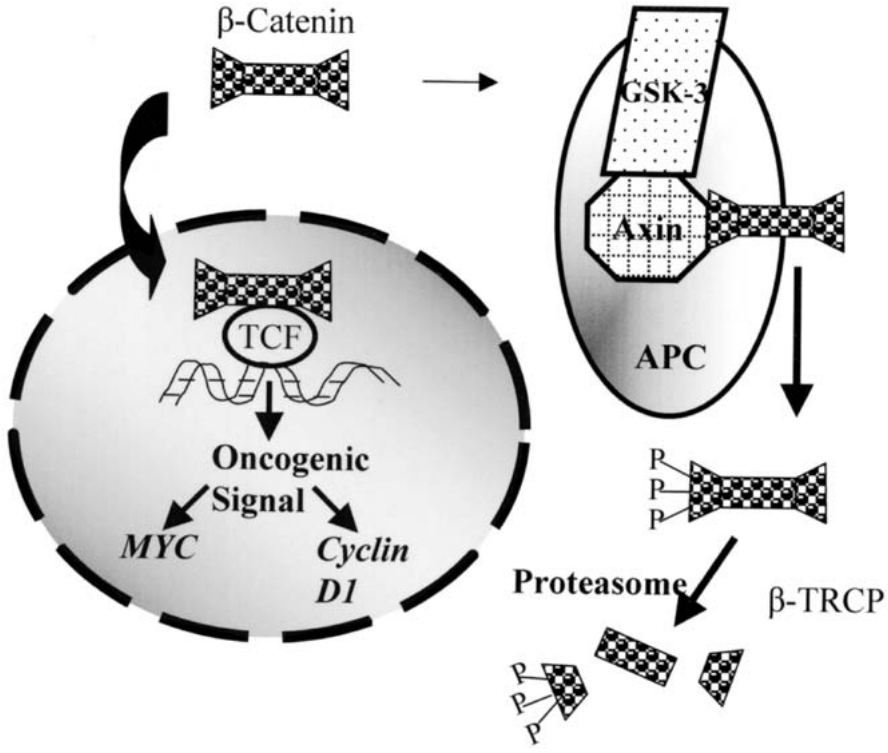


APC/β-Catenin Pathway. Fig. 1 – Schematic representation of the β-catenin, APC, and TCF proteins. a) β-catenin protein, showing the transcriptional activation domains, Armadillo repeats, and the N-terminal GSK-3-β phosphorylation domain (GSK). b) APC, showing armadillo repeats, β-catenin binding and down-regulation domains and the microtubule binding region. Also shown are the binding domains for EB1 and the human discs large protein. c) A simplified representation of the TCF family of proteins showing the β-catenin binding site, as well as the DNA-binding HMG domain.

gested to act as a scaffold for these phosphorylation events. APC, a protein believed to be exclusively cytoplasmic, was shown to be present in the nucleus. In addition, APC contains a nuclear export signal (NES) and may be involved in shuttling β-catenin from the nucleus to the cytoplasmic degradation complex. The regulation of the complex and the roles of the different proteins within the complex are incompletely understood. The multifaceted regulation of β-catenin likely reflects the importance of the pathway in development and in tumorigenesis.

Clinical relevance

The APC/β-catenin pathway is deregulated in several common human cancers. *APC* mutations are the cause of FAP [→ APC gene in Familial Adenomatous Polyposis], a familial colon cancer predisposition syndrome. Genetic testing of FAP families allows the identification of individuals at risk of colon cancer and the establishment of appropriate management options. In addition, *APC* mutations are found in 80% of all sporadic cases of colon cancer. Mutations of APC lead to a lack of regulation of the β-catenin protein and inappropriate activation of the downstream genes. Interestingly, in sporadic colon tumors without *APC* mutations, β-catenin itself is frequently mutated. The mutations typically affect GSK3β phosphorylation sites in the N-terminus of β-catenin, leading to a protein resistant to phosphorylation and subsequent degradation. While mutations in *APC* are not frequently observed in cancers other than colon, mutations in β-catenin have been reported in several human malignancies such as melanoma, colon cancer, prostate cancer and skin cancer. Axin, another member of the pathway is also found mutated in liver cancer. Cellular consequences of the activation of the APC/β-catenin pathway are unclear but several lines of evidence suggest that the pathway is important for the maintenance of stem cell characteristics, including longevity. In any event, it is clear that many human malignancies



APC/β-Catenin Pathway. Fig. 2 – APC/β-catenin signaling pathway. In the absence of signaling, β-catenin is targeted by the APC complex for proteasome degradation. When stabilized, β-catenin interacts with TCF to activate transcription of genes such as *c-Myc* and *Cyclin D1*. See text for details.

gain a selective advantage through activation of this pathway. Selective inhibition of β-catenin activation may represent a useful therapeutic strategy for a large number of cancers.

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Aphidicolin

Definition

Aphidicolin is a drug that specifically inhibits DNA polymerases, particularly DNA polymerase α. DNA polymerase α is necessary for proper synthesis of the lagging strand during DNA synthesis. Aphidicolin is frequently used at high doses to block cells in the S phase of the cell cycle and at lower doses to induce common → fragile sites. It is also used as a chemotherapeutic agent.

API3

Definition

AP13 (synonym:BIRC4) inhibits caspase 3 and caspase 7 (and possibly others), thereby suppressing → apoptosis. It is a ubiquitously expressed, cytoplasmic protein of 497 amino acids, and 56 kD, and the gene maps to Xq25.

APL

Definition

→ Acute promyelocytic leukemia.

APO-1

Definition

→ Fas.

Apopain

Definition

→ Caspase 3.

Apoptosis

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Synonyms

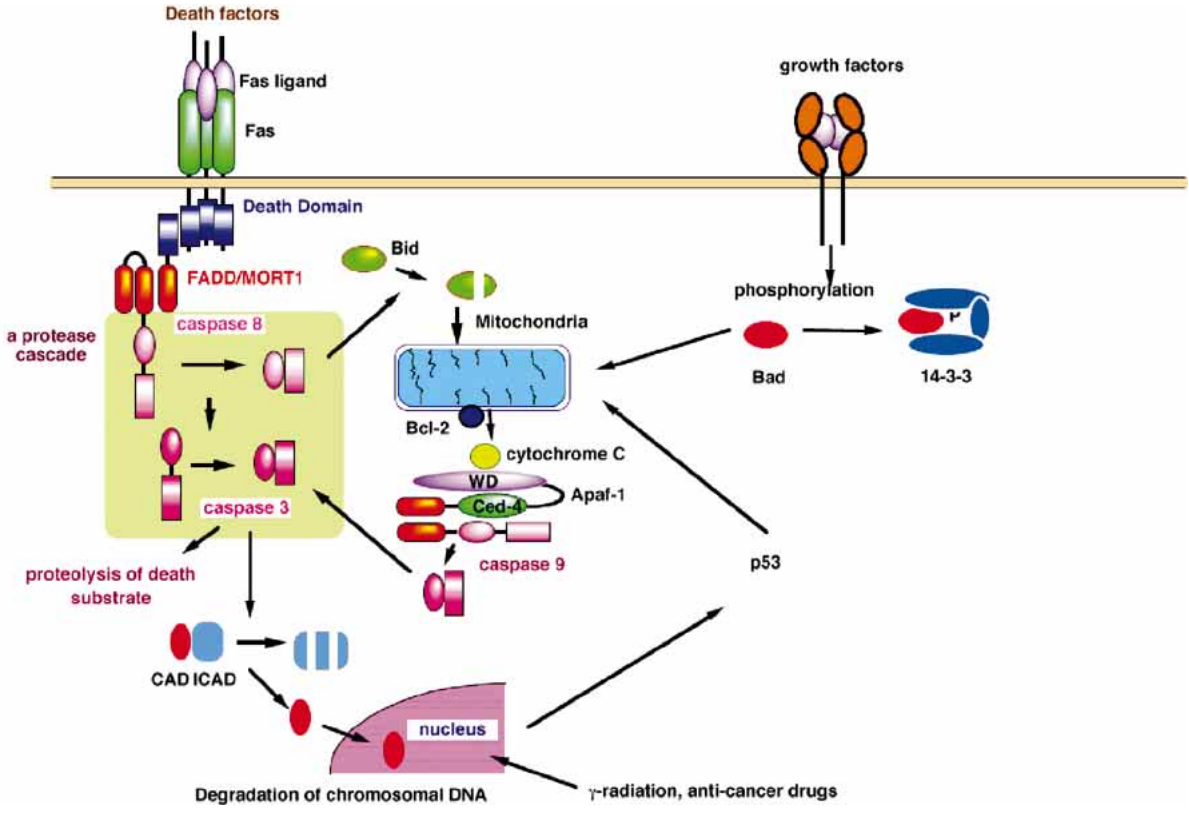
- Programmed cell death

Definition

Apoptosis is a cell death process which occurs during development and aging of animals. It is also induced by cytotoxic lymphocytes (CTL), anti-cancer drugs, γ- or UV-irradiation, a group of cytokines called death factors and deprivation of survival factors.

Characteristics

Apoptosis was initially characterized by morphological changes of dying cells. During apoptosis cells shrink, and microvilli on the plasma membrane disappear. The nucleus is also condensed and fragmented. At the final stage of apoptosis the cells themselves are fragmented



Apoptosis. Fig. – Signal transduction for apoptosis. Inducers of apoptosis are categolized into three groups (death factors, genotoxic anti-cancer drugs, and factor deprivation). Fas ligand, a representative of death factors, binds to Fas receptor, and causes its trimerization. The trimerized death domain in the Fas cytoplasmic region recruits pro-caspase 8 through a FADD/MORT1 adaptor, and forms a DISC. The pro-caspase 8 is autoactivated at DISC, and becomes a mature active enzyme. Two routes have been identified to activate caspase 3 by caspase 8. In one route, caspase 8 directly processes pro-caspase 3 in the downstream, and caspase 3 cleaves various cellular proteins including ICAD. CAD is released from ICAD, and degrades chromosomal DNA. In another route, caspase 8 cleaves Bid, a pro-apoptotic member of Bcl-2, which translocates to mitochondria to release cytochrome C into the cytosol. Bcl-2 or Bcl-xL, anti-apoptotic members of the Bcl-2 family, inhibits the release of cytochrome C, the mechanism of which is not well understood. The cytochrome C then activates caspase 9 together with Apaf-1, and caspase 9 in turn activates caspase 3. The genotoxic anti-cancer drugs such as etoposide and γ-radiation generate damage in chromosomal DNA. The signal seems to be transferred to mitochondria in a p53-dependent manner by as yet an identified mechanism. This releases cytochrome C from mitochondria, and activates caspase 9 as described above. The apoptosis induced by factor-deprivation is best studied with IL-3-dependent myeloid cell lines. In the presence of IL-3, the signal from the IL-3 receptor causes phosphorylation of Bad, a pro-apoptotic member of the Bcl-2 family. The phosphorylated Bad is trapped by an adaptor called 14-3-3. In the absence of IL-3, non-phosphorylated Bad is released from 14-3-3, and translocates to mitochondria to release cytochrome C to activate caspase 9.

with all cellular contents inside. One of the biochemical hallmarks of apoptosis is the fragmentation of chromosomal DNA into → nucleosome size units (180 bp).

Apoptotic cells can be recognized by staining of the condensed nuclei with fluorescence dyes Hoechst or DAPI. Apoptotic cells expose phosphatidyl-serine to the cell surface, which can be stained with fluoresently labelled annexin V. The fragmented DNA can be detected by → TUNEL (terminal deoxynucleotidyltransferase-mediated UTP end labelling) procedure, or by electrophoresis of the isolated DNA on an agarose gel, which yields a ladder of DNA fragments with a unit size of 180 bp.

Cellular & Molecular Regulation

Apoptosis is mediated by a family of proteases called → caspases that are activated by processing from its inactive precursor (zymogen). Thirteen members of the human caspase family have been identified. Some of the family members are involved in apoptosis, and these can be divided into two subgroups. The first group consists of caspase 8, caspase 9, and caspase 10, which contain a long prodomain at the N-terminus and function as initiators of the cell death process. The second group contains caspase 3, caspase 6, and caspase 7, which have a short prodomain and work as effectors, cleaving various death substrates that ultimately cause the morphological and biochemical changes seen in apoptotic cells. The other effector molecule in apoptosis is → Apaf-1 (apoptotic protease activating factor), which, together with cytochrome C, recruits pro-caspase 9 in an ATP (or dATP)-dependent manner, and stimulates the processing of pro-caspase 9 to the mature enzyme.

The other regulators of apoptosis are the → Bcl-2 family members. Eighteen members have been identified for the Bcl-2 family, and divided into three subgroups based on their structure. Members of the first subgroup, represented by Bcl-2 and Bcl-xL have an anti-apoptotic function. Members of the second subgroup, represented by → Bax and Bak [→ BAK1], as well as members of the third subgroup such as → Bid and → Bad are pro-apoptotic molecules.

The signal transduction pathway for a death factor (→ Fas ligand)-induced apoptosis has been well elucidated. Binding of Fas ligand to its receptor results in the formation of a complex (disc, death-inducing signaling comlex) consisting of Fas, FADD and pro-caspase 8. Pro-caspase 8 is processed to an active enzyme at the disc. There are two pathways downstream of caspase 8. In some cells, such as thymocytes and fibroblasts, caspase 8 directly activates 3. In type II cells such as hepatocytes, caspase 8 cleaves Bid, a member of the Bcl-2 family. The truncated Bid then translocates to mitochondria and stimulates release of cytochrome c, which activates caspase 9 together with Apaf-1. The activated caspase 9 causes processing of pro-caspase 3 to the mature enzyme. In addition to the death factors, anti-cancer drugs, γ-irradiation or factor-depletion induce apoptotic cell death. Although cytochrome C is released from mitochondria during apoptosis induced by these stimuli, the molecular mechanism that triggers the release of cytochrome C from mitochondria is not known.

Caspase 3 activated downstream of the caspase cascade activates a specific DNase (CAD, caspase-activated DNase). CAD is complexed with its inhibitor, ICAD (inhibitor of CAD), in proliferating cells. When caspase 3 is activated in apoptotic cells, it cleaves ICAD to release CAD. CAD then causes DNA fragmentation in the nuclei.

Clinical Relevance

Blocking of apoptosis by loss-of-function mutations of apoptosis-inducing molecules such as Fas, Fas ligand and caspases, or overexpression of apoptosis-inhibitory molecule such as Bcl-2, causes cellular hyperplasia. In some cases it leads to tumorigenesis, as evident in B-cell lymphomas, which over-express Bcl-2 due to the translocation of the Bcl-2 gene to the immunoglobulin gene locus. Some multiple myeloma and non-Hodgkin's lymphoma carry loss-of-function mutations in the Fas gene. Somatic mutation in the Fas gene can also be found in patients of autoimmune diseases called Canale-Smith syndrome or → autoimmune lymphoproliferative syndrome (ALPS).

Exaggeration of apoptosis causes tissue damage. For example, administration of Fas ligand, exposure to γ-irradiation, or treatment with a high dose of glucocorticoid kill test animals by causing massive apoptosis in the liver or thymus. Hepatitis, insulitis, graft-*versus*-host disease, and allergic encephalitis are due to the excessive apoptosis by Fas ligand expressed on CTL. Apoptotic cells are detected in the brain of ischemia or Alzheimer patients, suggesting that apoptosis is at least in part responsible for the disease manifestation in these patients.

Apoptosis. Table – The apoptosis factory.

worker	synonym	apoptosis job		chromosome
		pro	anti	
Fas	CD95 Apo-1	+		10q24
FADD	MORT-1	+		11q13
granzyme B	GZMB	+		14q11
Apaf-1	CED4	+		12q23
Casp 2	ICH1 NEDD2	+		7q35
Casp 3	CPP32 Yama apopain	+		4q33
Casp 4	TX ICH-2 ICE-rel-II	+		11q22
Casp 6	MCH2	+		4q25
Casp 7	MCH3 ICE-LAP3	+		10q25
Casp 8	MACH MCH5 FLICE	+		2q33
Casp 9	APAF3 MCH6 ICE-LAP6	+		1p36.3 – p36.1
Casp 10	MCH4	+		2q33
CAD	DFF40	+		1p36.3
Bak		+		6p21
Bax		+		19q13
Bcl-2			+	18q21
Bid		+		22q11
Bik		+		22q13.3
XIAP			+	Xq25
UBL1	SUMO-1 sentrin		+	2q32

A proper dose of anti-cancer drugs or γ-irradiation can kill cancer cells by activating the apoptotic death program in the target cells. Some cancer cells are resistant to these drugs by an unknown mechanism. It is hoped that elucidation of the molecular mechanism of apoptosis leads to development of an efficient cancer therapy.

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APT1

Definition

→ Fas.

Apudoma

Definition

Apudoma, refers to tumors capable of amine precursor uptake and decarboxylation; → neuroendocrine tumors.

ARE

Definition

Adenine- and uracil-rich elements (AU-Rich Element) are present in the untranslated region (3′-UTR) of mRNAs and have been described in the 3′-UTR of numerous mRNA of cytokines and proto-oncogenes. AREs comprise a major group of *cis*-acting elements that target these mRNAs for rapid degradation. AREs exert a post-transcriptional control of gene expression by interacting with cytoplasmic and nuclear RNA binding proteins.

ARF

Definition

ARF is an alternatively spliced product of the INK4a locus. When triggered by *E1A*, *myc* or *E2F* or other illegitimately activated oncogenes, ARF binds *MDM2*. This leads to a rise of the → p53 level, causing growth arrest and/or apoptosis.

Argentaffinoma

Definition

Argentaffinoma, also known as → neuroendocrine tumors, are related to positive reactions to silver stains.

Aromatase

Definition

Aromatase is a key enzyme converting a precursor into an → estrogenic hormone.

Asbestos

Definition

Asbestos is used for various incombustible silicate minerals that can be separated into long, thin flexible fibers that have been used for a number of commercial purposes, such as heating and insulation. Asbestos is a known carcinogen, and use of these minerals has been stopped or restricted in some countries because exposure to asbestos has been clearly linked with the development of certain cancers, particularly malignant mesothelioma.

ASCO

Definition

American Society of Clinical Oncology; (www.asco.org/)

ASK1

Definition

→ Mitogen-activated protein kinase kinase kinase 5.

Askin Tumor

Definition

Askin tumor is a → Ewing sarcoma or peripheral primitive neuroectodermal tumor of the chest wall.

Association Genetics

Definition

Association genetics establishes the relationship of certain genes to specific diseases.

Astrocytic Tumor

Definition

Astrocytic tumors comprise a wide range of neoplasms that differ in their location within the central nervous system, in their age and gender distribution, their growth potential, their extent of invasiveness and their clinical course. There is increasing evidence that these differences reflect the type and sequence of genetic alterations acquired during the process of transformation. The following distinctions can be made:

1. Diffusely infiltrating astrocytoma. These tumors are usually found in adults, arise at any site in the central nervous system but preferentially in the cerebral hemisphere. They show a diffuse infiltration of adjacent and distant brain structures, have a tendency for malignant progression with the glioblastoma [→ glioblastoma multiforme] as the most malignant phenotypic endpoint. The survival rate of patients ranges from more than five years for grade II to less than one year for the majority of patients with stage IV glioblastoma.
2. Diffuse astrocytoma. This type of astrocytic tumor is characterized by a high degree of cellular differentiation and slow growth. Typically it affects young adults with the tendency to undergo malignant progression to anaplastic astrocytoma, eventually developing into glioblastoma.
3. Anaplastic astrocytoma. A diffusely infiltrating astrocytoma with focal or dispersed anaplasia and a marked proliferative potential. This tumor type has an intrinsic tendency towards a malignant progression into glioblastoma.

Astrocytoma

Definition

Astrocytoma is the collective term for a class of tumors derived from astrocytic precursors; → brain tumors; → astrocytic tumor.

AT/RT

Definition

atypical teratoid/rhabdoid tumor (AT/RT); → rhabdoid tumor.

Ataxia Telangiectasia

Definition

Ataxia telangiectasia (AT; → ATM protein) is an autosomal recessive human disorder. The gene maps to 11q22. AT is a multisystem disease characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, radiosensitivity, predisposition to lymphoid malignancies and immunodeficiency with defects in both cellular and humoral immunity. Ataxia is the presenting symptom and is usually recognized at the age of 12-14 months, the patient is confined to a wheelchair before adolescence. Telangiectasia has a later onset, usually is observed between 2-8 years of age; telangiectasias can affect the eyes, the ears and the butterfly area of the face. The major debilitating features are the progressive neurological abnormalities and immunodeficiency. Chromosome instability with apparently random breaks is a characteristic cytogenetic feature, and it is a common notion that the genomic instability in AT is a major cause of cancer in AT patients, in keeping with the well-established link between cancer and genomic rearrangements.

ATM Protein

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Synonyms

- ataxia telangiectasia mutated (ATM)

Definition

ATM protein is a large protein kinase involved in the control of numerous signaling pathways, most notably cellular responses to DNA damage. ATM is a 'master controller protein' that exerts its pleiotropic effects via multiple interactions with other proteins and its catalytic activity. ATM is missing or inactive in patients with the multisystem genetic disorder ataxia telangiectasia (A-T), which is characterized by defects in the nervous and immune systems, genomic instability, sensitivity to ionizing radiation, and cancer predisposition. The complex and broad manifestations of A-T demonstrate the extensive involvement of the ATM protein in many cellular and developmental processes; → ataxia telangiectasia.

Characteristics

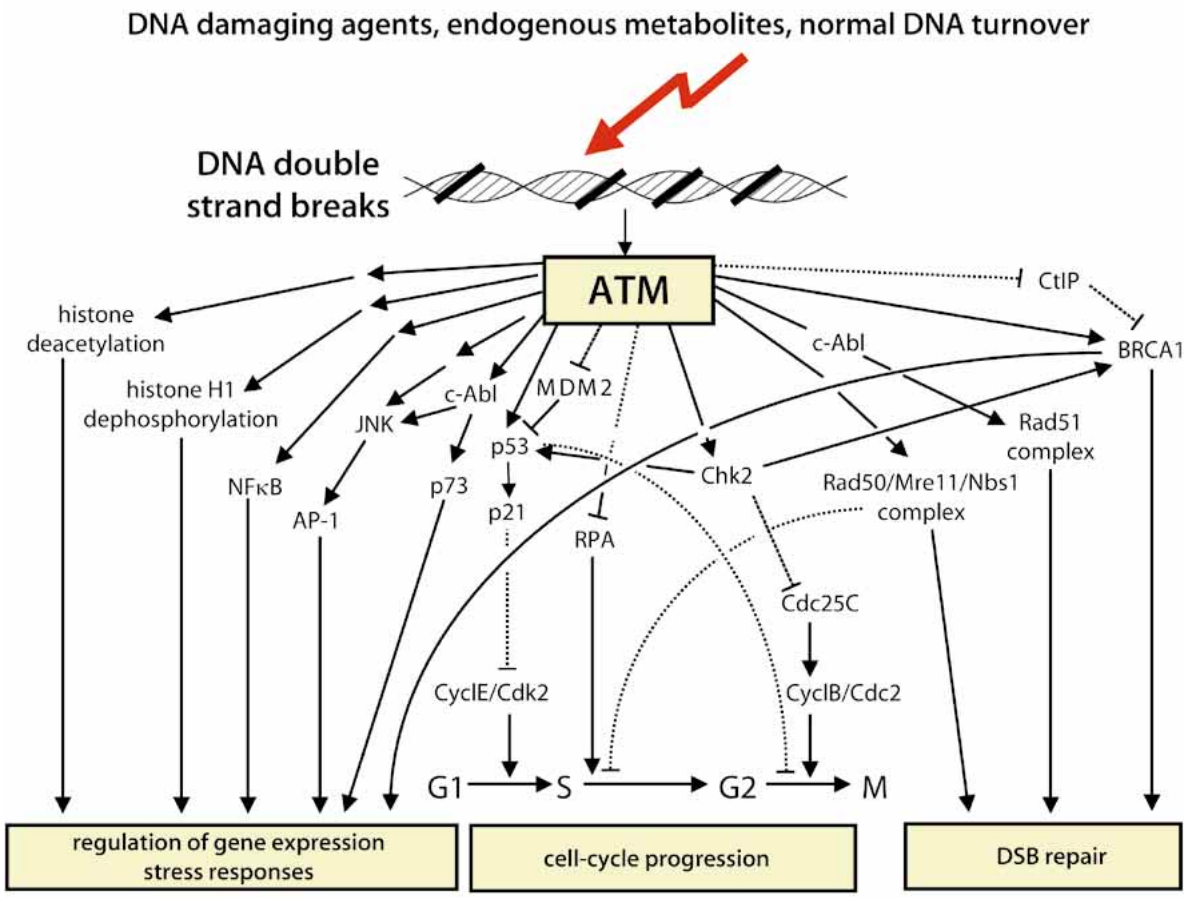
ATM is a large, heavily phosphorylated protein containing 3,056 amino acids. Its most prominent motif is a carboxy-terminal region of about 350 amino acids that is highly similar to the catalytic subunit of phosphatidylinositol 3-kinases (PI3-kinases). The large size and PI 3-kinase-related region are common to a family of proteins identified in organisms ranging from yeast to mammals, which are involved in maintaining genomic stability, cell cycle control and responses to DNA damage. Most members of this protein family were found to have a serine/threonine protein kinase activity, i.e., they phosphorylate other proteins on specific serine or threonine residues. A notable member of this group is the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), which is activated *in vitro* by DNA ends. Mice lacking DNA-PK activity show severe combined immunodeficiency (scid) due to defective maturation of the immune system genes via V(D)J recombination. They also exhibit radiation sensitivity, immunodeficiency and cancer predisposition, pointing to the involvement of DNA-PK in the processing of DNA cleavage products ocurring in normal somatic recombination as well as radiation-induced DNA double strand breaks. The mammalian PI3-kinase-like member most closely related to ATM is the ATR protein whose functions are partially redundant with those of ATM.

Cellular functions and molecular regulation

Cellular ATM is divided between the nucleus and the cytoplasm, the relative proportions varying in different cell types at different stages

of differentiation. Some cytoplasmic ATM is associated with peroxisomes. Most cellular ATM is associated with several protein complexes ranging in size from 500-2000 kD. Its involvement in a variety of cellular physiological processes is clearly evidenced by the characteristics of A-T cells: premature senescence, chromosomal breakage, extreme sensitivity to ionizing radiation and radiomimetic chemicals, and defective activation of an extensive array of signaling pathways normally activated by these agents (Fig.). A prominent DNA damage response that is defective in A-T cells is the delay in cell cycle progression caused by the activation of mechanisms called cell-cycle checkpoints, which temporarily halt the cell cycle at specific stages to allow time for DNA repair (Fig.). Defects were also reported in a number of other functions unrelated to stress responses, such as metabolic cascades activated in lymphocytes by the B-cell receptor, and potassium currents.

Mouse strains lacking the Atm protein were generated by targeted inactivation of their ATM gene, the murine ATM homolog. These animals recapitulate the human disease, with radiosen-



ATM Protein. Fig. – Signaling pathways mediated by ATM in response to DNA double strand breaks (DSB). Single arrows indicate direct physical interactions between proteins or enzyme-substrate relationships, leading to activation of downstream effectors. Two successive arrows point to functional relationships which may be mediated by additional, yet unknown proteins. Dotted 'T' lines indicate inhibitory interactions. A variety of stress responses (left side of the diagram) are mediated by chromatin modifications or activation of transcription factors such as AP-1, NF-κB and p73. Activation of the cell-cycle checkpoints (center) is obtained by interference with various components of the cell cycle machinery. Repair of DSB is carried out via activation of repair complexes and other proteins, some of which are involved also in other damage-induced pathways. Note the involvement of the BRCA1 tumor suppressor protein in these responses.

sitivity and a striking propensity to thymic lymphomas, but they do not show the profound cerebellar degeneration typical of human patients. ATM-deficient mice, like A-T patients, are sterile as a result of extensive chromosomal fragmentation during meiotic recombination. Indeed, in normal mice, the ATM protein is associated with the synapsed chromosomal axes during normal meiosis, pointing to its involvement in meiotic recombination. This indicates that ATM is involved not only in damage responses, but also in responding to and processing DNA strand breaks that take place during normal physiological processes. Another such process is the maturation of the immune system genes by somatic recombination. The marked immunodeficiency of A-T patients, as well as the appearance of chromosomal translocations involving these genes in patient lymphocytes, points to the possible involvement of ATM in this process as well.

Much of ATM's control of multiple pathways is attributed to its ability to phosphorylate a variety of cellular proteins. Importantly, this activity is enhanced immediately following DNA damage. A notable physiological substrate of ATM's kinase activity is the p53 protein, the product of a tumor suppressor gene that is mutated in more than 50% of human cancers. The p53 protein is a transcription factor that is involved in many cellular stress responses, most notably those activated by DNA damage. It plays a major role in the control of the G1/S cell-cycle checkpoint (Fig.). p53 activation following damage entails enhanced transcriptional activity and accumulation due to decreased degradation. This activation is accompanied by several modifications of the p53 molecule, some of which are phosphorylations on various residues such as the amino acid serine at position 15. Both p53's activation and phosphorylation on serine 15 are delayed in A-T cells. Indeed, ATM phosphorylates p53 *in vitro* on serine 15. ATM also acts on p53 by activating Chk2 protein kinase, which phosphorylates p53 on another site, serine 20, and phosphorylates another targets that activates a different checkpoint (Fig.). ATM also phosphorylates the Mdm2 protein, a tight negative regulator of p53, which also mediates its degradation. This intricate network of processes converging at the p53 protein, demonstrates the multiple ways in which ATM can control a single physiological pathway.

Several other proteins were found to be substrates of ATM's kinase activity: the Brca1 protein, the product of a tumor suppressor gene BRCA1, with a major involvement in predisposition to breast cancer, that plays a role in the repair of DNA damage; the Nbs1 (nibrin) protein that is directly engaged in strand break repair; and replication protein A (RPA), suspected to play a role in the cell-cycle checkpoint at the S-phase of the cell cycle (Fig.). Nbs1 is the product of a gene mutated in another human genetic disorder, the Nijmegen breakage syndrome (NBS). NBS patients exhibit immunodeficiency, acute cancer predisposition and radiation sensitivity, very much like A-T patients, but do not show cerebellar degeneration or telangiectasias. The finding that the Nbs1 protein is a substrate of ATM's kinase activity explains the similarity and functional relationship between these two human disorders.

Many of the numerous stress responses controlled by ATM end up in activation or downregulation of gene expression. A global look at gene expression profiles in the cell can be obtained using → microarray technology, which demonstrates the extent of expression of thousands of genes at the same time. This technique disclosed that ATM is involved in the majority of alterations in gene expression that follow treatments with ionizing radiation or radiomimetic chemicals. This finding further underscores the central role of ATM as a master controller of physiological processes in the cell.

Clinical Relevance

The clinical manifestation of ATM inactivation is a genetic disease characterized by a perplexing array of developmental and cellular defects. The phenotype attests to the centrality of ATM-controlled cellular pathways to the development and proper function of the immune system and specific parts of the nervous system, to genome stability, and to a network of cellular defense mechanisms activated in cells by DNA damage. The striking predisposition of A-T patients to lymphoid cancers (→ lympho-

ma) clearly shows that ATM controls an important intersection of functions necessary to prevent the development of malignancy in these cells. It is a common notion that the genomic instability in A-T is a major cause of cancer in A-T patients, in keeping with the well established link between cancer and genomic rearrangements.

It has long been suspected that carriers of A-T mutations bear a certain degree of predisposition to malignancies, primarily breast cancer. This notion stemmed primarily from epidemiological observations and is now being re-examined using molecular assays for the detection of carriers of *ATM* mutations. Recent observations indicate that some degree of cancer predisposition may be conferred by heterozygosity for specific types of *ATM* mutations. Since cells from A-T carriers exhibit a moderate degree of radiation sensitivity, heterozygosity for *ATM* mutations might also lead to adverse side-effects of radiotherapy, such as severe local responses to treatment or radiation-induced secondary cancers.

A further link between *ATM* sequence alterations and cancer comes from another line of research: In certain hematopoietic malignancies, most notably T-prolymphocytic leukemia, both copies of the *ATM* gene are inactivated due to somatic mutations and rearrangements. This phenomenon is typical of tumor suppressor genes and points to the role of ATM-dependent processes in guarding mammalian cells from malignant transformation.

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Atonal Family

Definition

Atonal family is a → bHLH (basic helix-loop-helix) protein family, comprising proteins Atonal (*Drosophila*), Lin-32 (*Caenorhabditis elegans*), Hath1, Math1, Math2, NeuroD, NeuroD2, NeuroD3, NeuroM. The members are involved in neurogenesis and pancreas development.

ATR

Definition

→ Ataxia telangiectasia (ATR) and Rad3-related are acronyms for a checkpoint kinase (AT- and Rad3- related) that is homologous to the → ATM protein mutated in Ataxia telangiectasia and the yeast RAD3 kinase. Activation of ATR is a proximal step in the cellular mechanisms that sense and signal DNA damage. The gene maps to 3q22-24.

Atrophic Gastritis

Definition

Atrophic gastritis is the inflammation of the ephitelial lining of the stomach and the loss of gastric glands.

ATRX

Definition

α-Thalassemia/mental retardation syndrome X-linked (ATRX), also known as RAD54, is a nuclear protein of 2375 amino acids and 269 kD. It is presumed to be a global transcription regulator that modifies gene expression by affecting chromatin, belonging to the helicase family. Defects of the gene at Xq13 result in thalassemia/mental retardation syndrome.

Atypia

Definition

Atypia is a term used to describe cells that have lost their normal appearance but have not reached the level of abnormality of cancer cells.

Autocrine

Definition

Autocrine is a factor acting on the same cells that produce it. Following interaction with the endogenous receptor(s), an autocrine activation (autocrine loop) is obtained; → paracrine; → receptor tyrosine kinases.

Autocrine Stimulation

Definition

Autocrine stimulation is stimulation by a factor that is produced by the same cell type that responds to it; → receptor tyrosine kinases.

Autograft

Definition

An autograft is the engraftment of organs, cells or tissues from one individual to themselves.

Autoimmune Disease

Definition

Autoimmune disease is an aggressive reaction of the immune system against self-antigens with massive infiltration of activated T cells (CD44v+) and macrophages into the reactive organ. Destruction of tissue results in the production of autoantibodies, which further augment tissue destruction. There are multiple induction mechanisms such as genetic factors, gender and infections.

Autoimmune Lymphoproliferative Syndrome

Definition

Autoimmune lymphoproliferative syndrome, also known as Canale-Smith Syndrome, is a childhood syndrome of autoimmunity with a phenotype of hemolytic anemia and thrombocytopenia with massive lymphadenopathy and splenomegaly within first two years of life. Lymphadenopathy is associated with mutations of the → FAS gene, and the accumulation of lymphocytes is mainly the result of failure of *FAS*-mediated → apoptosis. Autoimmune manifestations, such as hemolytic anemia and thrombocytopenia, which result from the production of autoantibodies against red blood cells and platelets, can persist into adolescence, and the *FAS* mutations are compatible with long-term survival. Patients have been detected lacking mutation in the *FAS* gene, and it is thought that in these cases the impaired apoptosis results from other defects of the *FAS* pathway (Ref.: Vaishnaw AK, Orlinick JR, Chu J-L, Krammer PH, Chao MV, Elkton KB. (1999) The molecular basis for apoptotic defects in patients with CD95 (Fas/Apo-1) mutations. J Clin Invest 103: 355-363).

Autonomic Nervous System

Definition

The autonomic nervous system is the 'involuntary' as opposed to the somatic or 'voluntary' nervous system. It is responsible for much of the unconscious regulation of body functions such as heart rate, blood pressure and movements of the gut.

Autophagosome

Definition

An autophagosome is a cytoplasmic organelle in eukaryotic cells that is formed by the engulfment of cytoplasmic material (including whole organelles) by ER-derived membranes. It either fuses with the → lysosome or matures to become a lysosomal compartment.

Autophagy

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Synonyms

- autophagocytosis

Definition

Autophagy is the intracellular uptake of cytoplasm (proteins, nucleic acids, small molecules, whole organelles, etc.) into the → lysosome and its subsequent degradation.

Characteristics

Lysosomal uptake and degradation of proteins by autophagy can be found in virtually all eukaryotic cells. It is responsible for the degradation of the majority of cellular proteins. Autophagy is regarded to be a largely non-selective bulk process. Some proteins or organelles, however, can be selectively transported into the lysosome. Autophagy helps to maintain cellular homeostasis by recycling cytosolic material. Autophagy may have more specialised roles in biogenesis of the lysosome (import of lysosomal hydrolases), cellular differentiation and cell death.

Cytosolic material can enter the lysosome via several routes:

- Macroautophagy involves the engulfment and inclusion of cytosol by membranes which probably stem from the ER (endoplasmic reticulum), thereby forming autophagosomes. These → autophagosomes fuse with lysosomes or are matured into lysosomes by acidification and aquisition of a set of lysosomal proteins.
- In microautophagy, cytosolic material is taken up by the lysosome through invaginations of the lysosome.
- Carrier-mediated uptake involves the recognition of cytosolic proteins by a → chaperone-related receptor and its subsequent translocation into the lysosome.

Model systems for the study of autophagy are hepatocytes (high metabolic rate) and the yeast *Saccharomyces cerevisiae*.

Cellular and molecular parameters

Autophagy is a constitutive and energy-dependent process, which ensures a permanent turnover and recycling of cytosol. Under conditions of stress or starvation autophagy is induced. When free amino acids are abundant, autophagic activity is reduced. In growth medium devoid of one of the regulatory amino acids (methionine, leucine, glutamine, proline, histidine, tryptophane, tyrosine, phenylalanine) the formation of autophagosomes is upregulated. The methylated derivative of the ATP precursor adenine, 3-methyladenine, is an inhibitor of autophagy.

No coherent view of the signal transduction involved in autophagy has been achieved so far. However, the → TOR (target of rapamycin) signalling pathway seems to be involved in the regulation of autophagy. This pathway transduces extracellular growth factor or hormone signals (e.g. insulin) through phosphatidylinositol 3-kinase, Akt/PBK, TOR, and p70S6 kinase, to ultimately regulate cell growth by translational (and also transcriptional) mechanisms.

The Hsc73 protein (heat shock cognate protein of 73 kD) is required for the direct uptake of proteins carrying the recognition peptide sequence KFERQ (lysine-phenylalanine-gluta-

mate-arginine-glutamine) into the lysosome. The recognition sequence is present in 25 to 30% of all cytosolic proteins.

Furthermore a novel protein conjugation system is required for autophagy. This conjugation system has been identified in the yeast *Saccharomyces cerevisiae* and is conserved in humans.

Clinical aspects

Certain forms of cancer are associated with decreased autophagic activity. It has been speculated that a reduced level of intracellular protein degradation confers a selective advantage to malignant cells over normal cells. Inhibitors of autophagy have been suggested as anti-cancer drugs.

→ Apoptosis and autophagy could be positively connected. In some cancer cell lines 3-methyladenine inhibits drug-induced autophagy and apoptosis. The Apoptosis-Specific Protein (ASP), which is induced in apoptosis, is identical to the Apg5 component of the conjugation system required for autophagy. → Beclin1 is a → Bcl-2-interacting protein involved in apoptosis and required for autophagy in yeast and mammalian cells.

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Autophosphorylation

Definition

Autophosphorylation is phosphorylation, most often is in *trans*, between subunits in a ligand-induced oligomeric receptor complex. It serves two important functions; autophosphorylation of a conserved tyrosine residue within the activation loop of tyrosine kinases activates the kinase and other phosphorylated tyrosines serve as docking sites for downstream components with → SH2 domains; → receptor tyrosine kinases.

Autosomes

Definition

Autosomes are all the chromosomes except the sex chromosomes; a diploid cell has two copies of each autosome.

Aza-Arenes

Definition

Aza-arenes are polycyclic aromatic hydrocarbons containing nitrogen at one of the ring positions.

B

BAC

Definition

Bacterial artificial chromosomes (BAC) are → cloning vectors.

Baculovirus IAP Repeat

Definition

Baculovirus IAP repeat (BIR) is a conserved protein domain of approximately 70 amino acids in members of the 'inhibitor of apoptosis' (IAP) protein family. BIR was originally identified to suppress apoptosis in baculovirus infected cells of the fruit fly *Drosophila melanogaster*. In mammalian cells some IAP proteins were identified as inhibitors of caspases, others apparently lack the ability to interact with caspases. It is likely that IAP proteins have also other functions, an indication supported by the fact that IAP proteins exist in yeast, which do not have caspases.

BAD

Definition

Bad (synonym: BAD) is a → Bcl-2 antagonist of apoptosis/death, a pro-apoptotic member of the Bcl-2 family of proteins. It is a 18 kD protein that binds to Bcl-xL and Bcl-2. It competes out the binding of Bcl-xL and Bcl-2 to Bax, thus promoting apoptosis.

BAGE

Definition

BAGE stands for B melanoma antigen. The BAGE protein (43 aa, 4 kD) is usuallyonly expressed in the testis, due to de-→ methylation of the *BAGE* gene the protein is also expressed in → melanoma and in a variety of other tumors. The function of BAGE is not known. The antigen is recognised on melanoma by autologous cytolytic T lymphocytes. Vaccination with BAGE is employed in immunotherapy of melanoma.

BAK1

Definition

Bak1 (synonym: Bak-1) is a → Bcl-2 antagonist/killer-1, a pro-apoptotic member of the Bcl-2 family of proteins. It is a 23 kD protein that binds to and antagonizes Bcl-2. It also forms heterodimers with anti-apoptotic → Bcl-xL and binds to → adenovirus E1b19k.

Balanced Translocation

Definition

Balanced translocation is the exchange of DNA material between chromosomes without gain or loss of DNA; typically found in leukemia and lymphomas.

Band

Definition

→ Chromosome band.

Barrett Adenocarcinoma

Definition

→ Esophageal cancer.

Barrett Esophagus

Definition

Barrett esophagus is the metaplastic epithelial lining of the distal esophagus, recognised as a premalignant condition; → esophageal cancer.

Basal Cell Carcinoma

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Definition

Non-melanoma skin cancers are the most common malignant neoplasms in the United States, representing one third of all cancers diagnosed every year. Basal cell carcinoma (BCC) represents 75% of non-melanoma skin cancers and has an estimated annual incidence of more than 700,000 cases in the United States outnumbering → squamous cell carcinoma (SCC) 4 to 1. Over half a million BCCs are diagnosed in the United States annually, outnumbering squamous cell carcinoma (SCC) 4 to 1. The US average annual incidence of BCC in whites is currently 191 per 100,000 and is increasing at a rate of 3%-7% per year. The peak incidence of BCC occurs in the seventh decade of life and is rare in children.

Characteristics

Basal cell carcinoma has multiple distinctive clinical forms, and these clinical subtypes can often be correlated with histologic subtypes. BCC is protean in its manifestations. For this reason, biopsy for histologic confirmation is necessary. The various clinical forms of BCC include nodular, morpheaform and superficial. Nodular BCC is the most frequent form of BCC. It usually presents as a waxy, pearly or translucent papule/nodule with overlying fine telangiectasias, with frequent ulceration or erosion of the surface. BCCs may occasionally be pigmented to varying degrees. Superficial BCCs most commonly arise on the trunk and extremities, but may be seen anywhere on the body. The tumors are characterized by an erythematous macule or patch, which may be variably pigmented. There may also be an overlying fine scale, a superficial erosion or hemorrhagic scale crust. Superficial BCC is the variant most frequently seen in chronic arsenism and as late sequelae of radiation therapy. Individuals may have broad areas of superficial BCC that are multiple and disconnected. Morpheaform or sclerosing has a scar-like appearance. It consists of a dermal plaque with overlying epidermal atrophy in a sun-exposed distribution. As with infiltrative BCC, subclinical extension is often great and treatment failures frequent.

Risk factors and therapy

Location, histologic subtype, clinical characteristics and size are predictive factors for the biologic behavior of BCCs. The typically indolent growth pattern of BCC accounts for the resistance and fusion planes of the central facial zone being a more significant determinant of subclinical extension. Size is also a good predictor of high risk BCCs. Cure rates with Mohs micrographic surgery (MMS) decreases as tumor size increases. A cure rate of 99.8% for tumors less than 2 cm in diameter, 98.6% for tumors between 2 and 3 cm and 90.5% for tumors greater than 3 cm has been reported.

Micronodular, infiltrative and morpheaform BCCs have a much higher incidence of positive surgical margins after surgical excision (18.6% to 33.3%) as compared with tumors with a nodular or superficial histologic pattern. Morpheaform BCCs may have significant subclinical extent, with the average subclinical extension being 7.2 mm. Similarly, significant subclinical extension in infiltrative BCC has been noted. BCC with marked squamous differentiation has been determined to be a more virulent tumor (a local recurrence rate of 45.7% and metastatic incidence 8.6% of 35 such tumors as compared to rates of 24.2%/0.09% for BCC). As with SCC, the perineural space can serve as a conduit for significant subclinical tumor extension.

Although BCC is rarely life threatening its capacity for local tissue destruction can result in significant functional or cosmetic morbidity. Untreated or inadequately treated BCCs have an insidious growth pattern and may result in death. Metastasis from BCC is a rare event, with estimates of metastatic incidence ranging from 0.0028% to 0.1%. Metastasis is associated with the metatypical (basosquamous) BCC and with duration and size of the lesion. The most frequent site of metastasis is the lungs, followed by bone, lymph nodes, and liver. For these reasons, great importance is attached to the early diagnosis and treatment of this malignancy.

BCC is related to chronic ultraviolet radiation (UVR) exposure. UVR exposure is partly responsible for both BCC and SCC, as evidenced by the preponderance of these lesions on sun-damaged skin after chronic exposure to sunlight. More than 99% of individuals developing BCC are Caucasians, and 85% of these tumors arise on the head and neck. The nose is most common of all sites, accounting for 25% to 30% of all tumors. Individuals of Scottish, Celtic, or Scandinavian ancestry are at higher risk. Affected persons usually have a history of significant occupational and/or recreational sun exposure. There is evidence that BCC arising before the age of 40 years corresponds with childhood or recreational sun exposure but does not correlate directly with cumulative sun damage. Thus, in areas of the world where the UV radiation is most intense, such as the sun belt in the United States, childhood sun exposure is at a maximum and younger patients are at a higher risk of developing BCC.

It is debatable whether BCC is more aggressive in children. As total incidence rates of BCC continue to rise, childhood cases may become more common. This increase in pediatric BCC may be especially true in areas of high UV radiation exposure. The percentage of sunny days during the year, higher altitude, and location closer to the equator may place children in these areas at increased risk. There exist other significant risk factors for the development of BCC: Prior injury such as trauma, burns, or vaccinations at the tumor site is frequently noted by persons with BCC. Carcinomas arising as a late sequelae of radiation therapy most frequently takes the form of BCC on the head, neck, and trunk, and SCC on the hands. Prior exposure to inorganic arsenic can also lead to the formation of BCC. In this setting, tumors are often multiple, truncal, and superficial lesions. Immunosuppressed individuals are also prone to the development of BCC, although their risk is greater for SCC than for BCC.

Basal cell → nevus syndrome (BCNS), → xeroderma pigmentosum, Baze syndrome and albinism represent inherited genetic disorders that predispose those affects to BCC and SCC. Patients with basal cell nevus syndrome are found to have a germline mutation in *PCTH*, a tumor suppressor gene located on 9q22.3. *PTCH* is the human homolog of *Drosophila* patched. Approximately 1/3 of cases result from a new germline mutation. Approximately 80% of *PTCH* mutations result in premature truncation of the patched protein. Inactivation of this gene was found in tumor tissue in 68% of BCCs examined and did not correlate directly with sun exposure or age. Typically, multiple BCCs develop at a young age in BCNS. Multiple BCCs, odontogenic keratocysts and palmo-plantar pits constitute the primary features of BCNS. Approximately 5% of infants with BCNS develop → medulloblastoma. Radiation treatment for the medulloblastoma can result in a crop of BCCs in the radiation port.

Xeroderma pigmentosum (XP) is due to a genetic defect in the biochemical pathway to

eliminate the carcinogenic potential caused by the damage of UVB to DNA. Several genes, those for XP groups B, D, and G and Cockayne syndrome groups B code for components of transcription factors, the protein complexes that bind the promoter regions and control gene trasncription. BCCs and SCCs occur at a much higher rate and a much earlier age. Keratoacanthomans, fibrosarcomas and melanomas are also common in patients with XP.

BCC can be treated with multiple modalities providing 90% cure rates for primary disease in most cases. Cure rates for ablative surgery and excisional surgery vary with a number of factors including the clinical size of the tumor, the location, the histological subtype and whether or not it is recurrent. Cure rates for ablative surgery are less than 90% for BCC exceeding 0.5 cm in diameter on the face and over 2.0 cm in diameter on the trunk and extremities. In these instances, consideration should be given for excisional surgery with adequate margin control. BCCs exceeding 0.5 mm in diameter of the central facial zone and aggressive growth pattern tumors with sclerosing stromas are best treated with Mohs micrographic surgery. The histologic subtype or growth pattern is a good predictor of cure rate. These tumors do not respond well to superficial or ablative surgery. Nodular and superficial BCC respond well to curettage and electrodesiccation, cryotherapy or shave excision can result in less morbidity than full-thickness excisional surgery. For adequate cure rates morpheaform or sclerosing, micronodular or infiltrative variants of BCC require excisional surgery with histologic margain control.

Squamous Cell Carcinoma

SCC is the second most common skin cancer, representing 20% of cutaenous malignancies. Over 100,000 cases of SCC are diagnosed annually in the United States, accounting for an incidence of 41.4 per 100,000. SCC of the skin is the fifth most common cancer among men and the sixth most common cancer among women in Sweden. SCC *in situ* or Bowen disease is the most common benign/precancerous tumor among men, while among women, it is second only to *in situ* cervical cancer. It most commonly affects individuals in mid to late life. SCC usually cause local tissue destruction and in advanced cases it may cause cosmetic and functional morbidity.

Clinically, typical SCC is a hyperkeratotic papule, nodule or plaque with variable erythema. Associated pain may suggest perineural extension. The central part of the face is the area at highest risk for recurrence. Tumors in this region tend to grow down or extend at various resistance planes such as the perichondrium of auricular and nasal cartilages. The tarsal plates of the eyelids or embryonic fusion planes at the junction of the nasal and nasolabial folds, and along the nasal columella or in the periauricular region. The size of the tumor also affects risk for recurrence. Tumors less than 1 cm have a 99.5% cure rate by Mohs micrographic surgery, compared with 82.3% for tumors 2 to 3 cm and 58.9% for tumors greater than 3 cm. Tumors under 2 cm of diameter have a local recurrence rate of 7.4% in contrast to a 15.2% recurrence rate for tumors greater than 2 cm. Therefore, margins of excision are adjusted according to size, with a 4 mm margin recommended for tumors less than 2 cm and 6 mm for tumors of 2 cm or greater.

Deeply invasive tumors have a greater tendency for local recurrence and metastases. Tumors with less than 4 mm in depth have a local recurrence rate of 5.3% compared with a rate of 17.2% for tumors 4 mm or greater. SCCs that penetrate though the dermis to the subcutaneous tissue have a recurrence rate of 19.8%. Tumors greater than 1 cm in diameter or a histologic grade 2 or higher are more likely to extend to subcutaneous tissue. The degree of histologic differentiation has a propensity for aggressive disease. SCC Broder grades 2 or higher usually require larger resections and has greater risk of local recurrence. Well differentiated SCC have a 13.6% recurrence rate in contrast to a 28.6% recurrence rate for poorly differentiated SCC. SCC with neurotropic growth pattern, which invade the perineural space, have a greater risk for local recurrence.

SCC usually have a low metastasis rates ranging from 0.3-3.7%. SCC arising in the lip, ear, penis, scrotum and anus have a higher risk for

metastases. There is a greater risk of metastases for SCC more than 2 cm in size, with depth of invasion to at least 4 mm, Broader's histologic classification of 2 or greater and perineural extension. SCC usually metastasis to the regional lymph nodes. The five year survival rates for patients with regional lymph node metastases is 26%, and 23% in patients with distant metastases.

Risk factors and therapy

The risk factors for SCC include exposure to UV light and arsenic compounds, immunosuppression and underlying genetic predisposition. Cellular atypia is often equally high among those with *in situ* SCC or invasive SCC, and it is difficult to use cytological criteria to define *in situ* SCC as a benign lesion, in spite of an intact basement membrane in histological specimens. Even when using molecular markers such as the expression of p53 gene, *in situ* and invasive SCC are indistinguishable. The incidence of invasive SCC is 2 times higher for men, whereas *in situ* SCC is more common among women. It is possible that there are close etiological links between*in situ* and invasive SCC, and *in situ* appears to be as important a marker for subsequent cancer risk as invasive SCC.

SCC can also be treated satisfactorily with different modalities. Histologic growth pattern is less important in SCC than clinical size and depth of invasion, with the exception of rare histologic subtypes such as adenosquamous cell carcinoma. SCC exceeding 1 cm in diameter and tumors that invade into the mid-dermis or deeper, particularly those involving cartilage and bone, are high-risk tumors. SCC on the lip, ear, temple, genitalia and those associated with preexistent conditions such as radiation or burn scars are all higher risk tumors. In these instances excisional surgery with careful margin control should be the treatment of choice. Postoperative radiation therapy may also be considered for these aggressive high-risk tumors on a case-by-case basis. Superficial or ablative procedures such as curettage and electrodesiccation, cryotherapy and shave excision should be reserved for SCC *in situ* (Bowen disease) or SCC that invades only the superficial dermis. The depth of the invasion can be measured with an adequate preoperative biopsy. Indurated tumors with an undermining infiltrative border are often deeply invasive and should be treated with excisional surgery.

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Basal Cell Nevus Syndrome

Definition

Basal cell nevus syndrome (BCNS), also known as Gorlin syndrome or NBCCS (naevoid basal cell carcinoma syndrome), is a heritable autosomal dominant tumor syndrome. It is characterized by a multitude of developmental abnormalities including basal cell carcinomas of the skin, keratocysts of the jaw, palmar and plantar pits, fibromas of the ovaries and heart, medulloblastomas and less commonly polydactyly, syndactyly and spina bifida. Approximately 2% of patients with BCNS develop medulloblastomas. The disease results from germline mutations in the *PTCH1* gene (→ patched) in chromosome 9q22.3.

Base Pair

Definition

A base pair (bp) is two nitrogenous bases (adenine and thymine or guanine and cytosine) held together by weak bonds. Two strands of DNA are held together in the shape of a double-helix by bonds between base pairs. The human genome contains an estimated 3 billion base pairs. One million bp is often referred to as 1 Mb, and one thousend as 1 kb.

Basement Membrane

Definition

The basement membrane (BM) is a thin mat of → extracellular matrix that separates epithelial sheets and many types of cells, such as muscle cells and fat cells, from connective tissue. The characteristic components of BMs are laminin, collagen type IV and → heparan sulfate proteoglycan.

BAX

Definition

Bax is a Bcl-2-associated X protein of 192 aa and 21 kD that is membrane-bound and expressed widely in different tissues. It has proapoptotic activity, by binding and antagonizing the anti-apoptotic → Bcl-2, thereby accelerating → apoptosis. The gene maps to 19q13.

BCC

Definition

→ Basal call carcinoma.

BCDF

Definition

B-cell differentiation factor; → interleukin-6.

B-cell Differentiation Factor

Definition

→ Interleukin-6.

B-cell Diseases of the Immunopoiesis

Definition

B-cell disease of the immunopoiesis is synonomous to chronic lymphoproliferative disorders. It consists of a group of leukemic malignant lymphomas at a developmental stage corresponding to B-cells, involved in immune reactions in secondary lymhoid tissues.

B-cell Non-Hodgkin Lymphomas

Definition

B-cell non-Hodgkin lymphomas are cancers that arise in mature B lymphocytes.

B-cell Stimulating Factor-2

Definition

→ Interleukin-6.

B-cell Tumours

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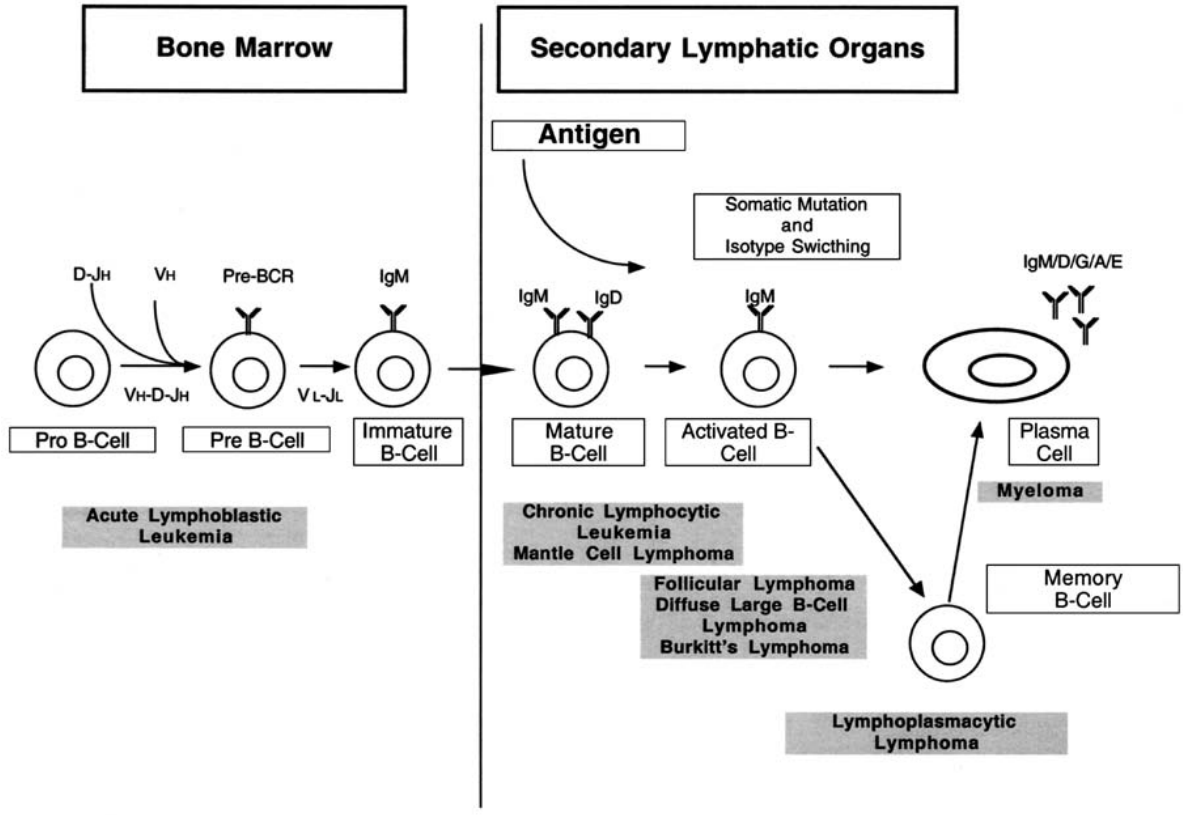
Synonyms

- B-cell lymphomas
- B-cell leukemias
- B-cell lymphoid neoplasm
- B-cell lymphoproliferative disorders/diseases
- Hodgkin and Non-Hodgkin lymphomas
- B-cell malignancy
- cancer of B-lymphocytes

Definition

B-cell lymphomas are malignant tumours of B-lymphocytes. They arise at all stages of B-cell differentiation, from immature B-lymphocytes in the bone-marrow through to terminally differentiated plasma cells (Fig. 1). It is now possible to use immunogenetic analyses to define more clearly the cell origin and clonal history of B-cell tumours.



B-cell Tumours. Fig. 1 – In the bone marrow first the D-JH then VH-D-JH recombination takes place. This heavy chain is expressed on the cell surface with the surrogate light chain to form the pre-B-cell receptor (pre-BCR). Next the light chain genes are rearranged. The B-cell now expresses surface Ig and leaves the bone marrow. Mature B-cells encounter antigen, and are stimulated to somatically mutate their V genes. Additionally class switching is initiated. Some B-cells then leave the germinal centre to become plasma cells, some become memory cells.
The grey blocks illustrate, at which stage of B-cell differentiation some B-cell tumours are thought to originate.

Characteristics

What is a B-lymphocyte?

B-lymphocytes are cells of the immune system that are destined to express immunoglobulins (antibody molecules). These immunoglobulins (Igs) play a central role in the recognition of foreign antigens like infectious organisms, which could threaten the integrity of the individual.

Igs are glycoproteins that can exist either as membrane-bound molecules on the cell surface or as secreted molecules in the serum. There are 5 classes of Ig with different size, structure and function; IgM, IgD, IgG, IgA and IgE. Each basic Ig molecule contains two identical heavy chains (μ, δ, γ, α or ε) and two identical light chains (κ or λ). A mature B-cell carries about 10^5 to 10^6 identical Igs on its cell surface. Both light chains and heavy chains can be subdivided into distinct regions. The N-terminal variable (V-) regions mediate antigen contact and their amino acid sequence is specific to each B-cell. The C-terminal constant regions are common to all antibodies of the same class.

The sequence variability, which is necessary to recognise the vast number of different antigens present in the environment, is created by two processes. The first is → immunoglobulin gene rearrangement and the second is somatic mutation. → Class switching changes Ig effector function.

Immunoglobulin gene rearrangement

During this remarkable process, double-stranded DNA breaks are created and repaired in a tightly controlled fashion. Rearrangement brings together one representative from different gene families: variable region (V_H) genes, diversity region (D) genes and joining region (J_H) genes for the Ig heavy chain, V_L and J_L for the Ig light chain (Fig.1). The process of V_H-D-J_H and V_L-J_L joining is imprecise; non-templated nucleotides (N-additions) can be inserted and the ends of the joined segments can be trimmed back. Thus the final products of rearrangement, the V_H-D-J_H and V_L-J_L, will have a unique nucleotide sequence.

The heavy chain variable (V_H) region is about 120 amino acids (aa) long and can be subdivided into discrete structural sections. Three complementarity determining regions form the classical antigen binding site; CDR1, CDR2 and CDR3. While CDR1 and CDR2 are encoded in the germline, CDR3 is created *de novo* in each B cell by V_H-D-J_H rearrangement. In the antibody molecule this sequence corresponds to the central part of the antigen recognition site. The CDRs alternate with four framework regions (FR1-4). Light chain variable regions (V_L) regions are about 10 aa shorter, but contain similar structural motifs.

In the bone marrow the Ig heavy chain genes are rearranged first followed by the light chain genes (Fig. 1). Ultimately a B-cell that successfully completes this process will have a unique immunoglobulin heavy and light chain gene sequence for antigen recognition. The nucleotide sequence in CDR3 can be viewed as its molecular marker or 'fingerprint'.

Somatic mutation and class switching

Following antigen encounter, further variability is introduced into the rearranged variable region genes by somatic mutation that occurs in secondary lymphatic organs. Somatic mutation can change the amino acid sequence of variable region genes and may therefore impact on the antigen binding of the resulting protein.

The process of class switching changes the effector function of the antibody molecule (complement activation, binding to Fc receptors or uptake by phagocytic cells). During class switching the DNA segment of one constant region (e.g. IgM) is deleted and the variable region of the heavy chain brought into the vicinity of another constant region gene (e.g. IgG or IgA). This process conserves the unique variable region.

What is a B-cell tumour?

In the broadest sense B-cell tumours are malignancies in which tumour cells have undergone rearrangements of their immunoglobulin genes. Analysis of the status of these genes provides information that defines origin and clonal history

of the tumour cell. Fig. 1 shows key steps in normal B-cell development, and gives examples of B-cell malignancies that may arise at a particular stage. Within each cancer, the tumour cells are clonally related, as revealed by the common CDR3 sequence in the tumour cell population.

In some lymphomas the tumour clone has non-functionally rearranged V_H genes, as appears to be the case in Hodgkin lymphoma. This sets these lymphomas apart from normal B-cells, which can only survive if they express immunoglobulins. The antigenic determinants, derived from the variable regions of the immunoglobulin molecule, provide us with a unique tumour antigen called → idiotype. This tumour antigen is now being exploited in new immunotherapeutic strategies.

Characteristics of B-cell tumours

B-cell tumours account for about 3% of all cancers. For unknown reasons their incidence is rising steadily at about 6% per year worldwide. B-cell tumours are the most common malignancies in childhood. In adults, the frequency of B-cell tumours increases steadily with age, with a median of 50-60 years. They occur more frequently in men than women.

The presentation of B-cell tumours at the clinical and morphological level can vary widely. Aggressive malignancies are at the one end of the spectrum, which if untreated will cause death in weeks but are frequently curable with combination chemotherapy. Indolent malignancies are at the other end of the spectrum, which are usually incurable but can remain untreated for decades. The diagnosis of B-cell malignancies relies on the clinical picture, histological analysis and immunophenotype of the tumour. Increasingly, hallmark genetic abnormalities are being defined in individual entities. They frequently involve translocations into the immunoglobulin loci of the heavy chain genes on chromosome 14 or the κ or λ light chain loci on chromosomes 2 and 22.

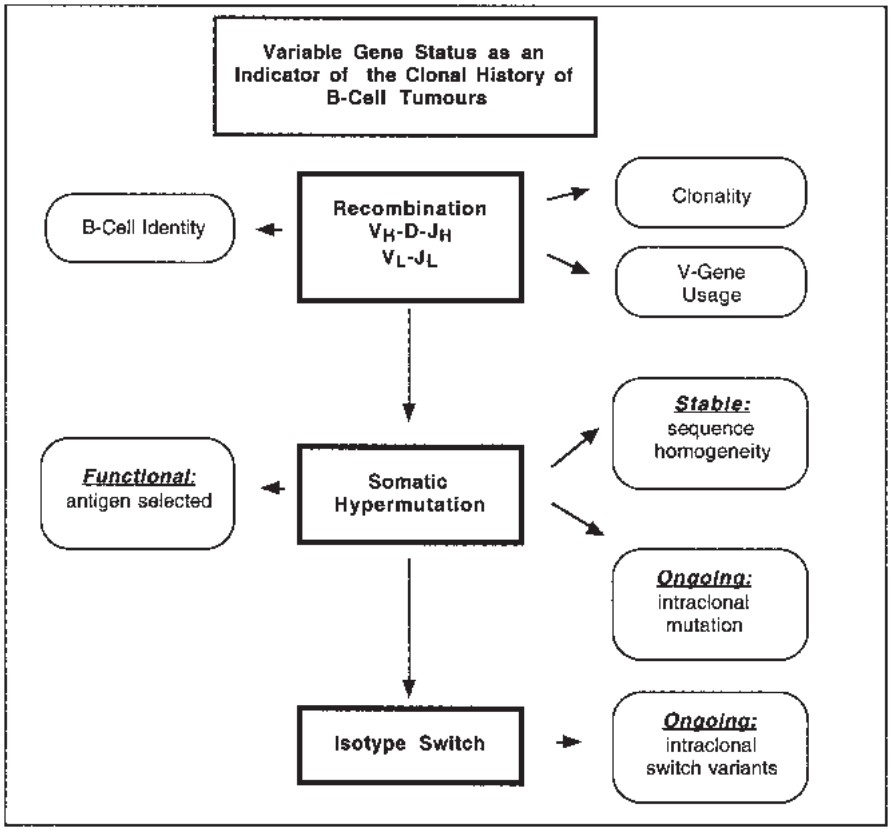
Different ways of grouping lymphoid malignancies in a logical fashion have been applied. They were based on the need of clinicians to determine a suitable course of treatment as well as the desire of pathologists to distinguish morphological similarities. Although these classifications were used in parallel, they are difficult to compare since similar entities were often attributed to different categories. In 1994, an attempt was made to divide lymphoid malignancies taking into account the combined available information from clinical patterns, morphology, immunophentoype and genetic characteristics. Also, as far as possible, the normal counterparts were attributed to each malignancy. This led to the 'Revised European-American Classification of Lymphoid Neoplasms' (REAL). This REAL classification provides the first truly international view of lymphomas and has been developed further in the form of the recently proposed WHO classification.

Immunogenetics of B-cell lymphomas

Genetic analysis of B-cell lymphomas has aided our understanding of malignant lymphoma. Specific chromosomal rearrangements in many of the lymphoma entities indicate that a particular type of genetic damage in the precursor cell is important for the development of the lymphoma. For example t(14;18) translocation is characteristic of → follicular lymphoma. The isolation of the same translocation in cells from healthy individuals suggests that this genetic change may be a necessary but insufficient condition for the development of follicular lymphoma.

More recently, the analysis of the status of the immunoglobulin genes in B-cell tumours has shed new light on the events which shape the malignant cell. Fig. 2 summarises the information that V-gene analysis of B-cell tumours can reveal. The presence of rearranged immunoglobulin genes defines the cells under investigation as being of B-cell origin (Fig. 2). In this way it could finally be established that in the majority of cases Hodgkin lymphoma is a B-cell tumour.

Sometimes it can be very difficult to assess if an abnormal population of B-cells represents a true malignancy. Examples include low grade → MALT 'lymphomas' in the stomach or lymphoproliferations after organ transplants. Here the



B-cell Tumours. Fig. 2

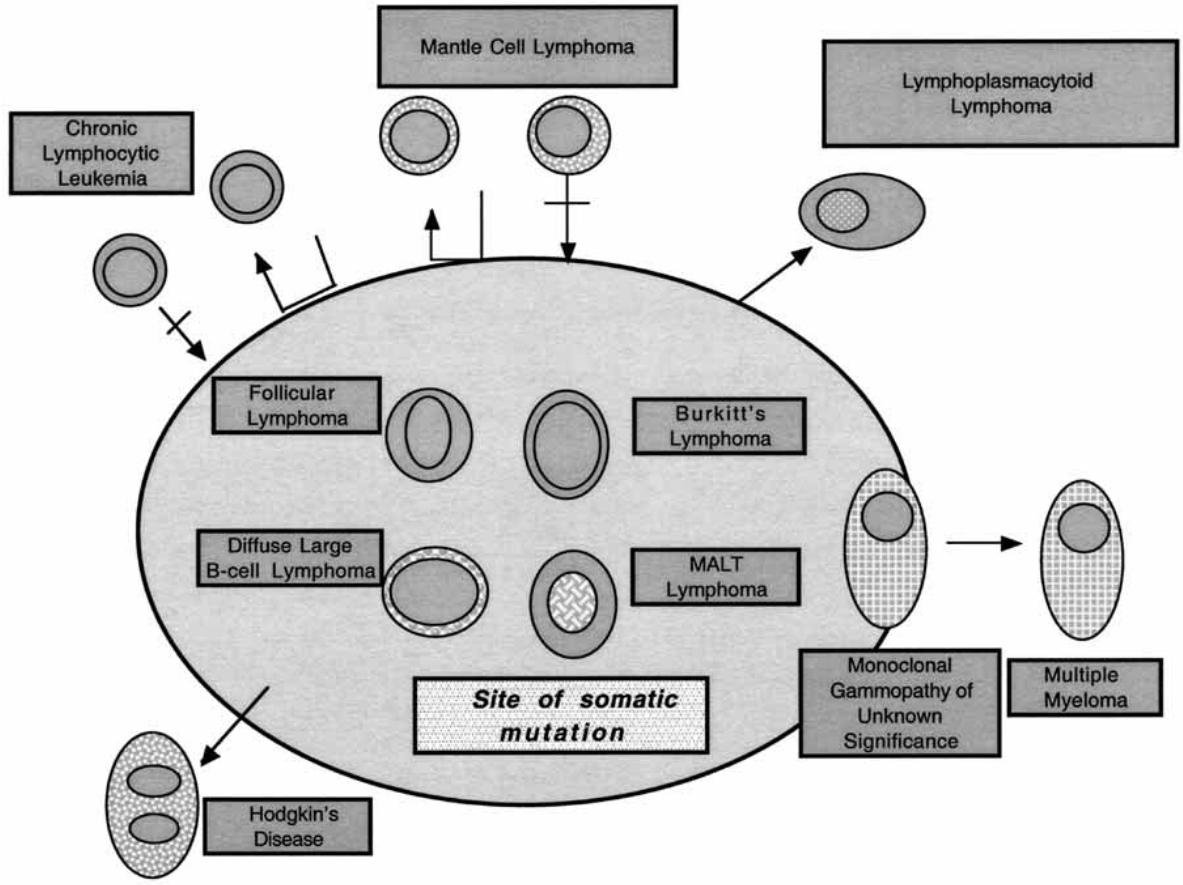
analysis of the Ig genes can help to separate a poly- or oligo-clonal and pre-malignant lesion from a truly clonal and cancerous one (Fig. 2).

In some B-cell lymphomas (follicular lymphoma, diffuse large B-cell lymphomas) the observed V_H gene usage is similar to that of normal B-cells. In other tumour types, however, a marked over- or under-representation of certain V_H genes has been detected. For example, a member of the V_H4 gene family, called V4-34, is used by about 6% of normal cells. In contrast, all known cases of → Waldenstrom macroglobulinemia with cold agglutinins of anti-I activity use the V4-34 gene. This suggests that B-cell superantigens may play a role in the pathogenesis of cancer in these B-cell lymphomas.

Since gene rearrangement, somatic mutation and class switching all leave their traces in the Ig-genotype of a B-cell, Ig analysis can provide important information about the clonal history of the malignant B-cell. V-gene analysis allows us to determine which processes the B-cell has been exposed to and also suggests which 'normal' counterpart the tumour cell may be related to.

The majority of B-cells in the periphery will have been exposed to somatic mutation in the germinal centre of the secondary lymphatic organs. Fig. 3 relates the origin and development of B-cell tumours relative to the germinal centre. The analysis of the tumour related V_H-D-J_H genes in tumours can reveal evidence that the tumour cell clone has entered this site, if somatic mutations are found in the V_H-D-J_H gene. Within the same tumour type some cases may show somatic mutation, while others do not. Chronic lymphocytic leukemia (CLL) segregates into two categories; patients with unmutated (pre-germinal centre) CLL have a significantly worse prognosis than those with mutated V_H-D-J_H genes.

Evidence for ongoing mutation can be identified by detecting micro-heterogeneity in clonally-related sequences from the tumour. While



B-cell Tumours. Fig. 3 – Origin and development of B-cell tumours in relation to the site of somatic mutation in the germinal centre (GC). V-gene mutational patterns can be used to classify tumours as follows: 1. not entering the GC (blocked arrows); 2. passing through the GC (arrows out); 3. remaining in the GC (no arrows). Monoclonal gammopathy of unknown significance may in some cases remain in the GC. CLL has subgroups, with different patterns of mutations, which thus possibly arise from different stages of B cell development.

the clonal fingerprint of the tumour is shared between all cells, some cells have acquired additional mutations that are not shared by other cells. This type of pattern is found in follicular lymphoma, → Burkitt lymphoma and diffuse large B-cell lymphomas (DLBCL) (Fig. 3). The tumour cells of DLBCL and hairy cell leukemias are also able to produce transcripts for more than one Ig isotype. This provides additional evidence that the malignant tumour cells are less frozen in their development than previously thought.

Malignancies like multiple myeloma (MM) have mutated V_H-D-J_H genes, but all sequences are identical (they are 'stable'). This suggests that MM has undergone somatic mutation but that the tumour cells have then left the site of somatic mutation (post germinal centre tumours). Monoclonal gammopathy of unknown significance (MGUS) can show or lack intraclonal heterogeneity.

The available data now allow us a detailed description of human B-cell tumours. Immunogenetics has contributed to the classification by providing information that is independent of the morphology and clarifies the developmental stage at which the final transforming event occurred.

It is likely that in the future new methods like the gene → microarray technology will help us understand which disease entities should be further subdivided. Additionally, we are likely

to predict better within lymphoma entities and tailor treatment according to more accurate prognostic factors.

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BCG

Definition

Bacille (synonym: bacillus) Calmette-Guerin (BCG) is used as immunostimulant in vaccination approches. Whole cell anti-tumor vaccines consist of X-rayed tumor cells that are administered together with an immunostimulant such as BCG. BCG immunotherapy is currently the most effective treatment for superficial transitional cell carcinoma of the urinary bladder and is also used worldwide in vaccination programs against tuberculosis.

BCL-2

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Synonyms

- B-cell leukemia/lymphoma-2 gene (*Bcl-2*)

Definition

The Bcl-2 family of proteins belong to a peculiar class of human cancer-related proteins; the genes coding for these proteins are neither dominant transforming → oncogenes (such as → *myc*), nor → tumor suppressor genes (such as → *p53*). They could be best defined as apoptosis-related genes, a definition that stresses the importance of → apoptosis (and of its derangement) in the genesis and development of human cancer. The Bcl-2 family encompasses about eighteen members divided in anti-apoptotic and pro-apoptotic proteins; among the anti-apoptotic are Bcl-2 and → Bcl-XL, whereas pro-apoptotic are → Bax, Bak [→ BAK1], → Bid and → Bad.

Characteristics

The first association between *Bcl-2* and human cancer was observed in follicular lymphomas bearing the t(14;18) chromosomal translocation by which the gene was cloned. This translocation brings the *Bcl-2* gene to chromosomal location 18q21 into juxtaposition with the immunoglobulin heavy-chain locus at 14q32, resulting in transcriptional de-regulation of the *Bcl-2* gene. This event does not involve alterations of the coding regions of the gene. Subsequently, *Bcl-2* over-expression was recognized as a general feature of various types of hematological and solid malignancies, and there are studies correlating *Bcl-2* over-expression with the neoplastic phenotype and disease prognosis. *Bcl-2* codes for a protein of relative molecular mass of 26,000 with a hydrophobic carboxyl terminus that is associated with all cel-

lular membranes. The main function of Bcl-2 is to promote cell survival by inhibiting the process of apoptosis or programmed cell death, thus being a key determinant of neoplastic cell expansion and resistance to anticancer treatments. Consistently, the amount of Bcl-2 protein has dramatic effects on cell fate; an increase of Bcl-2 protein is associated with prolonged survival and apoptotic protection, whereas its decrease is invariably associated with apoptosis or enhanced sensitivity to apoptosis-inducing agents.

Functions

Bcl-2 protein has multiple independent functions that can be grouped in two main categories, functioning as an ion/protein channel or a membrane adaptor/docking protein.

The three-dimensional structure of the Bcl-2 analogue, anti-apoptotic Bcl-XL, shows a surprising similarity to the pore-forming domains of some bacterial toxins that cause the formation of channels for ions, proteins or both. Bcl-2 and its homologues are localized to intracellular membranes, in particular, the outer mitochondrial membrane, the endoplasmic reticulum and the nuclear envelope. In these areas they might have a membrane transport function for calcium ions and proteins. The channels created by Bcl-2 insertion into membranes resemble the pores formed by bacterial toxins. Thus, the two long hydrophobic helices of the protein core insert deeply through the phospholipid bilayer, and the rest of the protein undergoes conformational changes resembling the opening of an umbrella with the five surrounding amphipathic helices resting on the top of the membrane. The ability to form channels, by insertion of the two hydrophobic helices, is essential for Bcl-2 anti-apoptotic function. However, by analogy with other channels, the Bcl-2 channels might be formed by two or more Bcl-2 proteins. Thus, there is the possibility that anti- and pro-apoptotic members of the Bcl-2 family form homo- or heterodimers. In fact, the pro-apoptotic members of the family also have channel forming activity, although the channels formed by these proteins might have different selectivities or subcellular localization. Heterodimerization of anti- and pro-apoptotic Bcl-2 family proteins might lead to the formation of different channels or, alternatively, the heterodimers might be unable to form channels at all. Schematically, the channels formed by Bcl-2 and the other anti-apoptotic members prevent apoptosis, possibly transporting back, and thus antagonizing, the pro-apoptotic factors that outflow through the channels formed by the pro-apoptotic members of the Bcl-2 family. For example, → Fas ligand, a well characterized inducer of apoptosis, activates a member of the → caspase family (caspase 8) that cleaves pro-apoptotic Bid. Once truncated, Bid translocates to mitochondria where it might function as a channel protein to release cytochrome c, activating cytosolic caspases that are the terminal effectors of apoptosis. Bcl-2 inhibits the release of cytochrome c either by plugging the channels opened by Bid or by transporting cytochrome c back to the mitochondria. Whatever the case, the level of expression and the ratio between anti-apoptotic and pro-apoptotic Bcl-2 family proteins is critical in deciding cell death or survival.

Besides the channel forming function, Bcl-2 and Bcl-XL but not the pro-apoptotic protein Bax, interact with a number of signal transducing proteins. These include the → protein kinase C homologue → Raf-1, the → G-proteins H-Ras and R-Ras, the p53-binding protein p53-BP2, the pro-apoptotic protein → CED-4 and the protein phosphatase calcineurin. The association between Bcl-2 and these proteins might be responsible for their translocation from the cytosol to intracellular membranes where Bcl-2 is anchored. This may lead to changes of their activity, such that they might be sequestered and inactivated, or targeted for interaction with other membrane-associated proteins. For example, Raf-1 is a serine/threonine kinase that transduces mitogenic signals from membrane receptors to the nucleus. Association between Raf-1 and Bcl-2 causes translocation of the kinase to the mitochondrial membrane where Bcl-2 resides. Once there, Raf-1 phosphorylates and inactivates Bad, one of the pro-apoptotic members of the Bcl-2 family. Phosphorylated Bad is sequestered in the cytosol, complexed

with another adaptor termed 14-3-3, and thus inactivated. In the absence of growth/survival factors (such as in → IL-3 deprivation of IL-3-dependent hematopoietic cell lines), Raf-1 is not activated and the non-phosphorylated Bad is able to perform its pro-apoptotic function. Another example of the Bcl-2 function as an adaptor protein derives from studies on CED-4, a pro-apoptotic protein from the nematode *Caenorhabitis elegans*. In this case, Bcl-2 binds to and sequesters CED-4, thus preventing CED-4-dependent activation of cytosolic caspases, a family of cell death cysteine proteases. Conversely, pro-apoptotic members of the Bcl-2 family heterodimerize with Bcl-2, as if competing with CED-4, thus causing its displacement from Bcl-2. Set free from mitochondrial confinement, CED-4 returns to the cytosol and activates cell death proteases. Bcl-2 family members compete with each other, and the final outcome (cell death or survival) clearly depends upon the anti-apoptotic:pro-apoptotic protein ratio.

Bcl-2 has also been reported to interact with chromosomes, thus leading to hypotheses suggesting a role in the regulation of the cell cycle. In a number of transformed human cell lines, Bcl-2 localizes on chromosomes in a cell cycle-dependent manner, accumulating in prophase and metaphase and disappearing during telophase.

Regulation

Whether through its function as a channel protein or as an adaptor/docking protein, the final result on cell fate, however, depends upon the level of expression of Bcl-2. Consistently, Bcl-2 expression control has been the object of numerous studies of transcriptional, translational and post-translational regulation. Over-expression of Bcl-2 in human cancer prevents the transformed cells from undergoing apoptosis so that they proliferate accumulating mutations, develop resistance to anti-cancer treatments and progress towards a more malignant phenotype. However, surprisingly little is known about the molecular basis of this over-expression. Thus, sequencing of the *Bcl-2* coding region in *Bcl-2*-over-expressing tumors revealed a notable absence of mutations. Another level of *Bcl-2* regulation is concerned with mRNA stabilization. *Bcl-2* mRNA is characterized by a conserved adenine- and uracil-rich element (AU-rich element, → ARE) present in the untranslated region (3′-UTR). AREs have also been described in the 3′-UTR of numerous mRNA of cytokines and proto-oncogenes. AREs comprise a major group of cis-acting elements that target these mRNAs for rapid degradation. AREs exert a post-transcriptional control of gene expression by interacting with cytoplasmic and nuclear RNA binding proteins, and ARE deletions are associated with the activation of proto-oncogenes such as *c-fos* and *c-myc*. Consistently, protein kinase C, the target of numerous tumor promoters, inactivates the destabilizing function of *Bcl-2* ARE, leading to increased *Bcl-2* expression and protection from apoptosis. Thus, transformed cells with increased level of diacylglycerol that activates protein kinase C, show increased resistance to the killing effects of ionizing radiations. However, apoptotic stimuli enhance the destabilizing function of *Bcl-2* ARE, thus being responsible for *Bcl-2* down-regulation during apoptosis.

Bioactivity

Oncogenes and tumor suppressor genes modulate *Bcl-2* expression with profound results on death or survival of neoplastic cells. The tumor suppressor gene *p53* can induce apoptotic cell death by down-regulation of *Bcl-2* and up-regulation of *Bax*. The p53-dependent negative response element on *Bcl-2* has the features of a transcriptional silencer, mediating inhibition of transcription in an orientation-dependent manner. In a variety of tumors, *p53* expression is associated with apoptosis and with sensitivity to DNA damaging agents (anticancer drugs and ionizing radiations), by enhancing the transcription of a gene that favours apoptosis (*Bax*), at the same time blocking the transcription of a gene that would protect cancer cells from apoptosis (*Bcl-2*). *Bcl-2* over-expression is able to hinder p53-induced apoptosis, but it is ineffective against p53-dependent growth arrest. However, when *Bcl-2* is expressed together with the proto-oncogene *c-myc*, both

p53-induced growth arrest and apoptosis are counteracted. Thus a model could be proposed on the basis of a reciprocal antagonism between *p53* and *Bcl-2* and of a cooperation between the latter and c-myc. The cooperation between a major dominant oncogene (myc) and an apoptosis protection gene (Bcl-2) leads to nefarious results in human cancer; it speeds up progression towards a more malignant phenotype (no more p53-induced growth arrest to repair mutations) with added resistance to apoptosis induced by anticancer treatments. On the other hand, the antagonism between p53 and Bcl-2 appears to be 'beneficial' in terms of anticancer therapy. Therefore, the prognosis of each human neoplasia appears to be dependent on the balance between dominant oncogenes, tumor suppressor genes and apoptosis-related genes of the *Bcl-2* family. It is foreseable that analysis of these genes will become routine practice in the development of specific therapeutic approaches for each individual tumor.

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BCL2L

Definition

→ BCL2L1.

BCL2L1

Definition

BCL2L1, also known as BCL2-like 1, BCLX, BCL2L, Bcl-X, bcl-xL and bcl-xS, is a dominant regulator of apoptosis. The long isoform bcl-xL forms heterodimers with → Bax and Bak [→ BAK1] and has apoptosis repressor activity. The short isoform bcl-xS promotes apoptosis and dimerizes with → Bcl-2. The gene encodes a protein of 233 aa and 26 kD. bcl-xS is expressed at high level in cells with high rate of turnover, such as developing lymphocytes, and bcl-xL is expressed in tissues with long-lived post-mitotic cells, i.e. in the brain.

BCL2L7

Definition

→ BAK1.

BCL2-like 1

Definition

→ BCL2L1.

BCL6

Definition

B-cell CLL/lymphoma 6, also known as Bcl-6, BCL5, LAZ3 and zinc finger protein 51 (ZNF51) is a zinc-finger potein of 706 amino acids and 78 kD. The human *BCL6* locus maps to 3q27 and the mouse *bcl-6* gene locus to chromosome 16 (13.90 cM). BCL6 is a transcriptional regulator that probably plays an important role in lymphomagenesis. It is involved in a form of B-cell non-Hodgkin lymphoma characterized by chromosomal translocation t(3;14)(q27;q32) and t(3;22)(q27;q11) that in-

volves *BCL6* and immunoglobulin gene regions, and also in a t(3;4)(q27;p11) chromosomal translocation with Arhh (Ttf).

BCL6 Translocations in B-cell Tumors

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Synonyms

BCL6 translocations in B-cell tumors are chromosomal translocations involving 3q27 chromosome band.

Definition

→ Mature B-cell tumors are often associated with chromosomal translocations that lead to the juxtaposition of cellular oncogenes with the → immunoglobulin gene (*IG*) loci. The 3q27 translocation is unique, fusing the *BCL6* gene on 3q27 to either one of the three *IGs* but also another non-*IG* partner. Cytogenetic and molecular analyses have demonstrated that alteration of 3q27 and/or *BCL6* is one of the most common genetic abnormalities in B-cell tumors.

Characteristics

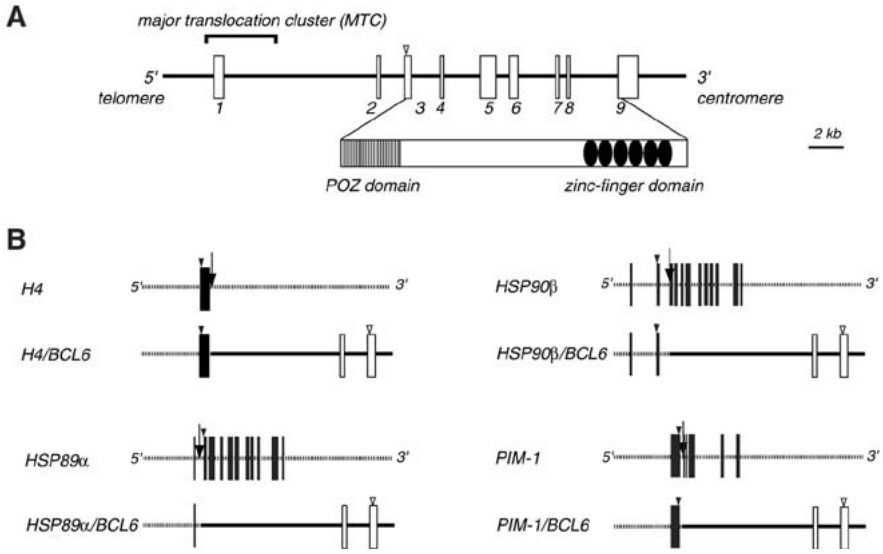
The *BCL6* gene and gene product

The *BCL6* gene spans 26-kb and contains 9 exons (Fig. A). At least two types of mRNA are produced by alternative splicing that may or may not contain exon 2. The ATG signal for initiation of protein synthesis is within exon 3 and is followed by an open reading frame, which encodes the Bcl-6 protein consisting of 706 amino acids with a calculated molecular weight of 79 kD. The Bcl-6 protein is a sequence-specific transcriptional repressor that contains two identified functional domains (Fig. A). The C-terminal region comprises six Cys2-His2 zinc finger motifs, each separated by a conserved stretch of seven amino acids. Hence, the Bcl-6 protein was classified as belonging to the Krüppel-like subfamily of zinc finger proteins. The N-terminal region contains a conserved protein-protein interaction motif, the POZ domain, which plays a role in homodimerization and heterodimerization. It has been shown that the repressing activity of Bcl-6 is exerted by the recruitment of both the → SMRT co-repressor and a SMRT/mSin3A/HDAC-containing complex. Targeted inactivation of *BCL6* in the mouse germline prevents germinal center formation in the lymphoid tissues and alters Th2-mediated immune responses, indicating that *BCL6* is an important regulator of lymphoid development and function.

BCL6 translocation affecting the *IG* loci

The most common type of 3q27/*IG* translocation is t(3;14)(q27;q32) involving *IG* heavy chain gene (*IGH*) on 14q32 (2, 3). The breakpoints on *IGH* are within the switch regions of *IGH*, and the *IGH* upstream sequences are juxtaposed to *BCL6* in the same transcriptional orientation. The breakpoints on *BCL6* are clustered within a 4 kb region spanning the noncoding exon 1 and intron 1 (major translocation cluster; MTC; Fig. A); in the majority of cases, breakpoints are localized immediately 3′ of exon 1 (hyper-cluster) (2). Thus, the coding regions of *BCL6* remain intact. On the reciprocal junction, the 5′-*BCL6* sequences are fused to downstream sequences of *IGH*. t(3;14) generates chimeric *IGH/BCL6* transcripts that initiate from the *IGH* germline transcript promoters (Iμ and Iγ), and are followed by the *BCL6* coding sequences (3).

Forms of the second, 'variant' type of translocation are t(3;22)(q27;q11), involving *IGλ* light chain (*IGLλ*) on 22q11 and t(2;3)(p12:q27), involving *IGLκ* on 2p12 (2). The last two 'variant' translocations lead to juxtaposition of the 3′ sequences of *IGLs* to the *BCL6* in divergent orientation. The breakpoints within the two *IGLs* are varied; 5′ of *V* genes, 5′ of *Vλ/Jλ* complex, at a point between the *Vλ2-1*, which is the most 3′ *V* gene of *IGLλ*, and the *Jλ1*



BCL6 Translocations in B-cell Tumors. Fig. – a Schematic presentation of the BCL6 gene and its protein product. **b** Non-IG/BCL6 translocations involving → histone H4 gene, HSP89α heat shock protein gene, HSP90β gene [see → HSP] and → PIM-1 proto-oncogene. Open (BCL6) and closed (partner genes) boxes indicate the exons, and arrowheads indicate the translation initiation sites of each gene. Arrows indicate breakpoints of each translocation. All the translocations occurred with the same transcriptional orientation.

segment, and between the *Jλ* and *Cλ* segments (2). Thus, the positions of breakpoints on the *IGLs* are not related to the regions in which *V/J* recombination normally occurs. Since *IG* translocations presumably occur as the result of errors in the recombination process, the positions of breakpoints on *IGs* can reflect the B cell stage where the translocation develops. While *IGH/BCL6* translocations are most likely associated with the isotype class switching process following completion of *V/D/J* recombination, this 'mis-class switch' mechanism may not be applicable to the variant *IGL/BCL6* translocations.

BCL6 translocations affecting non-*IG* loci

Non-*IG* partners have been cloned by 5′-rapid amplification of cDNA ends (5′-→ RACE) strategy. Another method to obtain a sequence of non-*IG* partner is long-distance → inverse PCR. The Table lists non-*IG* partners that have been identified using these two PCR-based approaches. These non-*IG* partners are not random but recurrently identified.

TTF is the first identified non-*IG* partner, which has been renamed *RhoH*. The *RhoH/TTF* product possesses many Rho-hallmarks and defines a new group within the Rho subfamily. Other non-*IG* partners were registered in the GenBank database, and the exon-intron structure as well as the sequence of the regulatory region was studied. Fig. B shows the representative organization of der(3) chromosome affected by *BCL6* translocation. The common molecular features of non-*IG/BCL6* translocations include:

- the gene fusion occurs in the same transcriptional orientation,
- the breakpoint on the partner gene is localized in close proximity to its promoter sequence,
- the complete set of the promoter is fused upstream to the coding region of *BCL6* on the der(3) chromosome.

As the result of the translocation, many types of regulatory sequences on each partner locus substitute for the 5′ untranslated region of the *BCL6* gene and the rearranged *BCL6* gene is presumed to be under the control of the replaced promoter activity (promoter substitution). In this regard, it should be noted that

partner genes are transcriptionally activated by a variety of stimuli, including cell-cycle control, changes in the physical environment and response to cytokines. As germinal center (GC) B-cells proliferate rapidly in response to antigen stimulation, it is likely that the *BCL6* gene affected by the translocation is inappropriately expressed during B-cell proliferation. A key experiment to establish the role of non-*IG*/*BCL6* translocation would be the creation of a transgenic mouse model, in which the translocation is re-created by placing *BCL6* under the control of these diverse promoters.

The 5′ non-coding region of *BCL6* undergoes somatic hypermutation

Somatic mutations within the 5′ non-coding region of *BCL6* have been described in a significant proportion of → GC/post-GC type B-cell tumors. The majority of the mutations cluster around the 3′ of exon 1, which has been referred to as the Major Mutation Cluster (MMC). These mutations are often multiple, are frequently biallelic and are independent of *BCL6* translocation or linkage to *IGs*. On the other hand, somatic mutations within the MMC were observed in a large proportion of memory B-cells isolated from normal individuals as well as GC B-cells from a reactive tonsil. The presence of cis-acting elements in *BCL6*, which are shared with *IG* and essential for targeting the mutation, has been suggested. Although the MTC and MMC apparently overlap, possible linkage between the two genetic alterations remains to be determined.

Clinical relevance

Large numbers of B-cell tumors with 3q27 translocation in association with the *IG* gene loci were first described in 1989. 3q27/*IG* Translocations were identified in 20 of 318 (6.3%) cases of B-cell non-Hodgkin lymphoma (B-NHL) with clonal chromosomal abnormalities. On the other hand, there are nearly 20 cytogenetically identifiable non-*IG* chromosomal sites,

BCL6 Translocations in B-cell Tumors. Table – Diverse partner genes of *BCL6* translocation in B-cell tumors.

Genes		Gene product	Chromosomal locus
IG genes	*IGH*	immunoglobulin heavy chain	14q32
	IGLκ	immunoglobulin κ light chain	2p12
	IGLλ	immunoglobulin λ light chain	22q11
Non-*IG* genes	*RhoH/TTF*	Rho GTP-binding protein	4p13
	BOB1/OBF1	B cell-specific transcriptional coactivator	11q23.1
	H4	H4 histone	6p21.3
	HSP89α	heat shock protein 89α	14q32
	HSP90β	heat shock protein 90β	6p12
	CIITA	MHC class II transactivator	16p13
	PIM-1	pim-1 proto-oncogene product	6p21.2
	eif4AII	eukaryotic initiation factor 4AII	18p11.2
	TFRR	transferrin receptor	3q29
	Ikaros	Ikaros	7p12
	LCP1	L-plastin	13q14
	α-NAC	α chain of the nascent polypeptide-associated complex	12q23-q24.1

some of which have been reported by several independent laboratories. The overall incidence of 3q27 abnormalities in NHL has been estimated to be 15.9% of all NHL types and 23.1% of DLBCL.

An initial study of *BCL6* rearrangement, using Southern blot analysis with a probe for MTC, indicated a specific correlation of the rearrangement with diffuse large B-cell lymphoma (→ DLBCL). However, later studies of panels of many NHL types invariably showed that a significant number of cases with follicular lymphoma (FL) carried such rearrangements. The range of *BCL6* rearrangements in B-NHL subtypes are 5 to 15% in FL, 20 to 40% in DLBCL and its variants, and 20% in acquired immunodeficiency syndrome-associated DLBCL.

The 3q27 translocation and/or *BCL6* rearrangement sometimes coexist with other *IG* translocations associated with B-cell tumors, i.e. t(8;14)(q24;q32) and t(14;18)(q32;q21) and their variants. There is evidence that, in some cases, alteration of the *BCL6* locus was not a primary genetic abnormality but may have occurred at the time of transformation from low- to high-grade disease.

IG translocations sometimes correlate with clinical features of B-cell tumors. For instance, B-NHL associated with t(8;14) and/or its molecular equivalent shows aggressive clinical behavior even though the translocation can occur not only in → Burkitt lymphoma but also in other types of B-cell tumors. An earlier study showed that *BCL6* rearrangement more frequently occurs in extranodal DLBCL than in node-based disease and is correlated with a favorable clinical outcome. However, later studies failed to confirm these observations. Another interesting issue is whether the diverse partner gene can affect the clinical features of NHL carrying a particular *BCL6* translocation. It has been recently observed that the overall survival of DLBCL patients with non-*IG/BCL6* translocation was significantly inferior to that of those with IG/BCL6 translocation. Although the total number of patients analyzed is quite small, additional studies of larger cohorts are warranted.

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BCL2L8

Definition

→ Bad.

BCL-X

Synonyms

- bcl-xS
- BCLX
- bcl-xL
- Bcl-X
- BCL2-like 1
- BCL2L
- → BCL2L1

BCL-XL

Synonyms

- → BCL2L1
- BCLX
- Bcl-X
- BCL2-like 1
- BCL2L
- bcl-xL

Definition

Bcl-xL is an anti-apoptotic isoform of BCL2L1 and a member of the → Blc-2 family of proteins. Like Bcl-2, it inhibits apoptotic cell death by controlling primarily the activation of → caspase proteases. Its three-dimensional structure reveals a striking similarity to the pore forming domain of diphtheria toxin and to the bacterial pore-forming colicins.

BCL-XS

Synonyms

- BCLX
- bcl-xL
- Bcl-X
- BCL2-like 1
- BCL2L
- → BCL2L1

BCNS

Definition

→ Basal cell nevus syndrome.

BCR-ABL1

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Definition

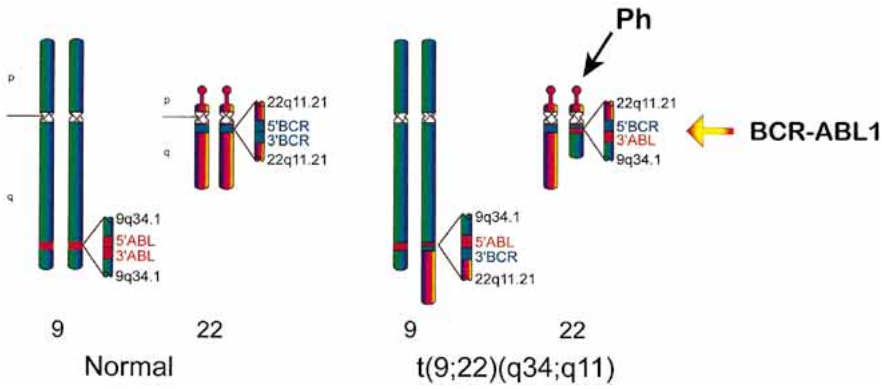
BCR-ABL1 is a hybrid (fusion or chimaeric) gene that arises when genomic DNA of the BCR gene on chromosome 22 and of the ABL1 gene on chromosome 9 breaks and recombines. The BCR-ABL1 hybrid gene is transcribed to produce a hybrid mRNA that is subsequently translated into a functional BCR-ABL1 protein. The BCR-ABL1 mutation causes and is diagnostic of human chronic myeloid leukemia and some acute leukemias, particularly acute lymphoblastic leukemia.

Characteristics

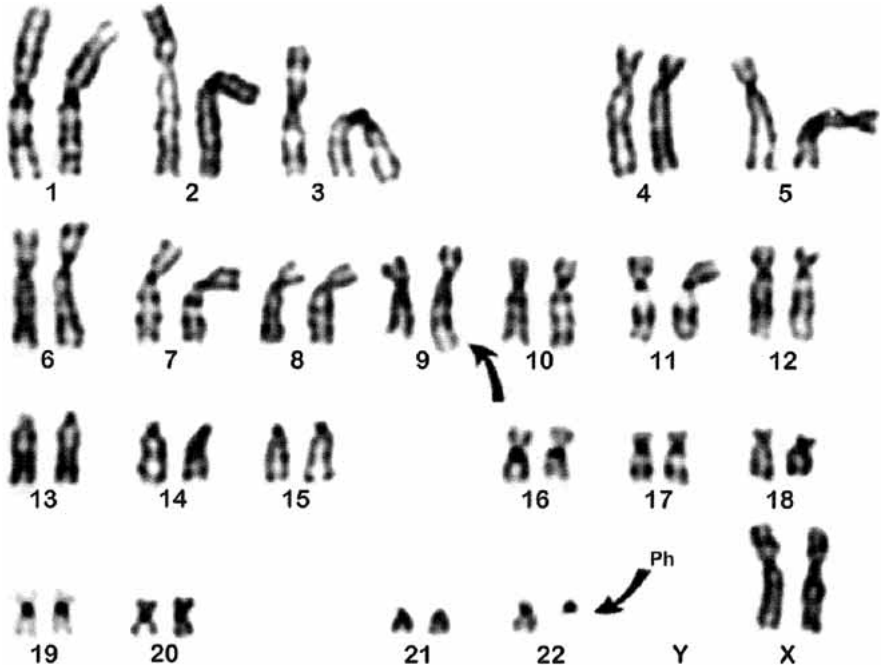
A somatic mutation of bone marrow progenitor cells

The BCR-ABL1 mutation is somatically acquired. Recombination between the BCR and ABL1 genes occurs in an early progenitor or stem cell of the bone marrow, and usually results in the microscopically visible chromosome translocation t(9;22)(q34;q11) (Fig. 1).

One product of this translocation is the well known Philadelphia (Ph) chromosome (Fig. 2), a shortened chromosome 22, identifiable in leukemic metaphase cells of ~90% of patients with chronic myeloid leukemia (CML) and in ~ 15%-20% of ALL cases, although with higher frequency in adult (15%-20%) compared with childhood (5%) acute lymphoblastic leukemia (ALL). The discovery of the Ph chromosome in 1960 was a milestone for cancer research, providing the first clear evidence that different cancers may be associated with specific genetic abnormalities.



BCR-ABL1. Fig. 1 – Ideogrammatic representation of chromosomes 9 and 22 before (left) and after (right) *BCR-ABL1* recombination.



BCR-ABL1. Fig. 2 – Karyotype of a leukemic metaphase cell showing the standard Ph translocation, 46,XY,t(9;22)(q34;q11).

Molecular features of BCR-ABL1 recombination

Recombination between the BCR and ABL1 genes usually generates two products: A 5′ABL1-3′BCR hybrid gene on the derivative 9q+ chromosome that is in some cases transcribed but so far has not been shown to be translated, and a 5′BCR-3′ABL1 product on the derivative 22q- or Ph chromosome that is both transcribed and translated. The 5′BCR-3′ABL1 protein has leukemia-causing properties in a variety of animal models, and it is to this product that the BCR-ABL1 acronym usually refers.

Both BCR (Fig. 3) and ABL1 (Fig. 4) are large genes, at approximately 152 kilobase pairs (kb) and 225-kb, respectively. Several viable in-frame BCR-ABL1 fusions have been reported or predicted. However, depending on the location of the breakpoint site within BCR, those

associated with leukemia generally differ according to the number of BCR exons that link with the constant ABL1 exons 2-11 (Fig. 5).

- p210 BCR-ABL1: Breaks occur within the 5-kb major breakpoint cluster region (M-Bcr) of BCR in most cases of CML, and in about 50% of BCR-ABL1 rearrangement-positive cases of ALL. In these cases, the BCR-ABL1 fusion gene is transcribed as a large chimaeric mRNA that is spliced into an 8-kb mRNA with BCR exon 13:ABL1 exon 2 (e13a2) and/or BCR exon 14:ABL1 exon 2 (e14a2) junctions. This hybrid mRNA is in turn translated to form a 210-kd BCR-ABL1 fusion protein.
- P230 BCR-ABL1: A larger 230-kd protein identifies a subgroup of patients with neutrophilic CML (CML-N) who present with a lower white cell count than usual and for whom progression to blast crisis is slow. In these cases, a breakpoint occurs in a region more 3′ in BCR (m-Bcr) to form a BCR exon 19:ABL1 exon 2 (e19a2) mRNA transcript in which almost the entire BCR gene is joined with ABL1.
- P190 BCR-ABL1: For the remaining 50% of BCR-ABL1 positive ALL cases, breakpoints usually occur at different sites across a wider ~35-kb region designated m-Bcr (minor breakpoint cluster region), which maps ~46-kb upstream of M-Bcr. A BCR exon 1:ABL1 exon 2 (e1a2) transcript is expressed in these cases, which is translated into a smaller 185-kd BCR-ABL1 protein. The e1a2 transcript is occasionally found in CML patients when it may be associated with a more aggressive clinical course.
- For all BCR-ABL1 leukemias, breakpoints in ABL1 are more variable than those in BCR and have been reported to occur at different sites within a >200-kb region that extends from a point 9-kb 5′ of the entire gene to exon 2.

Complex BCR-ABL1 rearrangements

About 10% of CML cases show more complex BCR-ABL1 rearrangements that involve other chromosomal sites, and which may be camouflaged by a normal karyotype. In all of these cases, the 5′ part of BCR is fused with the 3′ part of ABL1 to form the characteristic BCR-ABL1 fusion gene essential for the development of CML. However, the 3′ part of BCR, which unites with the 5′ ABL1 remnant in the standard t(9;22)(q34;q11), usually recombines with one of the additional chromosomes in the complex translocations or with a part of chromosome 9 outside of the ABL1 gene. Although patients show clinical features typical of BCR-ABL1 leukemias, the molecular and pathological significance of these complex translocations is still unresolved.

Detection of BCR-ABL1

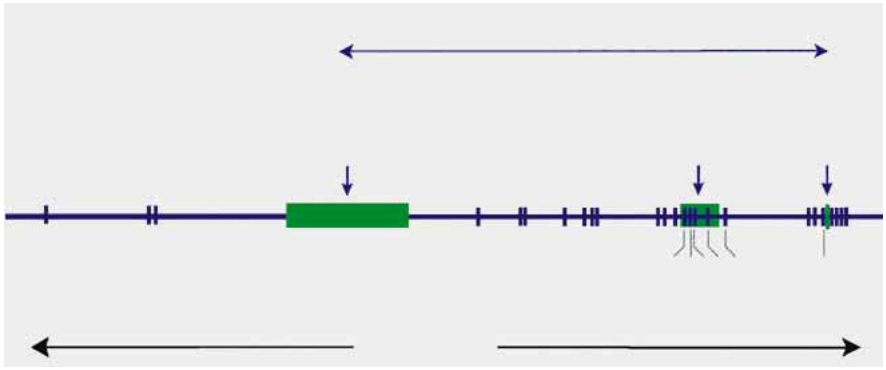
The BCR-ABL1 mutation may be detected by one or more of the following molecular procedures:

- Southern blotting using leukemic DNA digested with appropriate restriction enzymes and one or more probes from within M-Bcr or other relevant regions of BCR.
- Amplification of predicted e1a2, e13a2, e14a2, e19a2 or variant splice junctions using polymerase chain reaction after reverse transcription of leukemic cell mRNA (RT-PCR).
- Fluorescent *in situ* hybridisation (FISH) on single cells (metaphase or interphase) using a combination of large insert BCR and ABL1 probes.
- By immunoprecipitation and Western blot analysis of the chimaeric protein.

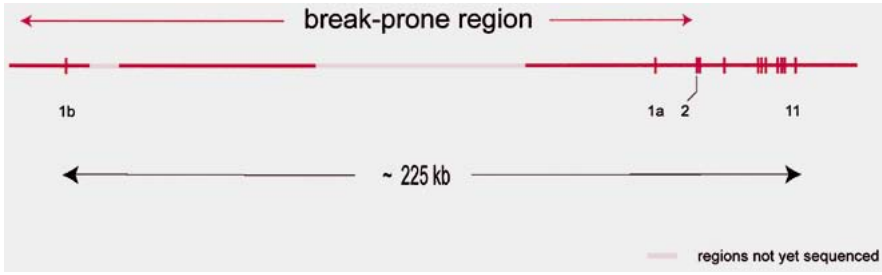
What causes the BCR-ABL1 mutation?

The cause of BCR-ABL1 mutations in most leukemias are unknown, but new and relevant clues are gradually emerging. For example, there is a clear association, both epidemiologically and in the laboratory, between exposure to ionizing radiation and the development of BCR-ABL1 leukemia. This increased risk is reflected in the increased incidence of CML in atomic bomb survivors compared to the general population, and in the increased occurrence of BCR-ABL1 mutations in cultured cells subjected to high-dose gamma-irradiation and X-

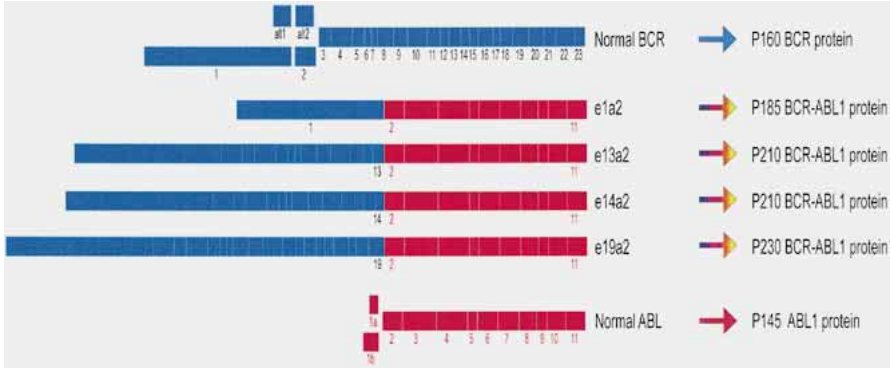
irradiation. Recent findings suggest that ionizing radiation can influence the generation of leukemia-specific fusion genes by juxtaposing genes normally distanced in the interphase cell nucleus, and that certain cell types with a lineage-specific 3-D chromatin distribution may be more or less susceptible to a particular fusion gene rearrangement than others.



BCR-ABL1. Fig. 3 – Genomic structure and features of the human *BCR* gene. Exons 1-23 and alternatives (a) are indicated as blue boxes; The minor breakpoint cluster region (m-Bcr), major breakpoint cluster region (M-Bcr) and micro breakpoint cluster region (μ-Bcr) are shaded in green. Disease subtypes associated with the different regions are shown as ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia and CML-N, neutrophilic chronic myeloid leukemia.



BCR-ABL1. Fig. 4 – Genomic structure of the human *ABL1* gene. Exons 1-11 and alternatives (a) are indicated as red boxed regions.



BCR-ABL1. Fig. 5 – Normal BCR and ABL1 transcripts and the most frequently detected variant BCR-ABL1 fusion transcripts. Corresponding protein products are shown to the right. Alternative (a) exons are marked above the normal transcripts.

BCR-ABL1 in healthy individuals

BCR-ABL1 transcripts have been identified, using RT-PCR, at very low levels in circulating peripheral blood granuloctyes of more than two thirds of healthy adults. The identification of other leukemia-associated fusion transcripts in different studies provides good evidence that aberrant recombination occurs ubiquitously at a baseline level in somatic cells of normal individuals. These findings also suggest that additional selective processes, such as immunological tolerance or cell type origin and stage of differentiation, are required to provide BCR-ABL1 cells with a proliferative advantage and produce the leukemic phenotype.

Why breakpoint cluster regions?

The molecular factors that determine preferential breakage sites in BCR, and precipitate BCR-ABL1 recombination are presently unknown, but the Alu repeat is a strong candidate to facilitate this process. Sequence analysis of M-Bcr has identified a single Alu element central within an approximately 3-kb region where more than 70% of the breakpoints occur. In addition, analysis of reciprocal BCR-ABL1 and ABL1-BCR breakpoint junctions from several cases of CML and ALL has identified sequence homology to Alu elements at, or close to, the sites of recombination. M-Bcr also recombines preferentially with Alu elements at chromosomal sites outside of ABL1 in complex BCR-ABL1 rearrangements, and in these cases an association with gene coding domains and Translin-specifc binding motifs was also suggested. Further research is needed to clarify the significance of these findings.

Molecular consequences of the BCR-ABL1 mutation

The leukemia-causing properties of the BCR-ABL1 protein have been demonstrated in a range of *in vivo* and *in vitro* laboratory models, including mice made transgenic for different forms of the hybrid oncogene or transplanted with BCR-ABL1 transfected stem cells. BCR-ABL1 cells are more proliferatively active, differentiate abnormally, show an increased resistance to apoptosis and have altered adhesion properties compared with their normal counterparts. Much recent work has sought to understand the mechanisms that precipitate these features and precisely how the BCR-ABL1 mutation activates cell transformation *in vivo*.

Normal BCR proteins are found in the cytoplasm and have at least two enzymatic activities, serine/threonine kinase at the N-terminal, and GAP activity at the C-terminal. The normal ABL1 protein is a non-receptor protein tyrosine kinase that is localized to the cytoplasm, where it is weakly associated with actin filaments, and the nucleus, where it is associated with chromatin. In BCR-ABL1 hybrid proteins, the fused BCR sequences block nuclear translocation and activate the actin binding function that is required for BCR-ABL1 to efficiently transform cells. Because of its heightened tyrosine kinase activity, the BCR-ABL1 protein can phosphorylate a range of different substrates, so activating multiple different cytoplasmic and nuclear signal-transduction pathways relevant to haematopoietic cell growth and differentiation. Examples of signalling cascades activated by BCR-ABL1 include the → Ras pathway, the Jun-kinase pathway, the phosphatidylinositol-3 kinase pathway, a variety of CRKL-linked signalling processes, the Jak-STAT [→ signal transducers and activators of transcription in oncogenesis] pathway and the Src pathway.

Clinical Relevance

- Chronic myeloid leukemia (CML) is a myeloproliferative disorder that develops after the BCR-ABL1 mutation occurs in a pluripotent bone marrow stem cell. The affected stem cell gains a proliferative advantage and a malignant leukemic clone becomes established. CML, characterized by overproduction of granulocytes in the bone marrow and peripheral blood, accounts for about 25% of all human leukemias, with an incidence of ~1 in 100,000 per year. CML affects both sexes and all age groups, but occurs most commonly at 40-50 years.
- → Blast crisis CML: CML is a biphasic disease, and usually progresses within 4 – 8 years of

diagnosis to an aggressive and terminal acute phase or blast crisis. The precise molecular events that determine blast crisis are still unknown, although there is much evidence from cytogenetic and molecular studies that non-random and lineage-specific accumulation of gene mutations may be important.

- BCR-ABL1 is also found in leukemic cells of patients with adult (10 – 20%) or childhood (5%) acute lymphoblastic leukemia (ALL L1 or L2), and in rare cases (~3%) of acute myeloid leukemia (AML, mostly M1 or M2).

Anti-BCR-ABL1 therapies

Bone marrow transplantation and/or alpha-interferon therapy have been the treatments of choice for BCR-ABL1 leukemias. But the identification of discrete cell signalling pathways through which BCR-ABL1 proteins interact, brings hope of the development of less life-threatening, simpler and more cost-effective therapeutic drug strategies that will specifically target these molecular pathways.

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Beckwith-Wiedemann Syndrome Associated Childhood Tumours

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Definition

Beckwith-Wiedemann syndrome (BWS) is a complex overgrowth disorder caused by a number of genes that are subject to genomic imprinting. A high incidence of solid childhood tumours is seen in patients that present with BWS.

Characteristics

Diagnostic criteria

Beckwith-Wiedemann syndrome is a disorder first described by Beckwith (1963) at the 11th annual meeting of the Western Society for Pediatric Research. Later, Wiedemann (1964) and Beckwith (1969) described the syndrome in more detail. BWS is characterised by a great variety of clinical features, among which are abdominal wall defects, macroglossia, pre- and post-natal gigantism, earlobe pits or creases, facial nevus flammeus, hypoglycemia, renal abnormalities and hemihypertrophy. BWS patients have a 7.5% risk of developing (mostly intra-abdominal) childhood tumours. Tumours most frequently found are → Wilms tumour (WT), adrenocortical carcinoma (ACC), rhabdomyosarcoma (RMS) and hepatoblastoma (HB). Patients can be classified as having BWS according to the clinical criteria proposed by Elliot or DeBaun, although cases of BWS are known that do not comply with either set of criteria.

(Epi)-Genetics

The syndrome occurs with an estimated incidence of 1:13,700 and most cases are sporadic (85%). The genetic predisposition for BWS lies

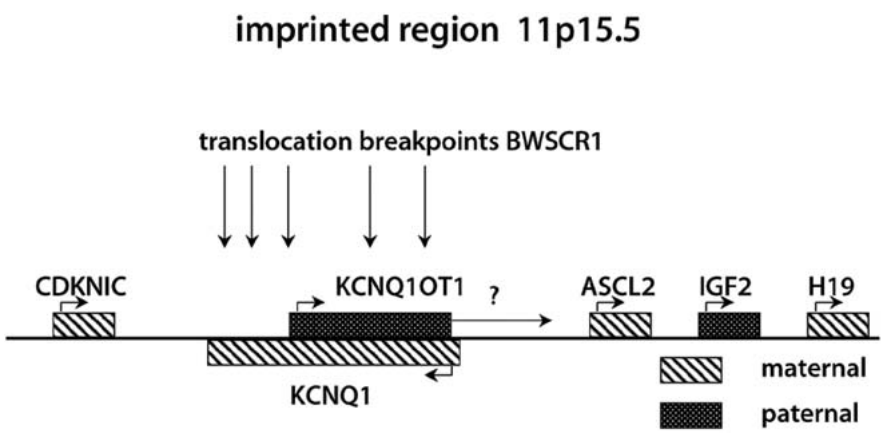
on chromosome 11p15 (linkage analysis, chromosome abnormalities, loss of imprinting (LOI), gene mutations). The syndrome is subject to genomic imprinting since maternal transmission seems to be predominant. In addition chromosomal translocations are of maternal origin, duplications and uniparental disomies (UPD) of paternal origin. All hitherto known causative genes are imprinted. The translocation breakpoints on chromosome 11 map to three distinct regions within 11p15.3-pter. Beckwith-Wiedemann syndrome chromosome region 1 (BWSCR1) near *INS*/*IGF2*, BWSCR2 5-Mb proximal to BWSCR1 and BWSCR3 2-Mb even more proximal. This already points to genetic heterogeneity, but also at the clinical level there seems to be heterogeneity. Chromosomal translocations in BWSCR1 and BWSCR3 are associated with the classical BWS phenotype, and BWSCR2 with minor BWS features but pronounced hemihypertrophy. BWSCR 1 and BWSCR2 have been cloned, and genes isolated from this region were shown to be involved in the development of this disorder. All genes involved are subject to genomic → imprinting.

BWSCR1. This region consists of a number of imprinted genes (Fig.). All known translocation breakpoints disrupt *KCNQ1*, a gene coding for a potassium channel involved in the Romano-Ward en Jervell-Lange-Nielsen cardiac arrhythmias syndromes. However, this imprinted gene is most likely not directly involved in BWS, but a gene transcribed in the antisense orientation of *KCNQ1* clearly is. This gene, *KCNQ1OT1*, shows aberrant methylation in 50 – 80% of BWS cases. It does not code for a protein and may function through its RNA. *CDKN1C* is an inhibitor of cyclin-dependent kinases. Heterozygous mutations have been identified in about 20% of BWS patients in two studies. Others, however, have not been able to confirm this mutation frequency. The gene is not a major cause of BWS. It is, however, possible that in certain countries the mutation frequency is elevated (e.g. Asia). In addition, it has been reported that this gene is more frequently involved in familial cases of BWS. *CDKN1C* mouse models revealed some of the clinical BWS features such as omphalocele and renal adrenal cortex anomalies. In humans, *CDKN1C* also seems to be more frequently associated with abdominal wall defects. Another strong candidate for involvement in the aetiology of BWS is the embryonic growth factor *IGF2*. Mouse models overexpressing *IGF2* displayed a phenotype overlapping with the BWS phenotype. Loss of *IGF2* imprinting is often seen in BWS patients. *H19*, another non-coding gene, lies downstream of *IGF2* and the expression of *IGF2* and *H19* seems to be linked. *H19* is important for the maintenance of the imprinting status of *IGF2*. Mouse studies underline the link between *IGF2* and *H19* expression and overgrowth phenotypes were found. *H19* loss of imprinting is frequently seen in BWS cases although not always in combination with *IGF2* loss of imprinting (LOI). Finally a gene called *ASCL2* is localised to the 11p15-imprinted region. Although no direct involvement in the BWS aetiology is known, this gene might account for the fact that most, if not all, BWS cases with uniparental disomy (UPD) present in a mosaic form. The mouse homologue codes for a transcription factor, which is expressed during early mouse development and is essential for the development of the placenta. Therefore, also in humans, complete lack of expression might be lethal.

BWSCR2. Two patients with balanced chromosomal translocations define this second chromosomal region, one of which developed a Wilms tumour. Both translocations in 11p15.4 disrupt a paternally imprinted zinc-binding finger gene *ZNF215*. Parts of the 3′ end of this gene are transcribed from the antisense strand of a second zinc-finger gene, *ZNF214*. Although putative mutations in these genes in other sporadic BWS cases were found, their involvement in BWS needs to be further elucidated by functional studies.

Diagnostics

BWS can be diagnosed in the laboratory with cytogenetics (< 5%) or DNA-diagnostics. The current major test involves methylation assays or LOI studies at the RNA level. The majority of cases (50 – 80%) exhibit aberrant methylation of *KCNQ1OT1* with or without aberrant methy-



Beckwith-Wiedemann Syndrome Associated Childhood Tumours. Fig. – Imprinted genes on 11p15 involved in BWS. The parental expression (imprinting) of these genes is indicated.

lation of *IGF2/H19*. These latter cases often show UPD for 11p15 (in a mosaic form) which explains this aberrant methylation for multiple genes. However, the majority of cases with *KCNQ1OT1* defects and some cases with *H19/IGF2* defects have no UPD 11p15. Therefore, an imprinting switch can be assumed, involving an imprinting centre analogous to the Prader-Willi and Angelman syndromes. The current data are most compatible with two distinct imprinting centres for either *KCNQ1OT1* or *IGF2/H19*. *CDKN1C* mutation analyses might be considered, especially in familial cases of BWS. The increased tumour risk for BWS patients seems to be associated with UPD in general and H19 methylation defects in particular. *KCNQOT1* methylation defects only seem to be a reliable prognostic factor since tumours are not associated with this group of patients. Recurrence risks for a second pregnancy can be assessed with UPD studies. In cases of a UPD in a mosaic form, there is no increased recurrence risk for BWS in a second pregnancy since the genetic defect occurred post-fertilisation.

BWS associated tumours

Although childhood solid tumours associated with BWS share some common genetic features, the spectrum of genetic changes found in these tumours is diverse and complicated with many genetic alterations seen.

Wilms tumour. The tumour most often found to be associated with BWS is Wilms tumour (WT) or nephroblastoma (59% of the tumours found in BWS patients). Overall it occurs with a frequency of 1 in 10,000 children, mostly in children under the age of 5 years. In patients suffering from BWS the incidence is 800-1000 times increased. A high percentage (38%) show loss of heterozygosity (LOH) of chromosome 11p. This region can be subdivided roughly into two parts: LOH of markers on 11p13 and LOH of markers on 11p15. The region on 11p13 has been shown to be deleted in patients affected by the WAGR. WAGR stands for the combined occurrence of sporadic aniridia, WT, genitourinary abnormalities and mental retardation. A gene in the candidate region (*WT1*) has been cloned. Mutations of this gene occur in only 10 – 15% of sporadic Wilms tumours, suggesting the existence of additional genes involved in the development of this tumour. The Denys-Drash syndrome, another syndrome associated with Wilms tumour, shows constitutional mutations of the *WT1*. The region on 11p15 showing LOH in WTs can be subdivided into two regions: An 800 kb region containing the *WT2* locus near *IGF2* and an additional locus of 336 kb proximal to *WT2*. *WT* can also be found in association with other syndromes, like the trisomy 18 syndrome, the Perlman syndrome and the Simpson-Golabi-Behmel, the Sotos syndrome and the Klippel-Trenaunay syndrome. The → Li-Fraumeni syndrome is a rare familial tumor syndrome and patients suffering from this disease contain germline point mutations in the

→ *p53* tumor suppressor gene. The tumours that develop in these patients show a deletion of the wild type *p53* allele. Although WT is not considered to be part of the Li-Fraumeni syndrome there have been few reports of the occurrence of WT in families affected by this syndrome. Mutations in the tumour suppressor gene *p53* have been found in sporadic WTs and seem to be associated with a histological subtype. In a series of 140 WTs, mutations were restricted to tumours of the anaplastic subtype, showing aberrations in 8/11 samples. This subtype is linked to a poor prognosis. In 10 to 25% of the Wilms tumours, LOH of 16q markers is found. It has been suggested that LOH of 16q is associated with an adverse prognosis. Another genetic abnormality, which seems to confer an adverse outcome, is LOH of 1p. This abnormality was found in 12% and 18% of the cases respectively. Chromosome 7 also seems to be involved in Wilms tumour. According to the literature in 23% of the cases chromosome 7 is rearranged. Another region found to be frequently involved in LOH (14%) is on chromosome 22q. In a study which quantified chromosome 12 allelic imbalance in a series of 28 Wilms tumours, duplications were detected in 18%. An inventory of all quantitative chromosome aberrations occurring in a series of 46 WTs was made using comparative genomic hybridisation analysis (CGH). Chromosome regions showing loss of DNA in 3 or more samples included 1p (11%), 11p (9%), 16q (13%) and 17p (7%). Regions showing gain of DNA in 3 or more samples included 1q (20%), 7q (9%), 8 (7%), 12q (17%), 17q (7%) and 18 (7%).

As expected, imprinting seems to play a major role in WT development since 11p15 LOH is always of maternal origin. This resulted in the hypothesis that a paternally imprinted tumour suppressor gene is involved in Wilms tumorigenesis. Alternatively, a maternally imprinted gene involved in stimulation of cell growth could be involved in the cases showing paternal UPD of (part of) chromosome 11. At present there are three candidate genes on 11p15 that show parent-of-origin-dependent monoallelic expression and belong to one of these two categories: the tumour suppressor genes *H19* and *CDKN1C* which are maternally expressed and the paternally expressed growth-promoting gene *IGF2*. Evidence for the involvement of these genes has been found i.e. loss of imprinting, or increased expression of *IGF2* or reduced expression of *CDKN1C* or *H19*.

Adrenocortical Carcinoma. The second most common tumour found in BWS-patients is adrenocortical carcinoma (ACC). It is found in 15% of patients that develop a tumour. In the general population ACC is found to be an extremely rare tumour with an incidence of 1.7 new cases per 1,000,000 per year. As in BWS, *IGF2* seems to be involved in sporadic ACC-tumorigenesis. A considerable proportion of the malignant tumors (~60%) display LOH of the 11p15.5 region, presumably all representing uniparental disomies. This is seen in both adult and childhood ACCs. In these cases a good correlation was found with overexpression of the *IGF2* gene. These phenomena were found in a much smaller percentage in the benign adenomas. It has been hypothesised that adrenocortical tumorigenesis is a multistep process with sequential progression from the normal to the adenomatous and then to the malignant cell. If this is the case then *IGF2* could be involved in the transition from adenoma to carcinoma. ACC is also found in association with other syndromes. One of these is the Li-Fraumeni syndrome, which is associated with mutations of the *p53* gene on chromosome 17p. In one study, in which sporadic ACC's were analysed for the presence of LOH at three different chromosome regions, chromosome 17p (containing the *p53* gene) had become homozygous in all informative samples. LOH of 17p was not found in adrenocortical adenoma, the benign counterpart of ACC. Again, if the hypothesis that adrenocortical tumours develop from normal tissue to adenomas to carcinomas is correct, this would mean that LOH of 17p could be a late event in ACC tumorigenesis. Two other groups identified mutations in the *p53* gene in ~30% of sporadic ACC's. In addition, CGH analysis showed loss of 17p in 50% of the (sporadic) cases. Another hereditary tumour syndrome associated with adrenocortical tumors is → multiple endocrine neoplasia type 1 (MEN1). In most cases associated with MEN1 adrenocortical adenomas are found. The dis-

ease is caused by mutation of the menin tumor suppressor gene (*MEN1*), located at 11q13.

Other regions found to be lost in ACC's include chromosome 13q, which was shown to have lost heterozygosity in 50% of informative patients, and chromosome 2. Genetic aberrations that were found in 38% of the tumours in this study were gains of chromosomes 12, 15q, 16q and 19p and losses of chromosomes 3p, 6q, 8p, 9p, 11p, 17q, 18q and 22q. There are numerous differences between the genetic aberrations found in adrenocortical adenomas and adrenocortical carcinomas. These differences may reflect various stages along the carcinogenic pathway.

Evidence for an involvement of imprinting in ACC again comes from LOH 11p15 studies (maternal loss) and LOI and expression studies for *IGF2* and *H19*. It should be noted that LOI of *IGF2* was associated with the malignant phenotype, since it was not detected in the adenomas but only in the carcinomas.

Rhabdomyosarcoma. Although rare, rhabdomyosarcoma (RMS) represents the most common soft-tissue sarcoma in children under the age of 15 years. It occurs with a frequency of 1.3-4.5 cases per million children per year. Based on their histology, rhabdomyosarcomas can be subdivided into three major subtypes: embryonal (E-RMS), alveolar (A-RMS) and pleomorphic (P-RMS) rhabdomyosarcoma, of which E-RMS is the subtype associated with BWS. Of all newly diagnosed cases 60% are E-RMS and 20% are A-RMS. Patients with E-RMS have a better prognosis than patients with A-RMS. LOH of chromosome 11p is an abnormality found frequently in RMS. In one study it was found in 72% of primary E-RMS and 20% of primary A-RMS. A gene located in this region, *GOK* (gene on chromosome 11) or *STIM1* (stromal interaction molecule 1) was postulated to be a candidate tumour suppressor gene in RMS. No expression was found in 7 RMS cell lines, and transfection of the gene into the RMS cell line RD was followed by growth arrest of the cells. LOH of 16q was also found in both types (in 55% of E-RMS and 40% of A-RMS). In total, LOH of 6p was found in 28% and LOH of 18p in 32% of the cases. Studies of A-RMS have shown that they often (~90%) contain a specific translocation. In most of these cases (68%) a t(2;13)(q35;q14) is found. In a smaller subset of A-RMS (14%) a variant translocation of t(1;13)(p36;q14) has been detected. Both these translocations cause the formation of a chimeric protein. In the case of the t(2;13) a PAX3-FKHR fusion product is expressed and in tumours with the t(1;13) a PAX7-→ FOXO1A product is detected. PAX3 and PAX7 are both transcription factors involved in embryonal myogenesis. In the chimeric proteins the DNA binding domains of the PAX genes are retained and fused to the C-terminal region of the FKHR gene containing a strong transactivation domain. It has therefore been proposed that both fusion proteins function as transcription factors that aberrantly regulate transcription of genes, controlled by PAX3 or PAX7 binding sites. The PAX3-FOXO1A fusion protein has been shown to be a strong transcriptional activator. In addition both PAX3-FOXO1A and PAX7-FOXO1A are over-expressed in A-RMS either by increased transcription (PAX3-FOXO1A) or by gene amplification (PAX7-FOXO1A). Although the presence of either translocation is considered to be a characteristic of A-RMS, some cases with the t(1;13) show mixed histology of both the embryonal and the alveolar type, and a case of E-RMS containing the t(2;13) has been described. In addition the age at diagnosis in patients with the t(1;13) is more consistent with E-RMS. Cytogenetic analysis of RMS showed a high incidence of trisomy 2 (in 9/9 E-RMS samples) and a high incidence of structural rearrangements of chromosomes 1 and 3 (both in 4/5 RMS samples). The alterations on chromosome 3 seem to cluster within 3p14-21. The presence of a der(16)t(1;16)(q21;q13) is also noted in both RMS types and has been categorised as a secondary structural abnormality. RMS was one of the first tumours found to be associated with the Li-Fraumeni syndrome. DNA amplifications have been identified for regions on chromosome 2p and 12q. Both A-RMS and E-RMS have been studied by CGH and the results showed clear differences between the two RMS subtypes. Aberrations found in E-RMS concerned gains and losses of whole chromo-

somes or large parts of chromosomes: Gains were most frequently found for chromosomes 2, 8, 12, 13 (in 6/10 cases), chromosome 7 (in 5/10 cases), and chromosomes 17, 18 and 19 (in 4/10 cases). Losses were identified most often for chromosome 16 (in 4/10 cases), chromosome 10 (in 3/10 cases) and chromosomes 14 and 15 (in 2/10 cases). One tumour showed an amplification of 12q13-q15. In the A-RMS samples whole (or part of) chromosome gains and losses were found to a much smaller extent. In 10 tumours and 4 cell lines gain of chromosome 17q was found in 4 cases. However, in a high percentage amplifications were present. Chromosome regions most often involved were 12q13-q15 (in 7 cases) and 2p25 (in 5 cases). The latter region contains the *N-MYC* gene which is known to be amplified in A-RMS. The regions containing the *PAX7* and *FKHR* genes on 1p36 and 13q14 were found to be amplified in 2 cases.

As for Wilms tumour, abnormal genomic imprinting of chromosome region 11p15 appears to play a role in the development of RMS (paternal LOH, LOI of *IGF2*). Increased expression of *IGF2* in tumours with monoallelic expression of the gene confirm the important role postulated for *IGF2* in the development of this tumour. The imprinting status of *H19* has also been examined in RMS and was found to be normal in both subtypes. However, the expression was reduced significantly in 13/15 E-RMS and 2/11 A-RMS. This phenomenon was associated with either loss of the maternal (expressed) allele or LOI of *IGF2*. In contrast to the situation for Wilms tumour, reduced expression of *H19* was not seen in all cases with LOI of *IGF2*.

Hepatoblastoma. Hepatoblastoma (HB) is a rare malignant epithelial tumor of the liver with an incidence of 1 case per million children. However, it is the most common malignant hepatic neoplasm of childhood, and occurs with a predominance in males. Although most cases are sporadic, some HBs are associated with either BWS or familial adenomatous polyposis coli (FAP [→ APC gene in Familial Adenomatous Polyposis]). Since FAP patients carry mutations in the adenomatous polyposis coli (*APC*) gene, sporadic HBs have also been analysed for the presence of mutations in this gene. Indeed, alterations of the APC gene were found in 69% of the sporadic cases. When FAP occurs in combination with extracolonic symptoms it is commonly referred to as Gardner syndrome. Patients suffering from this disease also have an increased risk for the development of HB. The trisomy 18 syndrome can also be associated with HB, as has been found in 4 patients. One of the phenotypic features of trisomy 18 syndrome is the presence of an omphalocele (also found in BWS patients). It has been suggested that this feature may be one of the factors important in the development of HB in cases in which part of the liver has herniated into the omphalocele. As was found for the other BWS associated tumours, LOH of 11p15 has also been found independently by several researchers for HB (up to 33%). An LOH study of chromosome 1 showed frequent loss of alleles in HBs. In 32 cases 34% had lost heterozygosity for (a part of) chromosome 1, of which 22% were homozygous for markers on the (distal) short arm. There has been a report of the occurrence of HB in the Li-Fraumeni syndrome, and in addition one study showed mutation of the *p53* gene in 1/3 sporadic HB samples. Cytogenetic analysis of HB revealed certain consistent chromosome anomalies. Extra copies of chromosomes 2q and 20 are most frequently found. There has also been a report about a recurring translocation: t(1;4)(q12;q34) that results in partial trisomy of most of chromosome arm 1q and partial monosomy of distal 4q. CGH analysis identified mostly gain of DNA. Chromosomes affected in more than 30% of the cases included 1, 2, 7, 8 and 17. When determining the parental origin of 11p alleles lost in HBs it became clear that in this BWS-associated tumour LOH of 11p15.5 was exclusively of maternal origin. When looking directly at the imprinting status of the *IGF2* and *H19* genes biallelic expression was detected. Two studies showed LOI of *IGF2* with normal imprinting of *H19* in 1/3 HBs and in 1/5 HBs. A third study showed LOI of both genes in 1/5 cases.

Common genetic pathways

When reviewing all genetic and epigenetic data it becomes clear that the most evident abnormality found in all BWS-associated tumours affects chromosome region 11p15. This is the region to which the syndrome has been linked. All 4 tumour types show LOH of markers in this region. To date, data has been published for all except ACC showing LOH affecting the maternal allele, with retention of the paternal allele (one ACC with paternal UPD has been described). This suggests the involvement of genomic imprinting. Indeed, abnormal imprinting was found for these tumours, as it was for BWS: They display LOI of the maternally imprinted *IGF2* gene. Therefore, this growth factor may play a central role in the development of the overgrowth syndrome and its associated tumours. Increased expression has been noted for WT, ACC and E-RMS, and LOI of *IGF2* has been associated with decreased expression of the supposed tumour suppressor gene *H19*. There is an additional genetic abnormality common between all 4 types of neoplasms. They all show mutations in the *p53* gene. However, this is found in a large proportion of all cancers and therefore is considered not to be specific for the development of tumours associated with the BWS.

Besides genetic evidence there are also pathological data indicating an association between these tumours. Both WT and HB may contain rhabdomyomatous tissue, whereas primary tumours of the liver have been shown to consist of ACC and RMS.

There are also several chromosome aberrations found in a subset of these tumours. When considering abnormalities found in three of the four tumour-types there seems to be a strong connection between WT, E-RMS and HB. They share 7 common genetic abnormalities. Besides the abnormalities already mentioned above, they all might contain extra copies of chromosomes 7q, 8 and 17q. Therefore, these chromosome regions may contain genes that play a role in the normal embryonic development of the affected tissues. Since these affected regions are large it would be very difficult to identify the genes involved. More interesting therefore is the abnormality of chromosome 1p that was found in these tumours. This presented either as LOH or structural abnormality of the short arm of chromosome 1. Since these aberrations affect small(er) regions of the chromosome they may be very helpful in the identification of genes. This applies especially to the analysis of translocation breakpoint regions, as has been shown for the regions involved in BWS. Extra copies of chromosome 12 have been identified in the subset consisting of WT, ACC and E-RMS. These tumours are also characterised by increased expression of IGF2.

When analysing the published data, it becomes clear that WT and E-RMS share most genetic aberrations, with a total of 12. Therefore, the genetic relationship is most evident between these two tumour-types. In addition to the abnormalities already mentioned they have both been shown to contain extra copies of chromosome 18, and in both tumour types decreased expression of H19 has been found. Further elucidation of the common genetic pathways involved in the aetiology of the BWS associated tumours awaits identification of the genes involved.

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Beclin1

Definition

The *beclin1* gene encodes a 60 kD coiled-coil protein that interacts with the apoptosis-inhibitor → Bcl-2. The *beclin1* gene maps to a region approximately 150 kb, centromeric to → *BRCA1* on chromosome 17q21 that is commonly deleted in breast, ovarian and prostate cancer. Co-expression of *beclin1* provides protection from sindbis virus infection. The *beclin1* gene is homologous to the yeast → autophagy gene *APG6*.

BECN1

Definition

→ Beclin1.

Benign Tumor

Definition

A benign tumor is a hyperplasia or non-malignant lesion that is often premalignant. It remains confined to its normal location and neither invades surrounding tissue nor spreads to other organ sites.

Berlin Breakage Syndrome

Definition

→ Nijmegen breakage syndrome.

Betel Quid

Definition

Betel quid, also known as *pan*, consists of the following four main ingredients: tobacco, areca nuts and slaked lime wrapped in a betel leaf. Betel quid chewing is widely practiced in South-East Asia, particularly in India and Taiwan, and there are about 600 million betel quid chewers in the world. Betel quid chewing serves as a stimulant and narcotic. It is associated with an increased risk of oral squamous cell carcinoma (OSCC) and oral submucous fibrosis (OSF), the latter being a pre-malignant fibrotic lesion of the mouth. Some preparations also contain safrole, a known rodent hepatocarcinogen, which in chewers may increase the risk of developing liver cancer.

BHD Syndrome

Definition

→ Birt Hogg Dube syndrome.

BHLH

Definition

Basic helix-loop-helix (bHLH) is a protein motif shared by a group of transcription factors, therefore named bHLH proteins (or E proteins). For DNA binding, mono- or heterodimerization is compulsory, which is mediated by the helix-loop-helix motif. The basic region is composed of basic amino acids and determines DNA sequence-specific binding of the dimer; → E-box.

BID

Definition

Bid is a protein of 195 aa and 21 kD which is a BH3-interacting domain death agonist that modulates → apoptosis. It forms heterodimers with the pro-apoptotic → Bax or the anti-apoptotic → Bcl-2 proteins. The gene maps to 22q11.

BIK

Definition

Bik is a → Bcl-2-interacting killer protein of 160 aa and 18 kD that induces → apoptosis. It accelerates cell death by binding to anti-apoptotic proteins, like Bcl-2, and does not interact with Bax.

Bilateral Acoustic Neurofibromatosis

Definition

→ Neurofibromatosis 2.

Biological Markers

Definition

A biological marker, or → biomarker, is a cellular, biochemical or molecular alteration that is measurable in cells, body fluids or excreta.

Biologically Effective Dose

Definition

The biologically effective dose is the amount of chemicals taken up by the body that have covalently interacted with subcellular macromolecular targets; → adducts to DNA.

Biomarkers

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Definition

Biomarkers are parameters that provide information on exposure to xenobiotics and to chemopreventive compounds, or on the effects of that exposure in an individual or in a group.

Characteristics

Biomarkers used for the detection of cancer risk factors and in studies of chemoprevention can reveal an overall body load of genotoxins (which should be avoided) or of chemoprotective components (which should be enhanced).

Background

Most forms of cancer are due to somatic alterations (mutation, amplification, recombination) in proto-oncogenes, in tumour suppressor genes or in DNA repair genes. These are acquired in the tumour target tissues during

life time, accumulate and produce a clonal selection of cells with aggressive and invasive growth properties. Only approximately 1% of all cancers are due to inheritance of these types of genetic alterations. Most other cancers are dietary related, are due to inhalation of tobacco smoke, or may be a consequence of inflammation or viral infections. Therefore the majority of all human tumours are considered to be preventable by avoiding exposure to risk factors. Biomarkers may be used in human trials and in studies of molecular cancer epidemiology to study these types of exposures and to identify measures to reduce cancer risks.

Types of biomarkers

The most straight forward determination of risk is to identify people already carrying the disease on account of having tumour cells in their body. These markers are of diagnostic value. In the context of exposure and health, however, other parameters that can be detected prior to manifestation of tumours are considered more feasible. These include:

- Susceptibility biomarkers (predetermining damage) to identify people at high risk, since they carry cancer prone genetic alterations (mutations, gene amplifications, or recombination) in cancer target genes (e.g. → APC deletions; hMSH mutations, K-→ ras → amplification).
- Susceptibility biomarkers (predisposing alterations) to identify people at different degrees of risk because they carry frequent alterations in genes that are more indirectly related to the process of carcinogenesis. These indirect mechanisms include features of carcinogen metabolism (→ metabolic polymorphisms) or pharmacological variations (e.g. receptors for micronutrients, sensory dispositions). There is some evidence available that single genetic polymorphisms, or a combination of these, can be associated with cancer risk.
- Biomarkers of early effects in cells and tissues to identify past exposure to risk factors by determining genetic damage (→ DNA adducts, DNA breaks, → oxidative DNA damage, genome instability) in somatic cells. This is based on the assumption that increased DNA damage is the result of a higher load of genotoxic agents that will cause the complex process of carcinogenesis. Additional cellular processes that may serve as biomarkers are cell proliferation or → apoptosis (intermediate endpoints). These may also be decreased on account of exposure to protective factors. Furthermore, the modulation of gene expression, such as induction of phase II enzymes may render the cell less vulnerable and more resistant to risk factors, and the measurement of these effects are thus novel biomarkers of chemoprevention.
- Biomarkers of exposure (risk and protective factors) to identify current exposure to risk compounds (e.g. carcinogens from tobacco [→ tobacco carcinogenesis] or food, reactive oxygen species, products of lipid peroxidation) or protective compounds (e.g. antioxidants, metabolites of chemopreventive agents, → fermentation products of the gut flora) by measuring their concentrations in urine or blood. For complex associations such as → diet and cancer, the shift of these groups of substances relative to each other can then be evaluated as contributing to an increase or to a decrease of risk.

Biomarker techniques and fields of application

Depending on the source of body fluid or cells analysed, the biomarkers will reveal systemic or tissue specific exposures. Specific endpoints will be more suited for → molecular cancer epidemiology studies, whereas non-specific end points are also of value for occupational types of exposure assessment or for dietary intervention studies. Non-invasive methods should be better suited for large scale studies, whereas invasive methods will be employed more selectively. In this context, largely depending on the degree of invasiveness, biomarkers may be categorised as follows:

- Non-invasive methods using body fluids or exfoliated cells include techniques such as the analytical detection of single compounds or of their metabolites. The methods are in-

Biomarkers. Fig. – Images of undamaged to damaged DNA from single human peripheral lymphocytes in the 'comet assay'. Cells were embedded into agarose on microscopical slides, lysed, subjected to alkaline electrophoresis and stained with ethidium bromide. Usually the proportion of damaged cells and degree of damage is quantified for 50 – 100 cells per slide, using an image analyser.

dicators of exposure. Also, a functional determination of mutagenic or genotoxic effects of body fluids using cultured cells as target organisms (e.g. determination of faecal water genotoxicity) are biomarkers for determining exposure. Other non-invasive methods are directed at analysing genetic alterations in isolated exfoliated cells from these body fluids. Examples are the analysis of micronuclei e.g. in sputum or urinary and buccal cells.

- Relatively non-invasive methods using cells of the peripheral blood stream are aimed at detecting exposure-related genotoxic damage. The endpoints include DNA-strand breaks, oxidised DNA bases (using the single cell microgelelectrophoresis assay, also referred to as the → comet assay), → DNA adducts (detected with → ^{32}P-postlabelling) and cytogenetic endpoints (micronuclei, sister chromatid exchanges, chromosomal aberrations). The development of the techniques for genetic damage has been largely based on their utilisation as methods to assess exposure in occupational, environmental settings or subsequent to tobacco smoke inhalation. They have only sporadically been used to study associations of diet and cancer.
- Invasive methods using cells from tumour target tissues make use of cells from biopsies (e.g. colon, breast, kidney) to determine functional parameters in potential tumour target tissues. The parameters indicate cellular responses and genetic alterations (proliferation, K-ras-, p53-mutations, APC-alterations and DNA damage). The end points are indicators of very early response to risk factors and are biomarkers of effect. However, they are invasive, and thus may be limited to studies on special exposures or in specific groups of patients. In any case, their utilisation and development will serve as basis for the refinement of non-invasive methods with exfoliated cells as outlined above.

In conclusion, a variety of biomarkers to assess the impact of risk and of protective factors is available. Already research has provided evidence that biomarkers can measure the efficacy of exposure as well as of exposure reduction. Many of the techniques, however, need to be further validated for their applicability, reliability and predictivity of potential tumour risks in human studies. Another set of techniques is available that can serve as a meaningful basis for the development of potentially new biomarkers. Altogether these methods are of value to serve as indicators of effects and indicators of exposure by risk and protective compounds. Depending on the specificity of the end point or on the technical feasibility, individual methods will be more or less suited for use in dietary intervention studies, in occupational exposure settings and/or in larger scale trials of molecular epidemiology.

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Biomonitoring

Definition

Biomonitoring, or biological monitoring, is the estimation of chemical or physical exposure as measured in biological materials.

Bioreductive Drug

Definition

A bioreductive drug is an agent that is reduced in the state of O_2 deficiency, usually to produce a more active metabolite that can be cytotoxic or that can be used for the detection of O_2-depleted tissue areas.

Biosynthesis

Definition

Biosynthesis is the production (usually enzymatic) of components in living cells.

BIR

Definition

→ Baculovirus IAP repeat.

BIRC4

Synonyms

- → HILP (human IAP-like protein)
- X-linked inhibitor of apoptosis protein
- X-linked IAP-like protein (XIAP)
- apoptosis protein inhibitor 3 (API3)

Definition

Baculovirus IAP repeat-containing 4. BIRC4 is a cytoplasmic apoptosis suppressor protein of 497 aa and 56 kD. The gene maps to Xq25. IRC4 is an inhibitor of caspase-3 and caspase-7, belongs to the → IAP family of proteins, which are also referred to as Baculovirus IAP repeat (BIR) family of proteins; gene maps to Xq25; → baculovirus IAP repeat.

Birt Hogg Dube Syndrome

Definition

Birt Hogg Dube syndrome is an inherited disorder characterized by a predisposition to develop multiple tumors of the hair follicle (fibrofolliculomas); the fibrofolliculomas usually occur on the face and neck. The Birt Hogg Dube

syndrome is associated with a predisposition to develop spontaneous pneumothorax and renal cancer. Pathologists have had difficulty classifying the renal tumors associated with the Birt Hogg Dube syndrome; the renal tumors appear to be either chromophobe renal carcinomas or renal oncocytomas (→ renal carcinoma).

B-K Mole Syndrome

Definition

B-K mole syndrome is a syndrome of large numbers of atypical moles (naevi) named after the two melanoma kindreds from the United States in which it was first recognised - 'family B' and 'family K'. These atypical naevi (often referred to as dysplastic naevi) are sometimes precursor lesions of melanoma.

Bladder Cancer

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Definition

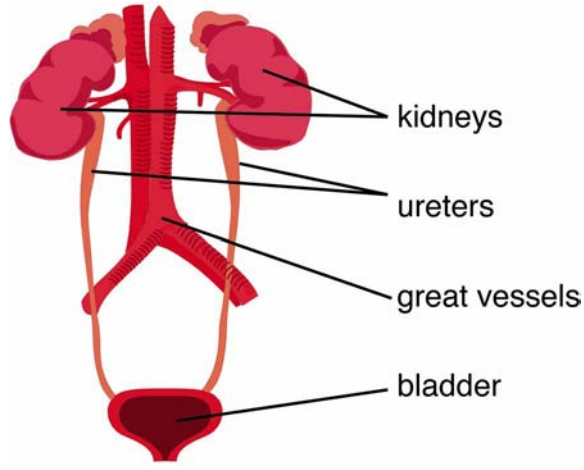
Bladder cancer is a malignant neoplasm that arises from the epithelial lining of the bladder (Fig. 1). Several histological forms have been identified. Transitional cell → carcinoma (TCC) comprises more than 90% of the neoplasms, while → squamous cell carcinoma (SCC) and → adenocarcinoma account for 5% and 2% respectively. In areas with endemic schistosomiasis, squamous cell carcinoma constitutes the predominant histological form. It is also not uncommon for transitional cell malignancies to have minor elements of adenomatous or squamous cell histology. However, from the clinical management standpoint, urinary neoplasms with minor components of these two histologic types are treated for their primary component. The clinical relevance of these minor components or the percentage at which a minor component becomes significant is unclear. An important prognostic criteria in transitional cell carcinoma is tumor grade. Tumor grading most commonly follows World Health Organization (WHO) guidelines in which Grades I - III represent well differentiated, moderately differentiated, and poorly differentiated tumors, respectively.

Characteristics

Clinical epidemiology and risk factors

Carcinoma of the urinary bladder is the second most common urologic malignancy. The worldwide incidence of bladder cancer is approximately 200,000 patients per year with 120,000 annual deaths. It affects more males than females by a 3:1 ratio. In the United States, the incidence is higher in whites than blacks, although survival is longer in whites and men than in blacks and women. The disease can affect all ages (even children) but the median age at presentation is 70 years. It is rarely found as an incidental finding at autopsy.

Environmental risk factors for transitional cell carcinoma include cigarette smoking, aniline dyes, pelvic radiation, benzidine, 2-naphthylamine and other aromatic amines.



Bladder Cancer. Fig. 1 – Anatomy of the urinary tract.

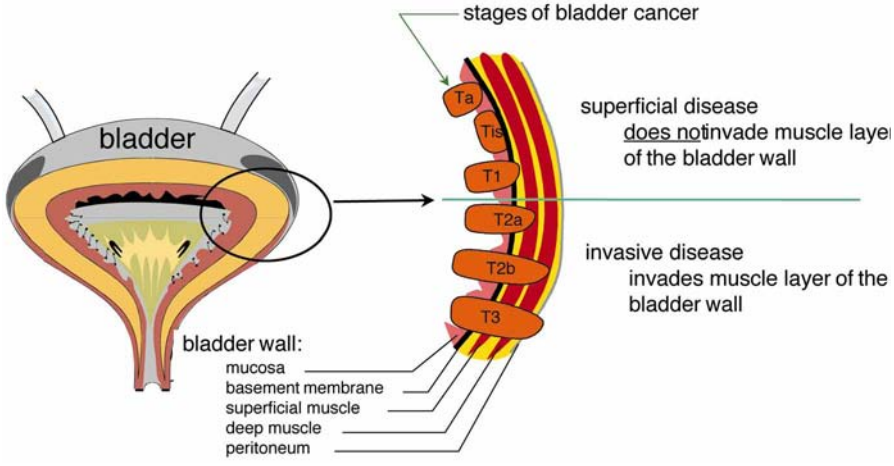
Acrolein, a metabolic product of cyclophosphamide, can increase the risk of bladder cancer 9-fold. Smoking increases the risk of bladder cancer 4-fold, and at least one quarter of cases can be attributed to smoking. Chronic cystitis and long-term bladder catheters increase the risk of squamous cell carcinoma. → Schistosoma haematobium infection not only increases the risk of squamous cell carcinoma significantly, but also increases the risk of transitional cell carcinoma.

Tumor biology and genetics

Transitional cell carcinoma is a → field change disease rendering the entire urothelium susceptible to malignant transformation. Both the → *TP53* and → *RAS* genes are known targets of chemical carcinogens. The most frequent genetic alterations in transitional cell carcinoma are monosomies of chromosome 9 (57%), and losses on chromosome arms 11p (32%), 17p (32%), 8p (23%), 4p (22%) and 13q (15%). Deletions specifically associated with higher grades and stages of cancer, indicative of tumor progression, have been identified at 3p, 4q, 8p, 10, 15, 17p, 18q among many others. Other studies utilizing immunohistochemical techniques have suggested that overexpression of p21Ras protein, mutated TP53 and the epidermal growth factor receptor (→ EGFR) in bladder tumors are related to bladder tumor progression. In addition, loss of → RB1, DCC, and E-cadherin (CDH1) expression has also been related to this transition. Tumors with p53 mutations tend to exhibit more aggressive behavior when present in both superficial and invasive disease, while p15/p16 are associated with low grade superficial disease.

Characteristics of non transitional cell carcinomas

SCC comprises only 1-3% of bladder tumors in the U.S. and Britain but represents 75% of tumors in Egypt. Most of the SCCs found in Egypt are due to Schistosoma haematobium ('bilharzial' bladder cancer) and are well differentiated, with lower risk of metastases than transitional cell carcinoma. Non-bilharzial squamous cell tumors are caused by chronic inflammation from infection, stones, indwelling catheters or bladder diverticuli. Although these tumors' prognosis is similar to transitional cell carcinoma by stage, nonbilharzial tumors tend to present with late-stage disease. Primary bladder adenocarcinomas represent approximately two percent of bladder tumors and are more common in extrophic bladders, urachi and intestinal conduits or augmentations. They may produce mucin and can be associated with cystitis glandularis. Most are poorly differentiated and present with advanced disease.



Bladder Cancer. Fig. 2 – Stages of Bladder Cancer.

Clinical presentation

Bladder cancer frequently presents with painless hematuria, although urinary frequency, urgency and dysuria can occur as well. Gross hematuria is common, and bladder cancer is rarely diagnosed in the absence of at least microscopic hematuria although this can be intermittent. Bladder cancer can also present with flank pain and hydronephrosis if the tumor obstructs the ureteral orifice.

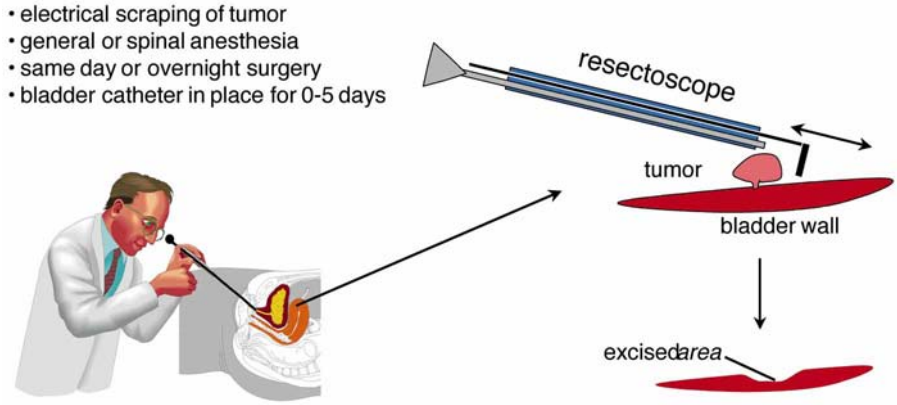
Diagnosis and staging

The diagnostic evaluation of bladder cancer begins with a history and physical examination including bimanual pelvic exam, urinalysis, cytology and cystoscopy. Intravenous urography is indicated in all patients with bladder tumors to evaluate the upper urinary tracts. Retrograde ureteropyelograms can also be performed at the time of cystoscopy if intravenous urography does not provide an adequate view of the upper tracts. Cytologic examination of bladder cells that slough off into urine is useful in the diagnosis of CIS or high grade tumors, but low grade tumors are more difficult to detect by cytology. Cytology is primarily used in the diagnosis and follow up of patients at risk for recurrent disease. Novel molecular markers such as the nuclear maxtrix protein (NMP-22) assay, telomerase activity, and the bladder tumor antigen (BTA) offer promise for enhanced detection of symptomatic patients and screening of high risk populations.

At presentation, 85% of patients with transitional cell carcinoma of the bladder have disease limited to the organ, while 10% have regional disease and 5% have metastatic disease. Of the 85% with localized disease, 80% have 'superficial' stage disease (stages Tis, Ta, T1) and 20% have invasive disease (stages ^{3}T2) (Fig. 2). Transitional cell carcinoma may grow in papillary, sessile, nodular or flat (Tis) forms. Papillary forms are more commonly associated with superficial disease while invasive tumors often look sessile or nodular. → Carcinoma *in situ* (= CIS/Tis) is flat, poorly differentiated transitional cell carcinoma involving only the urothelium. Although CIS can cause irritative voiding, it is frequently asymptomatic. Cystoscopy may be normal or exhibit erythematous patches, and urine cytology is 80 – 90% sensitive.

→ Transurethral resection (TUR) of bladder tumors not only provides tissue for pathologic diagnosis, but can represent definitive therapy in the majority of superficial tumors as long as the whole tumor was resected (Fig. 3). After the intraluminal portions of the tumor are resected, the tumor base is frequently resected as a separate pathologic specimen to ensure complete resection. Muscularis propria must be included in the specimen to define presence or absence of muscle invasion by the tumor and thus establish the stage of the cancer. The site is then fulgurated to prevent bleeding as well as destroy any residual cancerous cells. Random biopsies of the bladder and the urethra are sometimes taken to evaluate for CIS. If the tumor appears invasive (ie. sessile, solid configuration), the resection is tailored so as to accurately determine clinical stage and to optimize subsequent definitive therapy. For example, if the patient is likely to choose radical cystectomy as the treatment of choice then complete resection is not necessary. Conversely, if the patient is likely to select bladder sparing therapy with radiation and chemotherapy, then resection of as much tumor as safely possible should be carried out.

The staging of bladder cancer is based primarily on the specimen generated by the transurethral resection and is classified according to the 1997 international UICC/AJC system (Table). Pathologic exam of bladder specimens may be complicated by difficulty differentiating muscularis propria from the more superficial and thin muscularis mucosa, the latter which does not represent 'true muscle' and thus involvement of it by tumor does not consist of muscle invasion. If pathologic examination reveals tumor invasion into muscularis propria, CT or MRI of the abdomen pelvis is used to evaluate for gross extravesical spread, lymphadenopathy or hepatic metastases. These methods fail to detect lymph node spread in up to 40% of patients who have cancerous nodes at the time of surgery. A radionucleotide bone scan can be obtained to evaluate for bony metastases, but



Bladder Cancer. Fig. 3 – The Trans Urethral Resection (TUR).

the yield of a bone scan in the face of a normal alkaline phosphatase is quite low. Chest X-ray is obtained to rule out pulmonary metastases.

Management of superficial disease

The therapy of superficial (Ta/T1) bladder cancer consists of transurethral resection and fulguration. Because these tumors tend to recur and may progress to muscle invasion, follow up cystoscopy at regular intervals is critical. Tumors that invade the lamina propria (T1) should be considered potentially more aggressive, particularly if the tumor is high grade. Argon and Nd:Yag lasers have also been used successfully for ablation of superficial bladder tumors especially those that are multifocal or difficult to access via the resectoscope used for TUR. The disadvantage of these techniques is the lack of tumor specimen and thus only lesions with a high likelihood of being non-invasive should be treated in this way.

Patients with recurrent, high grade superficial bladder tumors or CIS may benefit from intravesical therapy with Bacillus Calmette-Guerin [→ BCG] (BCG) or Mitomycin C. These treatment can be given in two clinical contexts. They can be given for the treatment of residual disease that could not to be removed at TUR. Alternatively, they can be used to reduce the incidence of recurrence and progression in patients that have completely resected tumors. BCG is a live attenuated strain of *Mycobacter-*

Bladder Cancer. Table – Meanings of remarks: [1]suffix '*m*' after T stage to indicate multiple tumors [2]nodes of 'true pelvis' (i.e.: below bifurcation of common iliac vessels).

UICC/AJC 1997	description
Ta[1]	non-invasive papillary tumor
Tis	carcinoma *in situ:* 'flat tumor'
T1	tumor invades subepithelial connective tissue
T2a	tumor invades superficial muscle (inner half)
T2b	tumor invades deep muscle (outer half)
T3a	tumor invades perivesical fat microscopically
T3b	tumor invades perivesical fat macroscopically (extravesical mass)
T4a	tumor invades prostate or uterus or vagina
T4b	tumor invades pelvic wall or abdominal wall
N1[2]	single node <2 cm
N2	single node >2 cm to 5 cm or multiple nodes <5 cm
N3	any node >5cm
M1	nodal mets above bifurcation of common iliac vessels
M1	metastasis

ium bovis, which stimulates a local and possibly systemic immune response. BCG can often delay recurrence and progression of superficial disease and CIS. Side effects of BCG include bladder irritability, granulomatous prostatitis and systemic disseminated infection requiring anti-tubercular agents, and deaths are rare. Contraindications include: active tuberculosis, immunosuppression, traumatic catheterization and prior severe reaction to BCG. Mitomycin C is an antibiotic chemotherapeutic agent that inhibits DNA synthesis. Although a question for some debate, this agent appears to be as effective than BCG and relatively well tolerated. Recently, some controversy has arisen in regard to the long term (>15yrs) ability of BCG or Mitomycin C to reduce tumor progression. Other compounds for intravesical use exist but are thought to be generally less effective than the two mentioned above in delaying progression but may be equally efficacious in delaying recurrence in low grade tumors. Among patients with superficial disease, radical cystectomy is reserved for diffuse, recurrent or unresectable papillary tumors, or T1 or CIS lesions unresponsive to intravesical therapy.

Recurrence polychronotropism (multiple in space and time) in superficial bladder tumors is uniquely high relative to any other organ sites. 20 – 70% of patients suffer disease recurrence. While in the absence of progression recurrence per se is not life threatening, this phenomenon nonetheless constitutes a cause of significant morbidity and treatment expense. While less common, the progression of superficial tumors to muscle invasion is associated with a marked decrease in 5 year disease-specific survival. Progression risks vary widely by stage and grade, ranging from less than 5% for Ta grade 1 tumors up to 50% for T1 lesions with associated CIS.

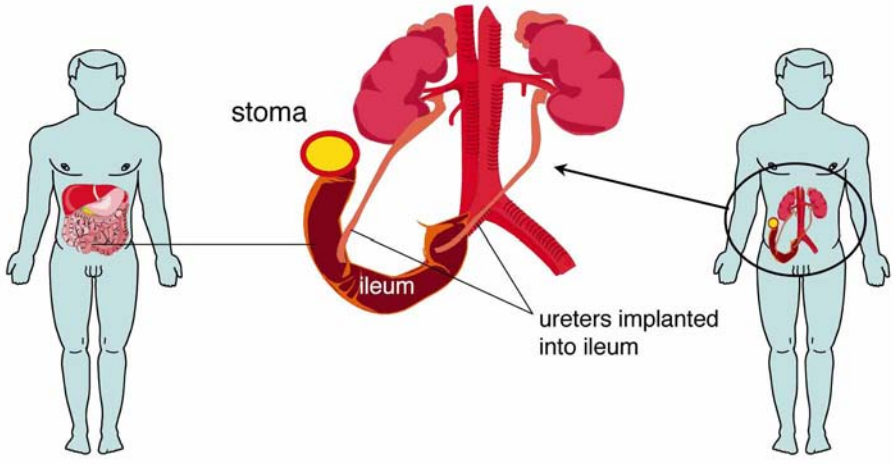
Management of invasive and metastatic disease

Radical cystectomy with urinary diversion or bladder sparing protocols, using a combination of radiation and chemotherapy, are the treatments of choice for patients who have resectable muscle invasive bladder cancer. Radical cystectomy includes wide excision of bladder and prostate in male patients and typically bladder, uterus, ovaries and anterior vaginal wall in females. Perioperative mortality from cystectomy is approximately 1% in most centers. The 5-year disease-free survival is 65 – 80% for pT2 and pT3A tumors and 37 – 61% for pT3B tumors. Microscopic involvement of local lymph nodes decreases 5-year survival to approximately 5 – 20% depending on the number and extent of nodal involvement. Pelvic recurrence rates after cystectomy range from 2 – 10% and depends on the stage of the primary as well as the presence of pelvic nodal involvement. Recurrence or persistence rates after bladder sparing protocols approach 50%. By careful patient selection these latter protocols can achieve comparable disease specific survival rates to those obtained by radical cystectomy.

Large tumors that are only minimally resectable by TUR and those causing hydronephrosis have a significantly worse response rate with such bladder sparing protocols. Complications of radiotherapy include dysuria, frequency, or diarrhea in up to 70% of patients.

Following cystectomy, multiple options in urinary diversion exist, most of which utilize intestinal segments. An → ileal conduit using a short portion of the terminal ileum to carry urine from the ureters to the anterior abdominal wall is the simplest and most commonly performed diversion. Patients then wear an external appliance on the stoma. Possible complications include parastomal hernia, stomal stenosis or stricture at the ureteroileal anastomosis. A cutaneous → continent urinary diversion such as the Kock (ileum) or Indiana (ileocecum) pouch forms an internal reservoir which can then be intermittently catheterized via a cutaneous stoma (Fig. 4). In selected patients, the continent reservoir can be anastomosed to the native urethra. The continent diversions are technically more difficult and require motivated patients to manage the post operative care required. → Ureterosigmoidostomies are now rarely performed because of difficulties with reflux, urolithiasis, electrolyte imbalance and increased risk of adenocarcinoma of the colon.

Metastatic transitional cell carcinoma has traditionally been treated with MVAC (Metho-



Bladder Cancer. Fig. 4 – The Ileal Conduit Urinary Diversion.

trexate, Vinblastine, Adriamycin and Cisplatin) with a response rate of 15 – 35%. Complete remission is seen in approximately 13% of patients and mean survival can be improved from 8 months to 12 months. Newer agents have recently been used with a significantly lower morbidity and mortality than MVAC and apparently equal or even superior efficacy. Large randomized trials are currently ongoing to determine the future role of these new agents.

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Blast Crisis

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Definition

Blast crisis is the aggressive and rapidly fatal terminal phase of → BCR-ABL1 positive → chronic myeloid leukemia (CML), characterized by the accumulation of immature myeloblasts or lymphoblasts similar to those found in patients with acute leukemia.

Characteristics

Clinical Features

Chronic myeloid leukemia (CML) is a biphasic disease. Although benign, and responsive to treatment with cytotoxic drugs such as busulphan and hydroxyurea, the chronic phase of CML is temporary without eradication of the BCR-ABL1 rearrangement positive cells. Within a mean period of 3-5 years, the natural course of the disease is to accelerate, then to transform to an aggressive and rapidly terminal acute phase or blast crisis. Features associated with transformation include an increasing number of leukocytes, particularly immature blasts, in the blood and bone marrow, progressive anemia, thrombocytopenia and lack of response to therapy (like that seen in some forms of acute leukemia). In a small proportion of patients, the blast transformation may occur outside the bone marrow (extramedullarly) in sites such as the lymph nodes, spleen, skin or meninges.

Blast crisis can be divided into two general forms: lymphoid and myeloid. Lymphoid blast crisis develops in about 30% of patients, and the blast cells are phenotypically similar to the common form of → acute lymphoblastic leukemia (ALL). In rare cases, T-cell morphology has been described. Myeloid transformation is heterogeneous, where myeloblasts are the usual blast cell type, but megakaryoblasts or erythroblasts have been frequently identified. Occasional patients show blasts with myelomonocytic, monocytic or very rarely, basophilic blast differentiation. It is important to differentiate between myeloid and lymphoid blast cells, as patients in lymphoid blast crisis respond better to treatment.

Cause

The precise molecular events that determine blast crisis are still largely unknown, although there is much evidence from cytogenetic and molecular studies that non-random and lineage-specific accumulation of gene mutations may be important. Cytogenetic changes that appear at transformation to blast crisis are highly specific, often diagnostic and include at least one of four major and six minor chromosomal alterations in ~80% of cases. Recurring molecular changes at transformation include mutation of the → *p53* and *RB* genes, activation of → *RAS* and, in lymphoid blast crisis cells, homozygous loss of the → p16 tumour suppressor gene. Loss of → imprinting at the insulin-like growth factor-II gene (IGF2 [→ insulin-like growth factors]) locus has also been detected in one patient series examined. These and other studies suggest that BCR-ABL1 cells are genetically unstable, and preferentially accumulate non-random genomic mutations that provide a proliferative advantage and are compatible with the BCR-ABL1 oncogene product. Non-random accumulation of chromosomal aberrations is also observed in advanced leukemias of BCR-ABL1 transgenic mice.

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Bloom Syndrome

Definition

Bloom syndrome is a prototypic genetic disease in which chromosomal instability is associated with predisposition to cancer, including those of haempoietic and epithelial lineages. Sufferers also exhibit growth retardation, telangiectasia and photo-sensitivity. The gene mutated in Bloom syndrome, *BLM*, encodes a member of the RecQ DNA helicase family implicated in the regulation of DNA replication and recombination; → Werner syndrome.

B Lymphocytes

Definition

B lymphocytes are cells that are responsible for synthesizing antibodies.

B-MYB

Definition

B-MYB is a member of → MYB family of oncogenes.

Body Mass Index

Definition

The body mass index (BMI) is a ratio between weight and height, a mathematical formula that correlates with body fat. BMI is determined by calculating your weight in kilograms divided by your height in meters squared (BMI=kg/m^2).

Bone Resorption

Definition

Bone resorption is calcium mobilization from bone.

bp

Definition

→ Base pair.

Brachytherapy

Definition

Brachytherapy is a kind of radiation therapy performed using a local origin of radioactive material placed into the body near the tumor. The word arises from the Greek word, brachys for 'brief ' or 'short' and refers to electromagnetic radiation delivered *via* insertion of radioactive materials a short distance from or within the tumor. It is a therapy that is delivered in close proximity to the target, typically through the use of indwelling radiation sources, allowing greater dose delivery to the target with limited toxicity to surrounding structures.

Brain Tumors

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Definition

Primary brain tumors most commonly arise in glial cells and embryonal precusors of the central nervous system. Primary cancer of the brain occurred in approximately 18,000 individuals in 1996, accounting for approximately 1.1% of newly occurring malignant tumors. In children and young adults, brain tumors are a major health problem second only to leukemia as a cause of cancer-related deaths. Tumors that metastasize to brain pose a major problem in the management of systemic cancer. About 15% of patients who die of cancer (80,000 individuals each year) have symptomatic brain metastases.

Characteristics

Classification and pathology

Tumors derived from astrocytes, → astrocytoma, are the most frequent primary intracranial neoplasms. Their neuropathological appearance is highly variable. The most widely used classification scheme is the World Health Organization (WHO) grading system, which is a four-tiered system of increasing malignancy. Grade I has an excellent prognosis following surgical excision, and Grade IV, → glioblastoma multiforme, which has multiple features of clinical aggressiveness, is typically incurable. Hypercellularity with evidence of high mitotic activity, nuclear and cytoplasmic atypia, endothelial proliferation and necrosis correspond closely to tumor virulence. The overwhelming majority of gliomas arising in adults are high grade and arise in a supratentorial location. High grade tumors do not have a clear margin separating neoplastic and normal tissue. This finding is consistent with the observation that tumor cells usually have extensively infiltrated adjacent normal brain.

Cytogenetic examination of chromosomes within the cells of a brain tumor has revealed characteristic regions frequently altered in various different tumor types (Table). Frequent sites at which chromosomal DNA is lost in astrocytic tumors include chromosomes 17p, 13q and 9. In oligodendroglioma, DNA from 1p and 19q is frequently lost, and in meningiomas, 22q is often lost. Molecular genetic analysis can also reveal evidence of tumor specific genetic alterations at sites where chromosomes appear normal upon cytogenetic analysis. Using a variety of molecular technologies it has been possible to document the alteration of a large number of different genes in brain tumors, particularly astrocytic tumors (Table). While the particular constellation of genetic alterations varies among individual brain tumors that appear to be histologically indistinguishable, an accumulation of mutations is typically associated with increasingly aggressive malignant behavior. Proto-oncogenes promote physiologic cell growth, but when inappropriately activated they mediate tumor cell proliferation. The most easily recognized of these genes function normally as growth factors or growth factor receptors. The epidermal growth receptor gene (EGFR) is the gene most frequently amplified in malignant astrocytomas. EGFR amplification occurs in approximately 5% of low grade astrocyotomas and in 40% of glioblastoma multiforme, indicating that this molecular change is principally associated with the progression from low or intermediate-grade neoplasia to high-grade astrocytic neoplasia. Other growth factor receptor molecules such as the platelet-derived growth factor and its receptor have also been implicated in the development of astrocytomas.

The protein products of tumor suppressor genes are proteins that act to regulate or suppress cell growth or promote cell death. These genes are inactivated during tumorigenesis and several such genes have been implicated in the development of astrocytoma. The p53 gene, located on chromosome 17p, has been found to influence multiple cellular functions thought to be important in tumorigenesis. Patients with Li-Fraumeni syndrome, caused by an inherited constitutional p53 mutation, have a predisposi-

tion for the development of brain tumors. p53 mutations have been reported in astrocytic tumors of all grades, occurring in approximately 40% of astrocytomas, 30% of anaplastic astrocytomas and in a slightly smaller fraction of glioblastoma multiforme. Deletion of the CDKN2 gene, which encodes the cyclin-dependent kinase inhibitor p16, has been reported to occur in approximately 40 – 70% of glioblastoma. The RB1 tumor suppressor gene is homozygously deleted or mutated in about 30% of high-grade gliomas.

The second most common primary brain tumor is oligodendroglioma [→ oligodendrocytoma], which has a more benign course than astrocytoma. Many → gliomas have mixtures of cells with astrocytic and oligodendroglial features. If this mixed histology is prominent, the tumor is termed a mixed glioma or an oligoastrocytoma. Many investigators believe that the greater the oligodendroglial component, the more benign the clinical course. The presence of such histologic characteristics as mitoses, necrosis and nuclear atypia generally are associated with a more aggressive clinical course. If these features are prominent, the tumor is termed a malignant oligodendroglioma.

Other malignant primary brain tumors include primitive neuroectodermal tumors (→ PNET) such as → medulloblastoma and → ependymoma, → germinomas and CNS lymphoma. Cerebral PNETs and medulloblastoma, a PNET that arises in the posterior fossa, are highly cellular malignant tumors thought to arise in neural precursor cells. These tumors most commonly occur in children and treatment includes surgery, radiotherapy and in most cases chemotherapy. If limited to the posterior fossa and completely resected, these tumors have a good prognosis. Ependymomas are rare tumors and when these occur in children they typically are within the fourth ventricle. In adults they arise more frequently in the spinal cord. Histologically, these tumors exhibit diagnostic ependymal rosettes. Following total excision of an ependymoma, the prognosis is excellent. However, many ependymomas cannot be totally excised. Germinomas arise most commonly during the second decade of life at midline locations. Both malignant and benign variants occur frequently. These tumors present with hypothalamic-pituitary dysfunction and visual field deficits. A stereotaxic biopsy should be performed for diagnosis. While surgery has a role in the management of some germinomas, radiation and chemotherapy typically play the central role in treatment.

Primary CNS lymphomas are most commonly seen in immuno-compromised patients, and have a clinical presentation similar to other primary brain tumors with signs and symptoms referable to cerebral and cranial nerve involvement. Imaging studies typically demonstrate a uniformly enhancing mass lesion. Stereotaxic needle biopsy can be used to establish the diagnosis. Secondary CNS lymphoma almost always occurs in association with the progression of systemic disease.

A large number of different tumors that are most often benign also occur in the nervous system. → Meningiomas are derived from cells of the arachnoid membranes. They are more frequent in women than men with a peak inci-

Brain Tumors. Table – Cytogenetic and genetic alterations in brain tumors.
* DMs, double minute chromosomes.

tumor type	chromosomal alteration	genetic changes	
		oncogene	tumor suppressor gene
astrocytoma	$1p^-$, 7^+,$9p^-$, de110, $13q^-$, $17p^-$, $17q^-$, 19q, $22q^-$, DMs*	*EGFR, CROS, MET, CDK4, NEU, RAS*	*P53, RB1, NF1, PTEN, GLI, MYC,* $P16^{INK4a}$, $P19^{ARF}$
oligodendroglioma	$1p^-$, $19q^-$	*EGFR*	*TP53, PTEN,* $P16^{INK4a}$, $P19^{ARF}$

dence in middle age. Meningiomas may be found incidentally on a CT ar MRI scans or they may present with a focal seizure, a slowly progressive focal deficit or symptoms of increased intracranial pressure. Meningiomas rarely have histological evidence of malignancy. Other tumors that have a benign clinical course include giant cell astrocytomas, pleiomorphic xanthroastrocytomas, neurocytomas and gangliogliomas. Colloid cysts, dermaid cysts and epidermoid cysts also occur in the brain.

Clinical presentation of brain tumor patients

The most common symptoms that bring patients with a tumor arising in the brain to their physician include a slowly progressive focal neurological disability, a non-focal neurological syndrome such as headache, dementia, or gait disorder or a seizure. Other systemic symptoms suggest a tumor from some other location that may have metastatasized to the brain, since patients with primary brain tumors typically do not exhibit systemic symptoms. Patients with primary brain tumors rarely have any biochemical abnormalities, but CT (Computerized Tomography) and MR (Magnetic Resonance) imaging are key diagnostic modalities for the identification of brain tumors. The characteristic imaging features of brain tumors are mass effect, edema and contrast media enhancement. Positron emission tomography (PET) scanning and single photon emission tomography (SPECT) have ancillary roles in the imaging of brain tumors. Brain tumors are also recognizable in many different inherited syndromes including von Aecklinghausen syndrome (Neurofibromatosis type1), Neurofibromatosis type 2, Li-Fraumeni syndrome, Multiple endocrine neoplasia type 1, Tuberous sclerosis, Turcot syndrome and Gorlin syndrome.

Clinical management of brain tumor patients and prognosis

The primary modality of treatment for most primary brain tumors is surgery. The goals of surgery are to obtain tissue for pathological examination, to remove tumor and to control mass effect. In the case of low grade and benign tumors, the removal of tumor tissue can be curative or contribute substantially to extending the time to symptomatic progression. In higher grade tumors, the role of surgery in contributing to curative therapy is less clearly defined, but in younger patients most surgeons aggressively pursue the removal of as much tumor as possible. Following surgery, radiation therapy has been shown to prolong survival and improve the quality of life of patients with high grade glioma, PNET, ependymoma and meningioma, when malignant histologic elements can be pathologically identified within the tumor. Up to half of patients with brain metastases have only a single detectable tumor mass, and such metastases are usually surgically excised as a palliative measure. However, radiation therapy is the primary treatment for brain metastases, since it is assumed that there are multiple microscopic deposits of tumor cells throughout the brain. Whole brain radiation therapy, though toxic, is usually recommended for sensitive tumors.

The medical management of most brain tumors is symptomatic, although a role for chemotherapy is clearly defined in medulloblastoma and oligodendroglioma. Patients whose brain tumors are associated with surrounding edema benefit symptomatically from the administration of high doses of glucocorticoids. Anticonvulsants are useful in the control of seizures. Some glioma patients receive anti-coagulation therapy to avoid complications of venous thrombosis that occurs in these patients.

The prognosis for patients with primary brain tumors varies greatly as a function of the histology and location of the tumor. Benign tumors are often cured by surgery alone. Germinoma and medulloblastoma are more sensitive to cytotoxic therapies than are other brain tumors, and the prognosis for patients with these tumors is generally better than it is for patients with high grade glioma. In modern studies, the median survival of patients with high grade gliomas is approximately 1-2 years.

Complications of therapy

The nervous system is vulnerable to injury by therapeutic radiation, and this is frequently

manifested by neuropyschological compromise and disability, particularly in very young children who have been treated with high doses of radiation. Pathologically, there is demyelination, hyaline degeneration of small arterioles, and eventually brain infarction and necrosis. Endocrine dysfunction is also commonly seen when the hypothalamus or pituitary gland has been exposed to therapeutic radiation. Depending on the radiated field, secondary tumors such as glioma, meningioma, sarcoma and thyroid cancer occur following radiation therapy.

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BRCA1

Definition

Breast cancer susceptibility gene 1; BRCA1/BRCA2; → breast cancer genes BRCA1 and BRCA2.

BRCA2

Definition

Breast cancer susceptibility gene 2; BRCA1/BRCA2; → breast cancer genes BRCA1 and BRCA2.

BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk

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Definition

Mutations in the → breast cancer genes *BRCA1* and *BRCA2* cause elevated risks to breast and ovarian cancer. *BRCA1* maps to chromosome 17 (band q21), *BRCA2* maps to chromosome 13 (band q12).

At the genetic level there are interesting analogies between the two genes, even though they are not detectably related by sequence. Both genes are large (coding regions of 5.6 and 10.2 kb, respectively), complex (22 and 26 coding exons, respectively), and span about 80 kb of genomic DNA. Both have extremely large central exons encoding >50% of the protein. The majority of the mutations in both genes detected to date lead to premature termination of protein translation, presumably resulting in an inactive truncated protein. Gene changes are distributed nearly ubiquitously over the coding exons and immediate flanking introns. Even though more than half of all mutations are found only once, many mutations have been detected repeatedly in certain populations. For most of these, this has been shown to be the result of a founder effect: these mutations arose a long time ago, and have since spread in the population. Typical founder mutations are the 1185delAG and 15382insC in BRCA1 and 26174delT in BRCA2 that have a joint frequency of about 2.5% among individuals of Ashkenazi Jewish descent.

Characteristics

Clinical characteristics

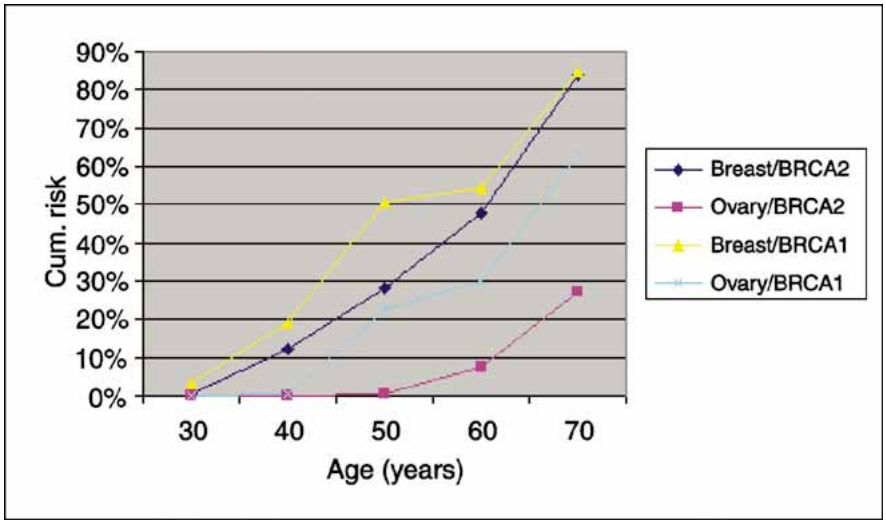
Female carriers of a deleterious BRCA1 mutation were estimated by the Breast Cancer Linkage Consortium (BCLC) to have an 87% cumu-

lative risk to develop breast cancer before the age of 70, and 40–63% risk to develop ovarian cancer before that age (Fig. 1). The gene frequency of BRCA1 was estimated at 1 in 833 women, implying that 1.7% of all breast cancer patients diagnosed between the ages of 20 and 70 are carrier of such a mutation. The estimated cumulative risk of breast cancer conferred by BRCA2 reached 84% by age 70 years. The corresponding ovarian cancer risk was 27% (Fig. 1). These estimates imply that BRCA2 mutations are about as prevalent as BRCA1 mutations. It has been suggested that the ovarian cancer risks are dependent on the position of the mutation in the gene, for BRCA1 as well as BRCA2 mutations. There is also some evidence that cancer risks can be modified by other factors. For example, a strong variability in phenotype can be seen among families segregating the same mutation. This can range from early-onset breast cancer and ovarian cancer, to late-onset breast cancer without ovarian cancer. Even within a single pedigree, ages of onset of cancer can vary substantially. It seems likely that environmental and hormonally related factors (smoking, oral contraceptives) importantly co-determine disease outcome in carriers.

Molecular and cellular characteristics

Tumor suppressor genes. The first clues to the roles of BRCA1 and BRCA2 in tumorigenesis were genetic. The fact that most germline mutations are predicted to inactivate the protein, and the observed loss of the wild type allele in almost all breast and ovarian cancers arising in mutation carriers, are strong indicators that BRCA1 and BRCA2 proteins act as tumor suppressors. This is supported by the finding that induced overexpression of wild type but not mutant BRCA1 in MCF-7 breast cancer cells leads to growth inhibition and inhibited tumor growth in nude mice.

Expression of BRCA1 and BRCA2. In normal cells, BRCA1 and BRCA2 encode nuclear proteins, preferentially expressed during the late-G1/early-S phase of the cell cycle, but down-

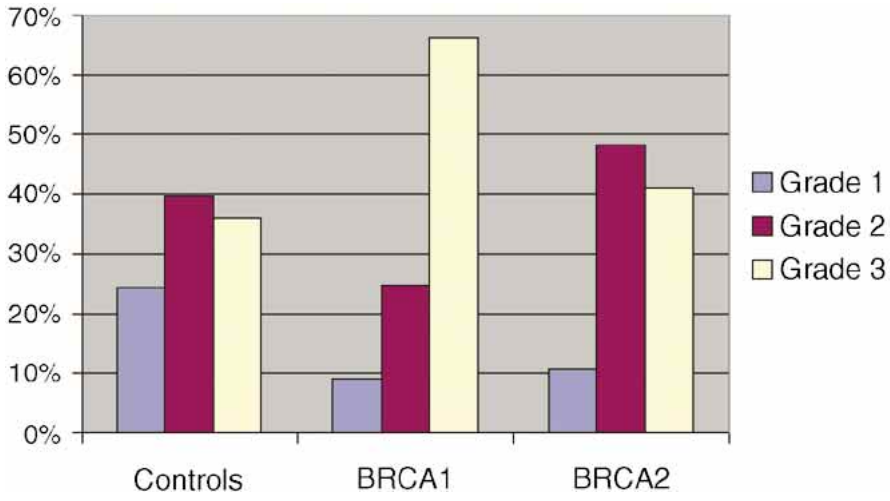


BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk. Fig. 1 – Overall penetrances of BRCA1 and BRCA2 for breast and ovarian cancer. Estimates were obtained by maximizing the LOD score with respect to all the different penetrance functions in those families with strong evidence of the breast and ovarian cancers being caused by the gene (done by linkage analysis). This is equivalent to maximizing the likelihood of the marker data, which is determined only by disease phenotype data. This will give an unbiased estimation of the penetrance irrespective of ascertainment of families on the basis of multiple affected individuals. Data were compiled from Ford et al. (1994) Lancet 343: 692-695, and Ford et al. (1998) Am. J. Hum. Genet. 62: 676-689. The graphs can be read in such a way that, for example, an unaffected carrier of a BRCA1 mutation has a 50% risk to develop breast cancer before age 50.

regulated in quiescent cells. While apparently at odds with the above-mentioned observations that BRCA1 expression inhibits cellular proliferation, the proliferation-induced expression could represent a negative feedback loop tending to decrease breast cancer risk. However, BRCA1 expression can also be up-regulated in a proliferation-independent way in mammary epithelial cells induced to differentiate into lactating cells by glucocorticoids. Hence, BRCA1 might also play a role in controlling mammary gland development. In mice, expression of BRCA1 and BRCA2 is coordinately up-regulated with proliferation of breast epithelial cells during puberty, pregnancy and lactation. Intriguingly, BRCA1 might suppress estrogen-dependent mammary epithelial proliferation by inhibiting ER-α mediated transcriptional pathways related to cell proliferation. Whatever the cellular function of BRCA1, it appears to be regulated by phosphorylation: it becomes hyperphosphorylated at G1/S with dephosphorylation occurring at M phase. BRCA1 might regulate the G1/S checkpoint by binding hypophosphorylated retinoblastoma protein. BRCA1 and BRCA2 have also been suggested to regulate the G2/M checkpoint by controlling the assembly of mitotic spindles and the appropriate segregation of chromosomes to daughter cells.

BRCA1- and BRCA2-related breast cancer. A close examination of the pathology of BRCA1- and BRCA2-related breast cancers has defined a typical pathology for each category, differing from that in sporadic cases. In general, cancers in carriers are of higher grade than age-matched controls (Fig. 2), and the BRCA1-cancers more frequently display a 'medullary'-like appearance. This is due to a higher mitotic count and lymphocytic infiltrate. BRCA2-related breast cancers generally show fewer mitoses and less tubule formation. For both BRCA1- and BRCA2-related cancers, greater proportions of the tumor show continuous pushing margins. Although a role for BRCA1 and BRCA2 in non-inherited sporadic breast cancer is unclear, protein expression of BRCA1 was found to be reduced in most sporadic advanced (grade III) ductal breast carcinomas.

BRCA1 and BRCA2 as caretakers of the genome. To date, several biological roles for



BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk. Fig. 2 – BRCA1- and BRCA2-related breast cancers are generally of higher grade than age-matched controls. Histologocal sections from 118 breast tumors attributable to BRCA1, and 78 attributable to BRCA2, were evaluated by five histopathologists, all experts in breast disease. Every slide was seen by two pathologists. An age-matched group of 547 apparently sporadic female breast cancer cases served as control. The overall grade of both BRCA1 and BRCA2 breast cancers was significantly higher than that of controls ($p < 0.0001$ and $p < 0.04$, resp.). For BRCA1 breast cancers this was due to higher scores for all three grade indices, whereas for BRCA2 breast cancers the grade was only significantly higher for tubule formation. Data taken from The Breast Cancer Linkage Consortium (1997) Lancet 349: 1505-1510.

BRCA1 and BRCA2 have been demonstrated, and a number of observations indicate that they function in a similar pathway. Both maintain genomic stability through their involvement in homologous recombination, transcription-coupled repair of oxidative DNA damage and double-strand break repair. These roles are suggested by interactions of the Brca1 and/or Brca2 proteins with proteins known to be involved in DNA damage repair, most notably RAD50 and RAD51. Murine embryonic stem cells and mice in which both copies of BRCA1 or BRCA2 have been mutated show a repair deficiency and defects in cell-cycle checkpoints. BRCA1 and BRCA2 play a role in transcriptional regulation, through interactions or complex formation with RNA polymerase II and various transcriptional regulators, although this is presently more firmly established for BRCA1 than for BRCA2. A transcriptional response to DNA damage is well-documented, and identification of downstream targets of BRCA1/2-mediated transcription regulation might help to further understand how BRCA1 and BRCA2 suppress tumor formation. Microarray-based screening of genes regulated by BRCA1 were recently found to fall into two categories, cell-cycle control genes and DNA damage response genes.

Clinical Relevance

When to take the DNA-test?

Diagnosis of gene defects became possible after the identification of BRCA1 and BRCA2 in 1994 and 1995, respectively. In many countries, testing for mutations is being offered to women with a high priori familial risk in Clinical Genetic Centres or multidisciplinary Cancer Family Clinics. A few studies have presented models to determine the prior probability that the counsellee is a BRCA mutation carrier, by combining breast and ovarian cancer family history data with results from comprehensive mutation-testing. These models enable the genetic counselor to decide when a DNA-test is indicated.

Why take the DNA-test?

A clear positive result of the DNA-test, i.e. the presence of a deleterious mutation, is being used to enter these women into early-detection cancer screening programmes or in the decision for or against prophylactic surgery. A woman in which breast cancer has just been diagnosed can benefit from knowledge about gene carrier status, since the risks to the contralateral breast and ovaria must be considered. The treatment of such cancer by lumpectomy will not reduce recurrence risks dramatically, as opposed to complete mastectomy. Healthy women who test positive can take action to prevent cancer developing, although the efficacy of the preventive options currently offered to a woman remains without formal supporting evidence. Chemoprevention is still controversial, and good prospective data on BRCA carriers will probably never become available, given the ethical and clinical difficulties surrounding randomisation. Prophylactic surgery, intuitively the most secure way to reduce breast cancer risk to below population levels, is socially ill-accepted in many parts of the world, and formal proof of its preventive effect in BRCA carriers is also lacking. Clearly, this area is frought with clinical dilemmas.

Interpreting a negative test result

Paradoxically, a negative test result (the absence of a deleterious mutation) presently still has limited power in excluding the presence of a strong susceptibility allele. A negative test result is presently being found in 70 – 80% of all probands tested in most non-Ashkenazi Jewish populations. Among probands with a family history for ovarian cancer, a negative test result is found less frequently (although still in 40 – 60% of the cases). There are several levels of uncertainty.

1. The first is technical: no single mutation-detection method is 100% sensitive, and therefore only exhaustive testing, using a range of different methodologies sensitive to various types of mutation-mechanisms, and investigating the entire coding regions and regula-

tory domains, can detect any changes. This is obviously very cost- and labour-intensive.

2. The second level of uncertainty relates to the interpretation of sequence changes that do not predict a truncated protein. Of the almost 5,000 BRCA1 and BRCA2 mutations submitted to the Breast Cancer Information Core (BIC) database, about one-third are either missense, in-frame deletions or insertions, base-substitutions not leading to an amino acid change (neutral changes) or intronic changes with unknown effect on mRNA-processing (Table). Only a small proportion of these have been unmasked as polymorphisms unrelated to disease outcome. They include missense changes and intronic variants, but, intriguingly, also a nonsense mutation in BRCA2. The K3326X mutation was found in 2.2% of over 400 controls tested. Only a few missense changes (e.g., BRCA1C61G) have been called a deleterious disease-related mutation, mainly because they reside in a validated functional domain of the protein or affect an evolutionary conserved residue. As a result, about 35% of all the distinct gene changes detected to date are lumped into the 'unclassified variant' category, meaning that their relevance to disease outcome is uncertain. Almost certainly, a substantial proportion of these represent rare polymorphisms but equally certainly, a number of them will turn out to be true deleterious mutations.
3. A third reason for a negative test result is that the familial clustering of breast cancer in a family is due to an unknown gene or in fact is a non-genetic chance event. The proportion of truly missed, deleterious mutations is therefore difficult to gauge. A study by the BCLC has suggested that a combination of incomplete testing and missed or misinterpreted gene changes, causes false-negative test results in over 30% of all family types with some evidence of being linked to BRCA1. This proportion was independent of the mutation-screening methodology used.

BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk. Table – Mutation types in BRCA1 and BRCA2 and their predicted effects. The entire Breast Cancer Information Core (BIC) database was down-loaded on March 1, 2000 from http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic. There were 3,086 *BRCA1* mutations and 1,892 *BRCA2* mutations. The total numbers of distinct changes were 724 and 670, respectively.

	BRCA1		BRCA2	
	% of total	% of distinct	% of total	% of distinct
mutation type				
frameshifting	47.1	38.7	33.7	36.5
nonsense	11.3	11.1	11.5	10.2
splice-site	4.4	7.9	2.2	3.6
in-frame del/ins	0.6	1.8	0.4	1.0
missense	28.4	28.4	44.3	35.4
neutral	3.5	3.9	3.1	5.5
intronic change	4.7	8.3	4.9	7.8
mutation effect				
protein truncating	62.6	56.9	41.4	47.9
missense	2.2	1.5	0.7	1.9
neutral polymorphism	11.0	7.2	14.4	13.7
unclassified variant	24.2	34.4	43.4	36.4

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Breast Cancer

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Definition

Breast cancer may originate from more than one cell type in the breast as a result of different subsets of molecular changes. It is therefore a collection of diseases with different characteristics, different risks and different treatments. It occurs predominantly in women but it may also occur in men (0.5% of cases). In addition to invasive disease, several benign pre-malignant and non-invasive forms exist. Although the broad pathological categories are generally accepted there are several alternative systems of sub-categorisation.

- **Benign conditions:** include sclerosing conditions and obliterative mastitis; mild moderate and severe hyperplasias and atypical hyperplasias; fibrocystic conditions, fibroadenomas and related conditions (note that the Oxford Textbook of Pathology presents a 'simplified nomenclature' of fifty subtypes of benign breast disease).
- **Non-invasive carcinoma:** generally divided into Lobular Carcinoma *in situ* (LCIS) and Ductal Carcinoma *in situ* (DCIS). DCIS was originally sub-divided into comedo, cribriform, papillary, solid and clinging but has more recently been categorised as well, moderately and poorly differentiated.
- **Invasive carcinoma:** broadly defined as of 'special type' (30% of cases) and 'no special type' (70% of cases).

Characteristics

Epidemiology

Breast cancer is the most common fatal malignancy in women in the Western world representing about 10% of all cancer deaths. It is however much less common in other countries probably as a result of environmental rather than genetic factors. Behavioral risk factors have been identified. Early pregnancy to term and multiple pregnancy are protective for breast cancer incidence probably due to a reduced exposure to estrogens. There are several reports of weak associations with diet and use of oral contraceptives.

Screening

In some countries mammographic screening is available to women to detect early disease, as it is likely that earlier treatment is beneficial for

survival. Educational programmes are also active, for instance in promoting self-examination as a method of early detection. The value of these approaches for improving patient survival is not yet fully established.

Genetics

About 5 – 10% of breast cancer cases are associated with a genetic predisposition to the disease. Recently three genes have been identified where the inheritance of variants is associated with a very high incidence (penetrance) of the disease. The → *BRCA1* gene was the first to be identified, and gene carriers are thought to have as much as a 70 – 80% chance of developing the disease, generally at an earlier age than women with 'sporadic' (not genetically predisposed) disease. Subsequently a second gene called → *BRCA2* was also found in other families that predisposes to breast and ovarian cancers. The particular risk in an individual gene carrier may be determined by the nature of the particular gene defect and by environmental and hormonal factors and by their background genetic makeup (→ breast cancer genes *BRCA1* and *BRCA2*). A further inherited condition, called → Li-Fraumeni syndrome is associated with increased risk to breast and many other types of cancers. This is due to the inheritance of a rare mutant copy of the → p53 gene.

Molecular biology

Breast cancer is the most studied form of human cancer, due to its common occurrence and to the availability of many immortal breast cancer derived cell lines that can be grown in tissue culture (in contrast to prostate cancer for instance). About 60% of breast cancers at diagnosis express the estrogen receptor. Several genes have been found to be altered by mutation or amplification in invasive cancer and in DCIS. The → *HER-2* or *ERBB2* gene (also known as *c-erbB-2* and *neu*) is amplified in about 25% of invasive breast cancers leading to overexpression of the growth factor receptor which it encodes. The *c-myc* gene is similarly found to be amplified in about 20% of breast cancer also resulting in overexpression of the c-myc protein. The → *cyclin D* gene, which specifies a protein important in regulating the cell cycle, is also amplified in a proportion of breast cancers. The *p53* gene has been found to be point mutated in approximately 20% of invasive breast cancers and to be overexpressed in about 50% of cases. Other point mutations have been found in E-cadherin gene, which encodes a cell adhesion molecule. In this case the mutations are most common in lobular cancers. More subtle changes occur in the expression of apparently normal proteins including growth factors such as those in the epidermal growth factor (EGF) family and the FGF family of proteins, the receptor tyrosine kinase c-erbB-3 and the Src tyrosine kinase. Some of these altered proteins represent targets for new forms of treatments.

Treatment

Three methods of treatment are available, surgery, radiotherapy and chemotherapy/hormonal therapy. Benign disease and lobular carcinoma *in situ* are rarely treated but are observed, as they are associated with an increased risk of developing breast cancer. DCIS of the breast has often been treated by mastectomy as it is frequently quite widespread within the breast but may also be treated by local surgery. It is possible that the different pathologically defined forms of DCIS may be associated with different relative risks of recurrence and recurrence as invasive disease. Invasive disease is generally first treated by surgery, and lymph nodes are sampled to determine, by pathological diagnosis, if there is evidence of tumour spread. This procedure may be limited to a single node (called the sentinel node) or may involve a greater degree of surgery. Patients with invasive breast cancer are often treated with radiotherapy to the breast to reduce the chances of local recurrence. Even if the cancer has not apparently spread, patients are sometimes offered preventative or 'adjuvant' therapy using drugs, as this helps to prevent recurrence of the disease. Chemotherapy or hormonal therapy are generally offered to patients where metastatic spread of the disease has occurred. Hormonal therapy is frequently offered to

women whose tumours express the estrogen receptor. Specific methods of treatment still vary depending on the patient and the institution where it is given, although more generally agreed protocols are becoming accepted.

New treatments

Breast cancer is frequently a hormonally-dependent disease. Thus treatments with drugs such as tamoxifen, which binds to the estrogen receptor and reduces its activity, or other drugs that suppress the production of estrogen, such as aromatase inhibitors, are frequently employed. However, new drugs directed to known molecular changes in the cancer cells are under development. These include signal transduction inhibitors directed to molecules such as the epidermal growth factor receptor, monoclonal antibodies to the c-erbB-2 receptor and drugs which inhibit proteolytic enzymes thought to be involved in the process of metastasis. Several of these are now in clinical trials that will determine their usefulness for the treatment of the disease.

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Breast Cancer Genes BRCA1 and BRCA2

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Definition

→ *BRCA1* and → *BRCA2* are recently identified genes, germline mutations in which confer predisposition to breast, ovarian and other cancers with a high penetrance. The function of their large, nuclear-localised protein products remains uncertain, but they have been implicated in the cellular response to DNA damage and in the regulation of gene transcription. BRCA1 and BRCA2 are highly distinct genes, despite the similarity in their acronyms.

Characteristics

About one-tenth of all → breast cancer cases exhibit a familial pattern of inheritance. Of these familial cases, germline mutations in either one of two genes, *BRCA1* or *BRCA2*, occur in 20 – 60% (that is, in 2-6% of all cases). These mutations are not a feature of non-familial (that is, sporadic) breast cancer.

BRCA1 and *BRCA2* were first identified in 1994-1995 through the analysis of families exhibiting a predisposition to early-onset breast cancer. Founder mutations affecting these genes occur in Iceland and amongst the Ashkenazim, where they confer a highly penetrant risk of breast, ovarian and other cancers (including cancers of the male breast, pancreas and prostate). In other populations, germline *BRCA1* or *BRCA2* mutations are found in the great majority (up to 80%) of families that suffer from multiple occurrences of breast plus ovarian cancer.

The *BRCA1* and *BRCA2* genes have been assigned to human chromosomes 17q and 13q, respectively. In both genes, exon 11 (approximately 3.4 kb in *BRCA1*, or 5 kb in *BRCA2*) encodes a large portion of the protein. Overall, the

murine and human genes are no more than 60% identical at the amino acid level, although small regions exhibit a much higher degree of conservation. Proteins encoded in alternative splice products may exist, but remain to be characterised.

Protein

BRCA1 and *BRCA2* encode large proteins (human BRCA1 is 1,863 amino acids long; and human BRCA2 is 3,418 amino acids) that localise to the nucleus in mitotic and meiotic cells (Fig. 1). They bear little resemblance to proteins of known function. At its N-terminus Brca1 protein contains a RING-domain known to mediate protein-protein interactions. At its C-terminus, BRCA1 includes two copies of a ~95 amino acid motif (the BRCT domain, for BRCA1 C-terminal) later detected in a number of different proteins implicated in DNA repair and cell cycle checkpoint control. This domain, whose atomic structure has been elucidated, also mediates a number of protein-protein interactions.

Eight repeated sequences (the BRC repeats), each of about 30 amino acids, are encoded in *BRCA2* exon 11. The BRC repeats, but not the intervening sequences, are conserved between several mammalian species suggestive of a conserved function. Indeed, the interaction of BRCA2 protein with Rad51, a mammalian homologue of bacterial RecA essential for genetic recombination, occurs through the BRC repeats.

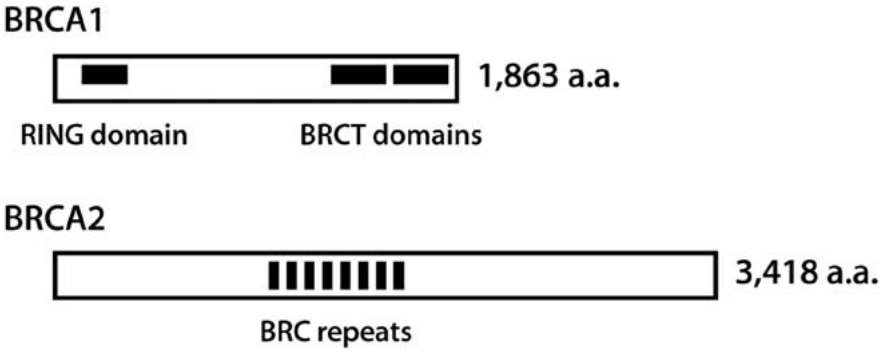
Several structural features of the BRCA1 and BRCA2 proteins - the lack of known catalytic domains, for example, and the presence of multiple sequence motifs implicated in protein-protein interactions - are consistent with the possibility that the molecules serve as scaffolds for the assembly of functional protein complexes with varied cellular roles. Recent progress in defining the biochemistry of proteins associated with BRCA1 and BRCA2 supports this notion.

Cellular and molecular regulation

The transcripts and protein products encoded by *BRCA1* and *BRCA2* are expressed in dividing cells of many types, with maximal levels found during the S-phase of the cell cycle. Expression is also high in meiotic cells. These expression patterns speak to the possible functions of BRCA1 and BRCA2 proteins (Fig. 2):

Role in the cellular response to DNA damage. Both BRCA1 and BRCA2 proteins localise to the nucleus. In meiotic cells, co-localisation has been reported to the synaptonemal complexes of developing axial elements. This is suggestive of a role in meiotic recombination, a process that is initiated by DNA double-strand DNA breakage. Similarly, there is increasing evidence that BRCA1 and BRCA2 are essential in mitotic cells for the repair of DNA double-strand breaks by homologous recombination.

Two major lines of evidence are indicative of such a role:



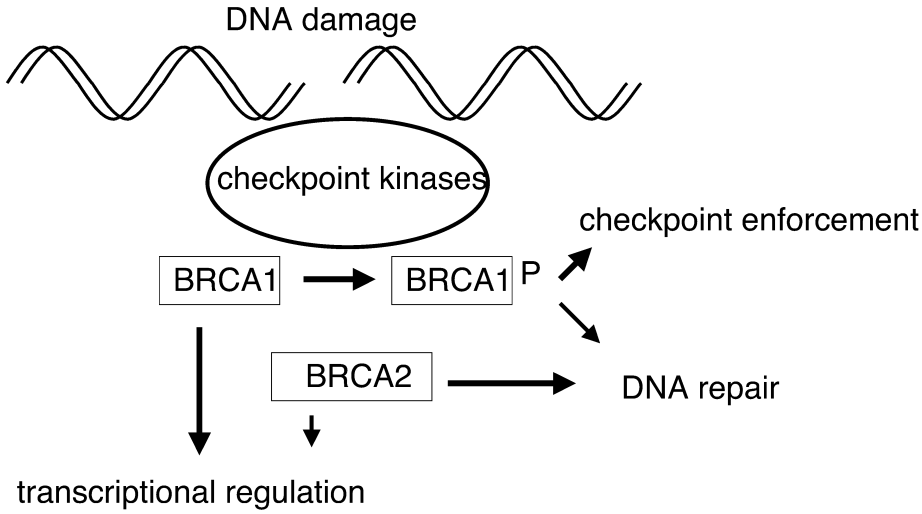
Breast Cancer Genes BRCA1 and BRCA2. Fig. 1 – Structural features of the BRCA1 and BRCA2 proteins (not drawn to scale). Known protein-protein interaction motifs (discussed in the text) are marked.

- First, murine cells in which the *BRCA1* or *BRCA2* homologues have been disrupted by gene targeting exhibit genotoxin hypersensitivity and chromosomal instability suggestive of defects in DNA double-strand break repair.
- Second, homology-directed repair of double-strand DNA breaks introduced into chromosomal substrates is impaired by the disruption of *BRCA1* or *BRCA2*, although pathways for non-homologous end joining remain unaffected.

The precise mechanisms that may underlie such a function remain to be determined. BRCA2 interacts directly, and at a relatively high stoichiometry, with Rad51, a protein essential for DNA repair by recombination. BRCA2 may therefore modulate Rad51 activity or availability. The interaction of BRCA1 with Rad51 is less well defined, although both proteins co-localise - along with BRCA2 - to discrete nuclear foci following DNA damage. BRCA1 may participate in the cellular mechanisms that sense and signal DNA damage, culminating in the activation of cell-cycle checkpoints and the machinery for DNA repair. The protein kinases ATM (encoded by the gene mutated in → Ataxia telangiectasia), ATR, chk1 and chk2 (mutated in → Li-Fraumeni syndrome) are proximal components of these sensing/signalling mechanisms. ATM, chk1 and probably the other checkpoint kinases, phosphorylate BRCA1 following DNA damage, a modification essential for its proper function. These observations are important because they place BRCA1 - and by extension, possibly BRCA2 - in the same pathway as genes such as ATM [→ ATM protein], germline mutations in which are also associated with an increased risk of breast and other cancers. Thus, a common DNA damage response pathway may be defective in a significant fraction of breast cancers.

BRCA1 and BRCA2 have also been implicated in the enforcement of cell cycle checkpoints during the G2 and M phases, and in the regulation of centrosome number. The evidence that these functions are direct, rather than a corollary of defects in proliferation or DNA damage responses, is limited. Nevertheless, it is likely that BRCA1 and Brca2 will be found to play multiple roles in preserving the genetic stability of dividing cells.

Transcriptional regulation. It is difficult to reconcile the disparate nature and severity of



Breast Cancer Genes BRCA1 and BRCA2. Fig. 2 – Possible functions of the BRCA1 and BRCA2 proteins in the biological response to DNA damage. BRCA1 is known to be phosphorylated (BRCA1-P) by checkpoint kinases after DNA damage.

the cellular and developmental defects induced by the disruption of murine homologues of *BRCA1* and *BRCA2*, with functions exclusively in the response to DNA damage. Indeed, evidence is accumulating that BRCA1, in particular, can control gene transcription. Several proteins that interact with BRCA1 are known to regulate transcription or mRNA processing. Moreover, at least a fraction of the total intracellular pool of BRCA1 is linked to the general transcription machinery - the RNA polymerase II holoenzyme - through its RNA helicase subunit. Also consistent with a role in gene transcription, BRCA1-deficient cells exhibit defects in the repair of DNA damage on the transcribed, but not the non-transcribed, DNA strand. Similar functions, albeit based on evidence that is far more limited, have also been attributed to BRCA2.

Tumour suppression by BRCA1 and BRCA2. Inheritance of a single defective copy of *BRCA1* or *BRCA2* confers cancer predisposition in humans. However, the second allele is almost invariably lost in the cancers that arise in predisposed individuals, indicating that *BRCA1* and *BRCA2* behave in some respects as → tumour suppressor genes.

Abnormalities in growth or in the maintenance of genetic stability have not yet been detected in murine or human cells heterozygous for *BRCA1* or *BRCA2* mutations. Thus, there is currently little to suggest that cancer predisposition is related to haplo-insufficiency, or a trans-dominant deleterious effect induced by a single mutant *BRCA1* or *BRCA2* allele. Rather, as has been proposed for other tumour suppressor genes, germline mutations in one allele may simply increase the likelihood that the gene is wholly inactivated by loss of the second allele through somatic mutation.

In this model, inactivation of *BRCA1* or *BRCA2* would initiate genetic instability, allowing the rapid evolution of tumours due to increased somatic mutation in genes that control cell division, death or lifespan. Thus, *BRCA* genes are proposed to work as '→ caretakers' of genetic stability. This 'caretaker' role is likely to arise through the function of the BRCA proteins in DNA repair and chromosome stability. Cells that harbour disruptions in *BRCA1* or *BRCA2* accumulate aberrations in chromosome structure, reminiscent of diseases like Bloom syndrome or → Fanconi anemia, where chromosomal instability is associated with cancer predisposition. This probably increases the frequency of somatic mutations throughout the genome.

It is unclear why carcinogenesis accompanied by loss of the second *BRCA* gene allele in individuals who inherit one mutant allele should occur preferentially in tissues such as the breast or ovaries. Both *BRCA1* and *BRCA2* are widely expressed and appear to perform functions essential to all tissues. Currently there is little evidence to help distinguish between the several possible explanations that can be advanced.

The chronology of the molecular events during carcinogenesis in *BRCA* gene mutation carriers is not known. Loss of the second allele is clearly very frequent, but it is unclear at what stage in tumour evolution this may occur. However, the catastrophic cellular consequences of homozygous inactivation of *BRCA1* or *BRCA2*, which quickly lead to cell death, does emphasise that other genetic alterations will be necessary. Current evidence favours the notion that the inactivation of cell-cycle checkpoint genes, particularly those that enforce mitotic checkpoints, is an important additional step during carcinogenesis in *BRCA* gene mutation carriers.

Clinical relevance

Germline mutations in *BRCA1* or *BRCA2* are frequently associated with familial, early-onset, breast and ovarian cancer, particularly in those families that suffer from multiple cases of cancer in both sites. This has obvious important implications for genetic testing and counselling in the clinic. The mutations have been estimated to carry a cumulative life-time cancer risk of between 40 – 70%.

There is some evidence that the pathological features of breast and ovarian cancers associated with *BRCA1* or *BRCA2* mutations differ from those of sporadic tumours. So far, these differences seem to be insufficiently well-marked to be of diagnostic significance.

It is also unclear if the prognosis of breast and ovarian cancers associated with *BRCA1* or *BRCA2* mutations will differ significantly from that of sporadic cases. Conflicting results have been reported in the literature, their interpretation made difficult by the varied study designs and by the relatively small numbers of cases that have been compared. Similarly, the value of prophylactic interventions, whether surgical or drug-based, in *BRCA* gene mutation carriers awaits evaluation.

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BRMS1

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Definition

BRMS1 (Breast Metastasis Suppressor 1) is a human →metastasis suppressor gene that, when overexpressed, suppresses metastasis of human breast carcinoma cell lines in immunocompromised mouse models.

Characteristics

BRMS1 is located on chromosome 11q13.1-q13.2. It is spread over 8.5 kb and is comprised of 10 exons, the first exon being untranslated. BRMS1 cDNA is 1485 base pairs and encodes a novel protein of 246 amino acids (Mr ~ 28.5 kD). It is fairly conserved across species with it mouse homolog (brms1) having 95% homology at amino acid level. BRMS1 protein sequence contains two nuclear localization sequences, two coiled-coil motifs and imperfect leucine zippers. It also contains an acid rich N-terminus and a potential endoplasmic retention signal. Thus, it shows characteristics of a transcription factor, but this function has yet to be definitively established. BRMS1 expression is not correlated with expression of other known metastasis suppressor genes viz, Nm23, Kai 1, KiSS1 and E-cadherin suggesting its involvement in a novel metastasis suppressor cascade.

Cellular and functional characteristics

BRMS1 shows almost ubiquitous expression in human tissues, with highest expression in kidney, placenta, peripheral blood lymphocytes and testis. Lowest expression is in brain and lung. Subcellular fractionation and immunofluorescence studies have determined that BRMS1 protein is predominantly nuclear. BRMS1 is involved in the establishment of cell-cell communication via gap-junctions, which is evident from the re-establishment of gap junctional intercellular communication in MDA-MB-435 and MDA-MB-231 human breast cancer cell lines. Wound healing studies performed in the same cell lines revealed an inverse effect of BRMS1 expression on cell motility. These results suggest that BRMS1 exerts complex changes on breast cancer cells. Recent studies also show that BRMS suppresses metastasis of human melanoma cells, implying that it may be active in a variety of tumor types

Clinical Relevance

Survival rates and quality of life in breast cancer patients is significantly worse for patients with stage IV (metastatic) disease. Thus, decreased morbidity or mortality depends upon prevention and /or effective treatment of metastatic disease. Karyotypic alterations such as deletions or translocations are frequent in cancerous cells. Among the most common changes (40 – 65% of cases) occurring in breast carcinoma are deletions and amplifications near band 11q13. This stresses the importance of the region encoding or adjacent to BRMS1. Now that BRMS1 has been demonstrated to suppress metastasis in animal models, further studies

will be needed to determine whether this is the only gene involved at that location.

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BSF-2

Definition

B-cell differentiation factor-2 (BSF-2); → interleukin-6.

Burkitt Lymphoma Cell Lines

Definition

Burkitt lymphoma cell lines are EBV-infected B cell [→ Epstein-Barr virus] lines established from Burkitt lymphoma biopsies; these cells are tumorigenic in nude mice.

Bystander Effect

Definition

In → suicide gene therapy the bystander effect is defined as the killing of cells that cannot activate the pro-drug, but are killed when in the presence of the pro-drug and cells, which are capable of activating the pro-drug; → HSV-TK/ganciclovir mediated toxicity.

bZip

Definition

bZip stands for the amino acid sequences of the two independently acting sub-regions of the DNA binding domain: the 'basic domain', rich in basic amino acids that are responsible for contacting the DNA, and the 'leucine-zipper' region characterised by heptad repeats of leucine being part of the well known '4-3 repeats' forming a coiled-coil structure, that is responsible for dimerisation and a pre-requisite for DNA binding; → E-box; → AP-1.

C

C. elegans

Definition

→ *Caenorhabditis elegans*, nematode worm.

CA19-9

Definition

→ Carbohydrate antigen 19-9.

CA125

Definition

CA125 is an antigen often secreted by → ovarian cancer cells, which can be used as an ovarian tumor marker. It is elevated in some cases of endometriosis.

CAAT Box

Definition

The CAAT box is part of a conserved sequence located upstream of the startpoints of eukaryotic transcription units. It is recognized by a large group of transcription factors.

Cachexia

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Definition

Cachexia is a complex metabolic syndrome characterised by marked weight loss together with poor appetite, weakness and often anemia, which is secondary to the growing malignancy.

Characteristics

Most cancer patients develop cachexia at some point during the course of their disease, and nearly one-half of all cancer patients have weight loss at diagnosis. Cachexia causes a poor prognosis: the severity of wasting is inversely correlated with survival. The causes of cancer cachexia syndrome are not fully understood, but it is evidently multifactorial.

Weight loss

The most apparent clinical manifestation of cancer cachexia is weight loss that affects both skeletal muscle (lean tissue) and body fat. Weight loss is not simply caused by competition for nutrients between tumour and host, as the tumour burden may be only 1-2% of total body weight, and patients with even smaller tumours are often markedly cachectic. The frequency of weight loss varies with the type of malignancy, from 31% in non-Hodgkin's lymphoma to 87% in patients with gastric carcinomas. Gastric and pancreatic cancer patients

may lose large amounts of weight, up to 25% of initial weight. Over 15% of weight loss in patients are likely to cause significant impairment of respiratory muscle function, which probably leads to premature death. Weight loss can arise from several metabolic changes that occur during malignancy, for example, reduced food intake, increased energy expenditure and tissue breakdown.

Poor appetite

Loss of the desire to eat or lack of hunger is common in cancer patients, and it can be related to the mechanical effect of the tumour (especially of the upper gastrointestinal tract), side-effects of chemotherapy or radiotherapy and emotional distress. Some tumours may secrete products that act on the brain to inhibit appetite. Regulation of food intake involves the integration of blood-borne and neural signals in the hypothalamus at the base of the brain. Cytokines such as interleukin 1 (IL-1) and tumour necrosis factor-alpha (TNF-α) have been proposed to be involved in cancer-related anorexia, possibly by increasing brain levels of corticotropin-releasing factor (CRF), a hypothalamic neurotransmitter that suppresses food intake at least in rodents, and/or inhibiting neurones that produce neuropeptide Y (NPY), a potent appetite stimulant.

Hypermetabolism and increased energy expenditure

Maintaining normal body weight requires energy intake to equal energy expenditure. In some patients with cancer cachexia, energy balance becomes negative as reduced food intake is not accompanied by a parallel decrease in energy expenditure. Indeed, some patients (for example, with lung and pancreatic cancers) have higher resting energy expenditure (REE) compared with normal control subjects; by contrast, REE is usually normal in patients with gastric and colorectal cancer. The source and mechanisms of increased energy expenditure are not certain, although excessive heat production can be demonstrated in various tissues of rodents with tumours, including their specialised 'brown fat' that is not normally present in adult humans. Cytokines and other tumour products may be responsible in rodents. In humans and animals with large tumour burdens, the metabolism of tumour-derived lactate via 'futile cycles' between the tumour and the host requires large amount of ATP and may generate heat.

Loss of body fat

Fat constitutes 90% of normal adult fuel reserves and depletion of adipose tissue together with hyperlipemia becomes a hallmark of cancer-bearing states. The activity of hormone-sensitive lipase, a rate-limiting enzyme of the lipolytic pathway, is increased in cancer cachectic patients, which results in elevated plasma levels of free fatty acids and triglycerides. Meanwhile, there is a fall in lipoprotein lipase (LPL) activity in white adipose tissue, thus inhibiting cleavage of triglycerides from plasma lipoproteins into glycerol and free fatty acids for storage, causing a net flux of lipid into the circulation. Finally, glucose transport and *de novo* lipogenesis in the tissue are reduced in tumour-bearing state that leads to a decreased lipid deposition.

Various factors produced by tumours (or the host's immune cells, responding to the tumour) can disturb lipid metabolism. TNF-α has been shown to decrease LPL activity in adipocytes, isolated human adipose tissue and experimental animals. TNF-α and IL-1 both are able to inhibit glucose transport in adipocytes and consequently decrease the availability of substrates for lipogenesis. Certain gut and pancreatic tumours secrete a lipid-mobilising factor (LMF) that causes rapid lipolysis *in vitro* and *in vivo*, possibly through activation of intracellular cyclic AMP. Recently, two new proteins have been identified with high homology to uncoupling protein-1 (UCP-1), the heat-producing protein of brown fat, UCP-2 and UCP-3. UCP-2 is expressed ubiquitously, while UCP-3 is expressed predominantly in skeletal muscle in humans and also in brown fat of rodents. These novel proteins have been suggested to provide a mechanism for lipid utilisation, and both UCP-2 and UCP-3 mRNA in

skeletal muscle are upregulated in tumour-bearing animals, which may accelerate lipid catabolism during cachexia.

Loss of muscle protein

Weakness, commonly seen in cancer cachectic patients, is directly related to wasting of muscle that accounts for almost half the body's total protein and bears the brunt of enhanced protein destruction. Reduced protein synthesis together with enhanced proteolysis have been observed in experimental animal models and in muscle biopsies from cancer patients with cachexia, and whole-body protein turnover can be markedly increased in cachectic cancer patients.

Some mediators and pathways of excessive protein breakdown have been incriminated in cancer cachexia. TNF-α appears to be involved, as treatment with recombinant TNF-α enhances proteolysis in rat skeletal muscle and activates the ubiquitin-proteasome system. Ubiquitin, an 8.6 kD peptide, is crucially involved in targeting of proteins undergoing cytosolic ATP-dependent proteolysis. There is an increase in ubiquitin gene expression in rat skeletal muscle after incubation with TNF-α *in vitro*. Recently, a proteolysis-inducing factor (PIF) has been isolated from a cachexia-inducing murine tumour (MAC16); it is also present in the urine of cancer patients with weight loss, but absent from those without weight loss, confirming the specificity to the cachectic state. This 24 kD glycoprotein can induce severe weight loss, attributable to stimulation of proteolysis and a reduction in lean body mass when given to non tumour-bearing mice.

Treatment

Simply increasing calories intake (oral or parenteral) has little effect and, critically, does not restore muscle mass or improve prognosis in cancer patients. Therapeutic approaches including anti-cytokines (thalidomide) and appetite stimulants (megestrol acetate and cyproheptadine) have also failed to reverse the cachexia. Eicosapentaenoic acid (EPA), a polyunsaturated fatty acid from fish oil, has been shown to be effective against the wasting induced by the MAC16 tumour. In randomised clinical trials, cachectic patients with unresectable pancreatic cancer receiving EPA showed a stabilisation in the rate of weight loss, fat and muscle mass as well as the REE.

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CAD

Definition

Caspase-activated DNase (CAD) is a DNase that is responsible for cleaving chromosomal DNA in cells dying by → apoptosis. CAD is complexed with its inhibitor (ICAD) in proliferating cells. Apoptotic stimuli such as death factors, factor-deprivation and anti-cancer activate caspase 3, which cleaves ICAD. CAD, released from ICAD, then degrades chromosomal DNA in the nucleus.

Cadherin

Definition

→ E-cadherin.

Caenorhabditis elegans

Definition

C. elegans is a small (about 1 mm long) anatomically simple nematode worm that is particularly amenable to the analysis of developmental mechanisms. Its advantage is the occurance of cell divisions and placements of cells with high precision. Therefore, the lineage relationships between cells forming the various parts of the body and the total somatic cell number are almost the same in each individual worm. In an adult worm the total number of somatic cells is about 1000. These features allow to follow the development of *C.elegans* cell by cell, (from the egg to the adult worm) by microscopic inspection. A number of developmental *C.elegans* genes have been identified and their homologs play also a role in the development of higher eukaryotes, including humans.

CALI

Definition

→ Chromophore-assisted laser inactivation.

Calpain

Definition

Calpains are a group of protein degrading enzymes (proteases), which include at least 15 members, expressed in different tissues.

cAMP

Definition

Cyclic adenosine 3′,5′-mono-phosphate (cAMP) is a second messenger that is regulated primarily by adenylyl cyclase, and activates protein kinase A.

Camptothecin

Definition

Camptothecin is a chemical class of drugs that inhibits DNA topoisomerase [→ topoisomerase enzymes as drug targets] I. It is also the name of the first compound of this class isolated from the Chinese tree *Camptotheca acuminata*. CPT-11, topotecan and 9-aminocamptothecin belong to this chemical class of compounds.

Canale-Smith Syndrome

Definition

→ Autoimmune lymphoproliferative syndrome.

Cancer

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Definition

Cancer is a deregulated multiplication of cells with the consequence of an abnormal increase of the cell number in particular organs. Initial stages of the developing cancer are usually confined to the organ of origin whereas advanced cancers grow beyond the tissue of origin. Advanced cancers invade the surrounding tissues that are initially connected to the primary cancer. At a later stage, they are distributed via the hematopoetic and lymphatic systems throughout the body where they can colonize in distant tissues and form metastasis. The development of cancers is thought to result from the damage of the cellular genome, either due to random endogenous mechanisms or caused by environmental influences.

The origin of cancers can be traced back to alterations of cellular genes. Genetic damage can be of different sorts:

- recessive mutations in → tumor suppressor genes,
- dominant mutations of → oncogenes,
- loss-of-function mutations in genes, involved in maintaining genomic stability and → repair of DNA (resulting in → genomic instability).

Characteristics

A large proportion of genetic changes appears to arise by mechanisms endogenous to the cell, such as by errors occuring during the replication of the approximately $3x10^9$ base pairs present in the human genome. Environmental factors have a major role as well, predominantly as:

- chemical carcinogens (e.g. aflatoxin B1 in liver cancer [→ liver cancer, molecular biology], tobacco smoke in lung cancer; → tobacco carcinogenesis),
- radiation,
- viruses (such as → hepatitis B virus in liver cancer or → human papillomavirus in cervical cancer).

Types of genetic damage

Damage to oncogenes and tumor suppressor genes can be of different sorts:

- Point mutations resulting in the activation of a latent oncogenic potential of a cellular gene (e.g. → *RAS*) or in the functional inactivation of a tumor suppressor gene by generating an intragenic stop codon that leads to
  a) premature translation termination with the consequence of an incomplete truncated protein (e.g. → p53) or
  b) the failure for maintaining genomic stability (→ mismatch repair genes in → HNPCC).
- → Amplification leading to an increase of the gene copy number beyond the two alleles normally present in the cell (copy number can reach 500 and more; example: → *MYCN* in human → neuroblastoma).
- Translocation, which is defined as an illegitimate recombination between non-homologous chromosomes, the result being either a fusion protein (where recombination occurs between two different genes such as *BCR-ABL* in CML) or in the disruption of normal gene regulation (where the regulatory region of a cellular gene is perturbed by the introduction of the distant genetic material such as → *MYC* in Burkitt lymphoma [→ Epstein-Barr virus]).
- Viral insertion by the integration of viral DNA into the regulatory region of a cellular gene. This integration can occur after a virus has infected a cell. Viral insertion is well documented in animal tumors (HBV integration in the vicinity of → *MYCN* in liver cancer in experimental animals; → liver cancer, molecular biology).

Cellular aspects

Cancer in solid tissues (solid cancer) usually develops over long periods (often 20 to 30 years latency period) of time. An exception are solid cancers (such as → neuroblastoma) in children, which often are dignosed shortly after birth. Malignant cancers are characterized by their ability to develop → metastasis (i.e. secondary cancers at distance from the primary tumor), often they also show multidrug resistance, which means that they hardly react to conventional chemotherapy. It is thought that the development of a normal cell to a metastatic cell is a continuous process driven by genetic damage and genomic instability, with the progressive selection of cells that have acquired a selective advantage within the particular tissue environment (→ multistep development). Studies of colorectal cancers have identified 6 – 7 genetic events required for the conversion of a normal cell to a cell with metatstatic ability. This is in contrast to leukemias, which usually require one genetic event, most often a translocation, for disease development.

Sporadic *versus* familial cancer

The vast majority of cancers are 'sporadic', which simply means that they develop in an individual. Descendants of this individual do not have an increased risk because the cellular changes that have resulted in cancer development are confined to this individual. In con-

trast, approximately 10% of cancer cases have a hereditary background, they show familial clustering (prominent examples include retinoblastoma [→ retinoblastoma, cancer genetics], → breast cancer, FAP [→ APC gene in Familial Adenomatous Polyposis] and → HNPCC as familial forms of colorectal cancer [→ colon cancer], → melanoma). The genetic basis for familial cancers mutations in the germ line have been identified affecting the corresponding genes. These germ line mutations do not always dictate cancer development, although they are considered 'strong' hereditary determinants. They represent susceptibility genes that confer a high risk for cancer development to the gene carrier. The relative risk of the individual carrying the mutant gene can vary considerably. For instance, the risk of carriers of one of the breast cancer susceptibility genes → *BRCA1* or → *BRCA2* for breast cancer development can vary between approximately 60% to 90%. In reality this means that the risk for cancer development is difficult to predict, and individuals may not develop cancer at all in spite of the presence of a mutated gene in their germ line. The molecular basis for the differences in risk are unknown. Formally the activity of modifying factors, either environmental or genetic, has been suggested. Such modifying factors appear to be less important for some other familial cancers, such as retinoblastoma, where the risk is constant between 90% to 95% for gene carriers.

Polygenic determinants of risk

The relative risk of the individual for cancer development can also be determined by so called 'weak' genetic factors. Normal cells contain a number of genes involved in → detoxification reactions. Different allelic variants of these genes exist in the human population that encode proteins with slightly different enzymatic activities. Although the exact contribution of individual allelic variants to cancer development is difficult to assess, it is reasonable to assume that individuals that have inherited 'weak' enzymatic activities in different detoxification systems are likely to have a higher risk. It is likely, therefore, that the risk for such cancers is 'polygenic'.

Cancer Chemoprevention

Definition

Cancer chemoprevention is the use of a chemical agent to either prevent the initiation of carcinogenesis or to reverse/halt the progression of neoplastic disease.

Cancer Vaccines

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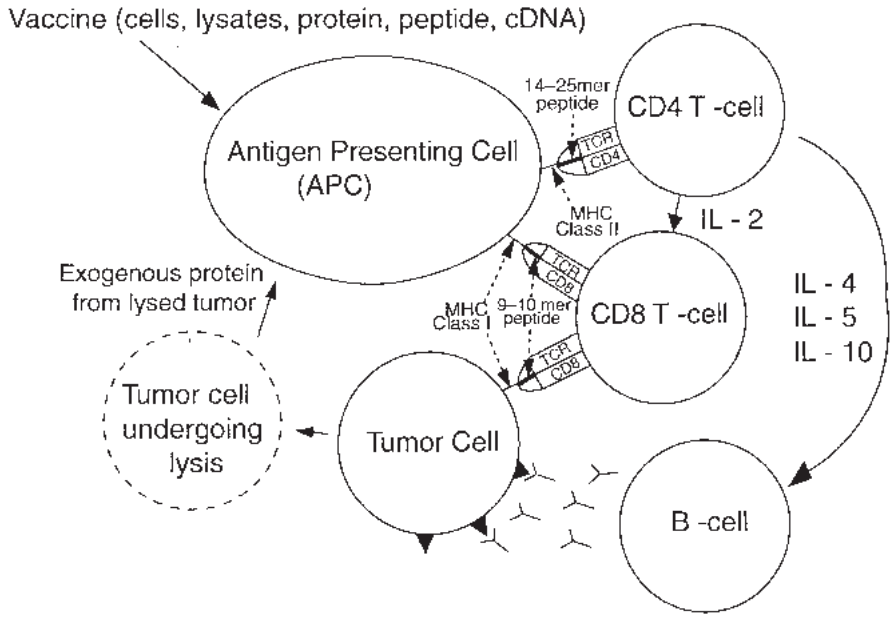
Definition

A vaccine should activate a unique lymphocyte (B and/or T cell) response, which has an immediate anti-tumor effect as well as memory response against future tumor challenge (Fig.). The primary role of a cancer vaccine is the treatment of cancer or in prevention of recurrence in a patient with surgically resected cancer, rather than 'prevention' of cancer in a person who has never had cancer. Therefore, cancer vaccines are not thought of in the traditional sense of vaccines that are used for infectious diseases. If the current cancer vaccines prove to be useful in the above repects, then they may have a future role in preventing cancer in persons who have never had cancer but are at high-risk for a particular type of cancer.

Characteristics

The first and most obvious types of vaccines are prepared from autologous or allogeneic tumor cells. Alternatively, membrane preparations from tumor cells may be used. In some instances, tumor cell vaccines have been combined with cytokines such as granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-2 (IL-2). More recently, with advances in molecular biological approaches, gene modified-tumor cells expressing



Cancer Vaccines. Fig. – T-Cell Activation. T-cells recognize antigens as fragments of proteins (peptides) presented with major histocompatibility complex (MHC) molecules on the surface of cells. The antigen presenting cell processes exogenous protein from the vaccine or from the lysed tumor cell in to a peptide, and presents the 14/25 mer peptide to CD4 helper-T-cells on a class II molecule. There is also data that suggests that exogenous proteins can be processed into 9/10 mer peptides that may be presented on MHC class I molecules to CD8 cytotoxic T-cells. Activated Th1 CD4 helper T-cells secrete Th1 cytokines such as IL-2 that upregulate CD8 cytotoxic T-cells. Activated Th2 CD4 helper T-cells secrete Th2 cytokines such as IL-4, IL-5 and IL-10 that activate B cells.

antigens designed to increase the immune response, or gene modified to secrete cytokines have been an additional tool used in vaccination. In addition, increase in our knowledge of tumor associated antigens (TAA) have led to the use of purified TAAs, DNA-encoding protein antigens, and/or protein derived peptides. All of these approaches are currently being tested in the clinic.

Mechanistically, the ultimate aim of a vaccine is to activate a component of the immune system such as B lymphocytes, which produce antibodies or T lymphocytes, which directly kill tumor cells. Antibodies must recognize antigens in the native protein state on the cell's surface. Once bound, these molecules can mediate antibody-dependent cellular cytotoxicity or complement-mediated cytotoxicity, both mechanisms which are capable of destroying tumor cells. T lymphocytes, on the other hand, recognize proteins as fragments or peptides that vary in size, presented in the context of major histocompatibility (MHC) antigens on the surface of the cells recognized (Fig.). The proteins from which the peptides are derived may be cell surface or cytoplasmic proteins. MHC antigens are highly polymorphic, and different alleles have distinct peptide binding capabilities. The sequencing of peptides derived from MHC molecules have led to the discovery of allele-specific motifs that correspond to anchor residues that fit into specific pockets on MHC class I or II molecules.

T lymphocytes

There are two types of T lymphocytes, helper T lymphocytes and cytotoxic T lymphocytes (CTL) that recognize antigens through a specific T cell receptor (TCR) in close conjunction to the CD3 molecule, which is responsible for signaling. CD4-helper T cells secrete cytokines and lymphokines that enhance immunoglobulin production as well as activate CD8-CTLs.

CD4-helper T cells are activated by binding via their TCR to class II molecules that contain 14-25 amino acid (mer) peptides in their antigen-binding cleft. Extracellular proteins are endocytosed and degraded into 14-25 mer peptides and bind to newly synthesized MHC class II molecules. The MHC peptide complex is transported to the cell membrane, where it can be recognized by specific CD4-helper T cells. In most cases, the MHC class II antigen containing peptide is presented to the CD4-helper T cells by a specialized cell called an antigen presenting cell (APC). More specifically, a variety of cells are capable of processing and presenting exogenous antigen including B cells, monocytes, macrophages and the bone marrow derived dendritic cells (DC). DCs are the most efficient APCs and express high levels of MHC class I and II molecules, co-stimulatory molecules such as CD80 and CD86, and specific markers such as CD83. After antigen uptake DCs migrate peripherally to lymph nodes where antigen presentation to CD4-helper T cells takes place.

Helper T cells

There are two types of CD4-helper T cells capable of generating either antibody or cell-mediated immune responses, based on the type of signaling they receive. Th1 CD4-helper T cells stimulate cell-mediated immunity by activating CTLs through the release of lymphocytokines such as IL-2. Th2 CD4-helper T cells mediate an antibody response through the release of lymphocytokines such as IL-4 and IL-10.

The CD8-positive CTLs are activated in most cases by peptides derived from intracellular proteins that are cleaved to 9-10 mer-peptides and the cytosol of tumor cells or APCs. The peptides are then transported via specialized transporter molecules to the endoplasmic reticulum, where they become associated with newly synthesized MHC class I molecules. The complex is then transported to the cell surface membrane where it is recognized by CD8-CTL via a specific TCR.

Tumor cells

The most straightforward means of immunization is the use of whole tumor cell preparations (either autologous or allogeneic tumor cells). The advantage of this approach is that the potential TAAs are presented to the immune system for processing and presentation to the appropriate T cell precursors. The difficulty with this approach lies in the availability of fresh autologous tumor material and the sparcity of well-characterized long-term tumor cell lines. Regardless, whole tumor cell vaccines have been an area of intense interest. A variety of trials using autologous tumors for colon cancer and malignant melanoma have been reported. In one trial, freshly thawed autologous colon cancer cells were inactivated with radiation, mixed with → BCG (bacille Calmette-Guerin) and injected into patients who had their primary colon cancer resected but were at risk for recurrence. This study did reveal disease-free survival and overall survival trends in favor of the vaccine arm. In a melanoma study, autologous tumor cells were mixed with dinitrophenyl (DNP) and mixed with BCG. Promising results were reported for patients with metastatic disease and for patients with locally resected melanoma.

The weakness of autologous cell vaccines can be overcome with the allogeneic approach: First an allogeneic vaccine is generic and developed from cell lines selected to provide multiple TAAs and a broad range of HLA expression. Second, allogeneic cells are more immunogeneic than autologous cells. Third there is no requirement to obtain tumor tissue by surgical resection for a prolonged course of immunotherapy.

A polyvalent melanoma cell vaccine called CancerVax developed for allogeneic viable melanoma cell lines has demonstrated promising results for patients with resected metastatic disease and for resected local disease. Randomized phase III studies are ongoing in the United States comparing CancerVax plus BCG versus BCG for patients with stage III melanoma.

Another variation of cell vaccines is using ‘shed’ antigen vaccines. These are vaccines that are prepared from the material shed by vi-

able tumor cells into culture medium. The potential advantage is that it contains a broad range of antigens expressed on the surface of melanoma cells and the shed antigens are partially purified. Trials of such vaccines in melanoma patients have demonstrated specific humoral and cellular immune responses in patients and promising early clinical results.

Another approach to tumor cell vaccines is the introduction of foreign genes encoding cytokines such as IL-2 and GM-CSF into tumor cells. Alternatively, molecules designed to increase the immunogenicity of the tumor cell such as CD80 and CD86. Gene transfer can be accomplished by transfection of plasmid constructs (electroporation) or transduction using a viral vehicle such as a retrovirus or an adenovirus. Another option tested for gene transfer is physical gene delivery in which a plasmid or 'naked' DNA is delivered directly into tumor cells. There are a number of mechanisms to carry this out including liposomes as gene carriers, use of a 'gene gun', electroporation and calcium phosphate-mediated gene transfer. In one phase I trial, 21 patients with metastatic melanoma were vaccinated with irradiated autologous melanoma cells engineered to secrete human GM-CSF. Metastatic lesions resected after vaccination were densely infiltrated with T lymphocytes and plasma cells and showed extensive tumor destruction.

Peptides and carbohydrates

An advantage to peptide vaccines is that they can be synthetically generated in a reproducible fashion. The major disadvantage is that they are restricted to a single HLA molecule and are not of themselves very immunogenic. To increase their immunogenicity, peptides may be injected with adjuvants, cytokines or liposomes or presented on DCs. Whole proteins have the advantage over peptides in that they can be processed for a wider range of MHC class I and II antigens.

Mucins such as MUC I are heavily glycosylated high molecular weight proteins abundantly expressed on human cancers of epithelial origin. The MUC I gene is over-expressed and aberrantly glycosylated in a variety of cancers including colorectal cancer. MUC 1 is being widely used as a focus for vaccine development.

Using expression-cloning techniques, several groups have cloned the genes encoding melanoma antigens recognized by T cells and have identified the immunogenic epitopes presented on HLA molecules. Ten different melanoma antigens have been identified. Direct immunization using the immunodominant peptides from the tumor antigens or recombinant viruses such as adenovirus, fowlpox and vaccinia virus encoding the relevant genes have been pursued to immunize patients with advanced melanoma. Initial results have demonstrated increased anti-tumor T cell reactivity in patients receiving peptide immunization. Immunization in melanoma patients with melanoma antigens have been reported. One study showed that immunization of melanoma patients with MAGE-1 peptide pulsed on DCs induced melanoma-reactive and peptide specific CTL responses at the vaccination sites and at distant tumor deposits. Administration of the gp-100 molecule in conjunction with high-dose bolus IL-2 to 31 patients with metastatic melanoma revealed an objective response of 42%. This is compared with the typical response of high-dose systemic IL-2 without peptide of only 15%. Based on these data, a randomized trial was initiated to compare the peptide vaccine plus IL-2 versus IL-2 alone in metastatic melanoma patients.

Immunization against tumor-associated carbohydrate antigens has also been attempted. Carbohydrate antigens typically bypass T cell help for B cell activation. Investigators demonstrated that some carbohydrates may activate an alternative T cell pathway. Vaccine studies have been reported using the GM-2 ganglioside vaccine. Patients were pretreated with low dose cyclophosphamide. After a minimum follow up of 72 months, there was a 23% increase in disease-free interval and a 17% increase in overall survival in patients who produced antibody against GM-2. This suggested a benefit to the GM-2 ganglioside vaccine which has led to a current phase III trial.

Recombinant vaccines expressing tumor antigens

The carcinoembryonic antigen (CEA) is highly expressed on colorectal cancer and on a variety of other epithelial tumors, and is thought to be involved in cell-cell interactions. A recombinant vaccinia virus expressing human CEA (rV-CEA) stimulates specific T cell responses in patients. This was the first vaccine to demonstrate human CTL responses to specific CEA epitopes and class I HLA-2 restricted T cell mediated lysis, and demonstrated the ability of human tumor cells to endogenously process CEA to present a specific CEA peptide in the context of a MHC for T-cell mediated lysis.

Anti-idiotype vaccines

The idiotype network offers an elegant approach to transforming epitope structures into idiotypic determinants expressed on the surface of antibodies. According to the network concept, immunization with a given TAA will generate production of antibodies against these TAA, which are termed Ab1; the Ab1 is then used to generate a series of anti-idiotype antibodies against the Ab1, termed Ab2. Some of these Ab2 molecules can effectively mimic the three-dimensional structure of the TAA identified by the Ab1. These Ab2 can induce specific immune responses similar to those induced by the original TAA and therefore can be used as surrogate TAAs. Immunization with Ab2 can lead to the generation of anti-anti-idiotypic antibodies (Ab3) that recognize the corresponding original tumor-associated antigen identified by Ab1. The anti-idiotype antibody represents an exogenous protein that should be endocytosed by APCs and degraded to 14-25 mer peptides to be presented by class II antigens to activate CD4-helper T cells. Activated Th2 CD4-helper T cells secrete cytokines such as IL-4 that stimulate B cells that have been directly activated by Ab2 to produce antibody that binds to the original antigen identified by Ab1. In addition, activation of Th1 CD4-helper T cells secrete cytokines that activate T cells, macrophages and natural killer cells that directly lyse tumor cells, and in addition, contribute to ADCC. Th1 cytokines such IL-2 also contribute to the activation of a CD8-CTL response. This represents a putative pathway of endocytosed anti-idiotype antibody. The anti-idiotype antibody may be degraded to 9/10 mer peptides to present in the context of class I antigens to activate CD8-cytotoxic T cells, which are also stimulated by IL-2 from Th1 CD4-helper T cells.

Several anti-idiotype antibodies that mimic TAAs on colorectal cancer cells and melanoma cells have been reported. One of these anti-idiotype antibodies, CeaVac, is an anti-idiotype murine monoclonal antibody that mimics CEA. Among 23 patients with advanced colorectal cancer, 17 generated an anti-anti-idiotypic Ab3 response and 13 of these responses were proven to be true anti-CEA responses. Median survival for the 23 evaluable patients was 11.3 months with 44% one year survival. Toxicity was limited to local swelling and minimal pain. The overall survival of 11.3 months was comparable with other phase II data in which advanced colorectal cancer patients were treated with a variety of chemotherapy agents. Thirty-two patients with resected colorectal cancer were randomized to treatment with CeaVac. All 32 of these patients generated high titer polyclonal anti-CEA responses that mediated ADCC. The predominant Ab3 immunoglobulin was IgG and the major subclasses were IgG1 and IgG4. All patients generated idiotypic-specific T cell responses, and 75% were CEA specific. These data demonstrated that 5-fluorouracil (5-FU) based chemotherapy regimens do not adversely affect the immune response to CeaVac. In addition, high titer anti-CEA immunoglobulin and Th1 helper cell responses can be maintained indefinitely with monthly boosts of CeaVac. Injections were well-tolerated with only minor local reactions and minimal systemic side effects. Although longer follow up is required, there appeared to be a biological effect in tumor progression suggested by the 17 patients with resected and incompletely resected metastatic colon cancer who continued on study from 6 to 29 months. This vaccine is to be studied in resected Dukes' C colon cancer patients in a prospective randomized phase III trial.

Conclusion

There exist several promising immunologic approaches to vaccine therapy of cancer. The challenge of immunotherapy research is to determine which combination of approaches leads to a favorable clinical response and outcome. Several studies have shown enhanced survival of patients receiving vaccines; however, a randomized phase III clinical trial has yet to show a statistically significant improvement in the survival of such patients.

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Cap

Definition

A cap is a structure at the 5′ end of eukaryotic mRNA, introduced after transcription by linking the terminal phosphate of 5′-GTP to the terminal base of the mRNA. The added G (and sometimes some other bases) are methylated, giving a structure of the form 7MeG5′ppp5′Np.

Carbohydrate Antigen 19-9

Definition

Carbohydrate antigen 19-9 is a cell surface antigen consisting of a sialylated lacto-N-fucopentose that is frequently found in increased levels in the serum of patients with various types of malignancy.

Carcinoembryonic Antigen

Definition

Carcinoembryonic antigen (CEA) is a cell surface glycoprotein detectable immunohistochemically in limited amounts in benign cells and more heavily expressed in fetal and some types of neoplastic cells.

Carcinogen

Definition

A carcinogen is any agent, chemical, physical or viral that causes cancer or increases the incidence of cancer.

Carcinogen Macromolecular Adducts

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Definition

Carcinogen-macromolecular adducts are chemical modifications (‘addition products’) of nucleic acids and proteins that form in tissues and cells exposed to reactive chemical species.

Characteristics

Endogenous and exogenous agents produce macromolecular adducts

Chemical carcinogens that induce macromolecular damage may be endogenous or exogenous. The endogenous formation of → reactive oxygen species and other free radicals may cause both appropriate and inappropriate chemical modification of nucleic acids. Exogenous chemicals, including environmental pollutants or

drugs, require activation to reactive species through metabolism. Following covalent binding of reactive chemical species to DNA, a mutation can result if the DNA is not correctly repaired. When reactive chemical species bind covalently to a protein, the resulting adducts persist for the lifetime of the protein. Formation of → DNA adducts is considered necessary for carcinogenesis, while protein modification is considered an indicator of exposure and a surrogate for DNA adduct formation.

Some normal physiological (endogenous) processes result in chemical modification of nucleic acids. For example, selective 5-methylation of cytosine in DNA regulates normal gene expression, and 7-methylation of guanosine in 5′ cap structures of mRNA is necessary for efficient protein synthesis in eukaryotes. Normal endogenous metabolic processes, including lipid peroxidation, nitric oxide metabolism and endogenous nitrosation can produce oxygen free radicals, oxidative-DNA adducts, etheno-adducts and nitrosamine adducts.

Exogenous carcinogenic agents that form macromolecular adducts can be direct-acting if they are highly reactive. Examples are the nitrosoureas, some nitrosamines, ethylene oxide and ozone. However, most are inert, like the polycyclic aromatic hydrocarbons (PAHs), and require metabolic activation. Exogenous carcinogens include some plant and fungal products (aflatoxins, ochratoxins, hydrazines), pyrolysis products from cooking (heterocyclic amines, PAHs), industrial combustion products (aromatic amines, PAHs, nitro-PAHs, benzene, vinyl chloride, nitrosamines, ethylene oxide), urban pollution contaminants (PAHs, nitro-PAHs, aromatic amines) and contents of tobacco smoke (PAHs, nitosamines and aromatic amines).

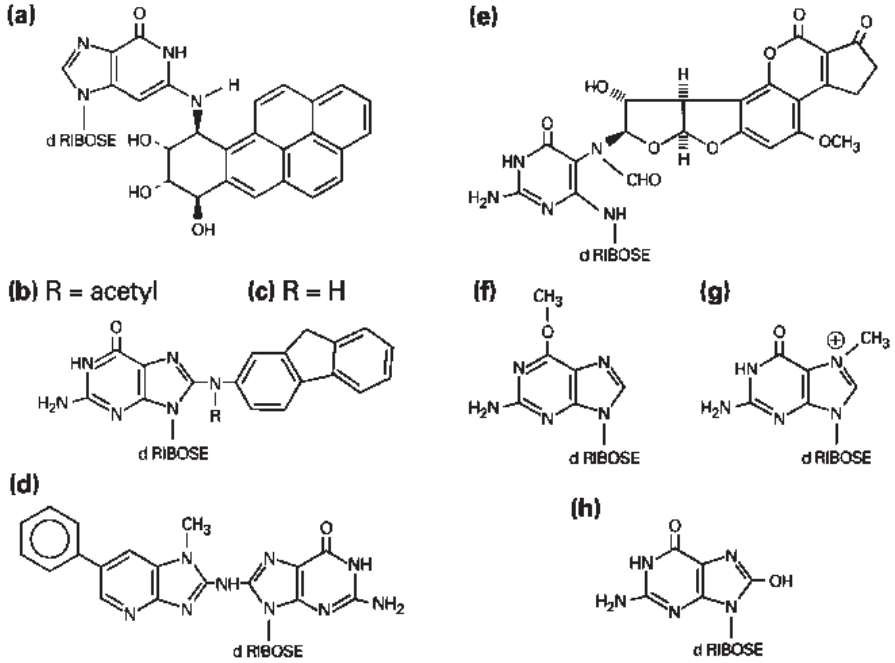
Carcinogen metabolism leading to macromolecular adduct formation

In order to form macromolecular adducts, exogenous agents that can be inhaled, ingested or absorbed through the skin, are altered metabolically by families of enzymes. These enzymes convert a small fraction of the initial dose to highly reactive intermediate metabolites that become bound covalently to specific bases in DNA or amino acids in protein.

Polycyclic aromatic hydrocarbons, such as benzo[a]pyrene (BP) are composed of variable numbers of fused benzene rings and are chemically unreactive and insoluble in water. These compounds are ubiquitous environmental contaminants, contained in cigarette smoke and produced by many industrial processes (→ tobacco carcinogenesis). To form macromolecular adducts in the body they are metabolized to simple epoxides by cytochrome P450, hydrated through the action of epoxide hydrolase and subjected to epoxidation (cytochrome P450) to form unstable dihydrodiol-epoxides. The unstable metabolites spontaneously convert to a positively charged, highly reactive free radical called a carbocation (the ultimate carcinogen), which binds covalently to DNA and protein. The structure of the major BP DNA adduct with deoxyguanosine is shown in Fig. 1a, and this compound also forms adducts with protein.

Aromatic amines are characterized by the presence of benzene rings and an exocyclic nitrogen. A prototypical aromatic amine, 4-aminobiphenyl (4-ABP), is implicated in human bladder cancer. In addition, another class of environmental contaminants, nitrated polycyclic aromatic hydrocarbons are related to aromatic amines by nitroreduction. The presence of the amino-group, which can be either acetylated or non-acetylated, contributes to the complexity of aromatic amine metabolism. Activation of aromatic amines proceeds by N-oxidation with sulfotransferase catalysis that results in the formation of acetylated and non-acetylated guanine adducts as shown in Fig. 1(b and c) for the carcinogen N-2-acetylaminofluorene. Aromatic amines also readily form adducts with protein.

Heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PHIP), are formed from the pyrolysis ($>150^{\circ}C$) of amino acids, creatinine and glucose that occurs during cooking. They have a similar structure to aromatic amines and undergo N-hydroxylation (cytochrome P450) and enzymic O-esterification. The major guanine adduct of PHIP is shown in Fig. 1d.



Carcinogen Macromolecular Adducts. Fig. – DNA Adduct Structures.

a) (7R)-N^2-{10-[r-7, t8, t-9-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene]-yl}-deoxyguanosine
b) N-deoxyguanosin-(8-yl)-2-acetylaminofluorene
c) N-deoxyguanosin-(8-yl)-2-aminofluorene
d) 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
e) aflatoxin B_1-N^7-deoxyguanosine
f) O^6-methyl-deoxyguanosine
g) N^7-methyl-deoxyguanosine
h) 8-hydroxydeoxyguanosine

Fungal micotoxins, like aflatoxin B1 derived from *Aspergillus flavus*, contaminate cereals, grain and nuts. Aflatoxin B1 ingestion is correlated with a high incidence of liver cancer. Aflatoxins are hetercyclic and contain several endocyclic oxygen molecules. They are activated by simple epoxidation (cytochrome P450) across the olefinic double bond at the 8,9-position giving rise to a carbocation. However, some addition products with DNA are unstable and lead to non-mutagenic depurination. The major → aflatoxin-guanine adduct is shown in Fig. 1e. Serum and albumin adducts of aflatoxin have been characterized and used frequently as human biomarkers.

Carcinogenic N-nitrosamines can be found in many substances including food, alcoholic beverages and tobacco. N-nitrosodimethylamine is activated (cytochrome P450) through α-hydroxylation to form an unstable α-hydroxy-nitrosamine, which forms formaldehyde and methyl diazohydroxide. The methyl diazohydroxide becomes a free radical and powerful methylating agent, which produces multiple DNA modifications (Fig. 1 f and g). Some nitrosamines such as the tobacco-specific nitrosamine, 4-[methylnitrosoamino]-1-[3-pyridyl]-1-butanone (NNK), are assymetrical and give rise to the formation of bulky DNA adducts. Nitrosamines are also known to form protein adducts.

Oxygen radical damage produced by both endogenous and exogenous events can result in the formation of macromolecular adducts. Pathways that lead to the formation of oxygen radicals include degradation of organic perox-

ides (catechol, hydroquinone, and 4-nitroquinoline-N-oxide), hydrogen peroxide, lipid peroxidation and the catalytic cycling of some enzymes. Treatment with certain drugs or exposure to plasticizers can stimulate peroxisome proliferation, also giving rise to oxyradicals. Exposure to tumor promoters indirectly increases oxyradical formation, for example through the action of phorbol esters, mediated by protein kinase C, and in inflammation mediated by nitric oxide. Oxygen free radicals produce multiple DNA adducts including 8-hydroxydeoxyguanosine (Fig. 1 h).

Stuctural modification of DNA to form carcinogen-DNA adducts

The structure of DNA can be modified through various mechanisms. These include oxidation, alkylation, dimerization, deamination and reaction with large, bulky aromatic-type carbocations. Endogenous and exogenous pathways lead to the formation of oxygen free radicals and the formation of oxidative DNA damage. Examples of oxidative DNA adducts include thymine glycol, 8-hydroxydeoxyguanosine, uracil glycol, 5-hydroxyuracil, 5-hydroxy-methyluracil and 6-hydroxy-5,6-dihydrocytidine.

Alkyl-radicals form during the metabolic activation of certain N-nitrosamines or spontaneously in the case of N-alkylureas (N-methyl-N-nitrosourea) or N-nitrosoguanidines. Protonated alkyl-functional groups, which become available to modify DNA, attack nucleophilic centers. There are ten of these: N1, N3, and N7 of adenine; N3 of cytosine; N2, O6 and N7 of guanosine; O2, N3, and O4 of thymidine. Repair of some of these lesions is correlated with mutagenicity. For example, O^6-methyldeoxyguanosine (Fig. 1f) can be repaired and is a promutagenic lesion, whereas N^7-methyldeoxyguanosine (Fig. 1g) is neither repaired nor mutagenic.

Larger, bulky aromatic-type adducts bind to DNA producing three-dimensional structures that reside either in the minor or the major groove of the DNA helix. Activated BP binds preferentially to the exocyclic (N2) amino group of deoxyguanosine (Fig. 1a). While guanine is a preferred site for PAH modification, covalent binding to deoxyadenosine and deoxycytosine are also possible. Aromatic amines form adducts at the C8, N2, and O6 positions of deoxyguanosine and deoxyadenosine but the major aromatic amine adducts form at the C8 of deoxyguanosine (Fig. 1b and c). Evidence suggests that activation of aflatoxin B1 produces adduction primarily at the N7-position of deoxyguanosine (Fig. 1e).

Methods to measure carcinogen-DNA adducts

Single methods currently in use for carcinogen-DNA adduct detection include radiolabeling, immunoassays, immunohistochemistry, ^{32}P-postlabeling, fluorescence and phosphorescence spectroscopy, mass spectrometry, atomic absorbance spectrometry and electrochemical conductance. Single methods are typically not able to chemically characterize specific adducts in human tissues, although they work well for animal models exposed to one agent. Using human samples, greater success in DNA adduct characterization has typically been obtained by combining preparative methods (immunoaffinity chromatography, high performance liquid chromatography or gas chromatography) with immunoassays, ^{32}P-postlabeling, synchronous fluorescence spectrometry or mass spectrometry. These assays are typically able to detect as little as 1 adduct in 10^9 nucleotides using ~5-100 μg of DNA. A relatively recent method, accelerator mass spectrometry, can detect 1 adduct in 10^{12} nucleotides but requires administration of exceedingly low levels of radioactively-labeled compounds.

Importance of DNA adducts in chemical carcinogenesis

The presence of a DNA adduct in a critical gene provides the potential for occurrence of a mutagenic event, resulting in subsequent alterations in gene expression and a loss of growth control. A substantial period of time is required for a tumor to become evident, and DNA damage is considered to be necessary but not sufficient for tumorigenesis since other events, such as mutagenesis and cell proliferation, must also take place. DNA adduct levels, mea-

sured at any point in time, reflect tissue-specific rates of adduct formation and removal, which depend upon carcinogen activation, DNA repair, adduct instability and tissue turnover. In experimental models dose-response associations have been observed for DNA adduct formation, mutagenesis, and tumorigenesis induced by chemicals, while reductions in DNA adduct levels have been associated with chemoprevention. However, some adducts are highly-mutagenic and associated with carcinogenesis, while others are not. Studies in animal models have demonstrated an association between mutation 'hot-spots' in proto-oncogenes and tumor suppressor genes, and specific adducts. Mutations considered potentially carcinogen-specific have been observed in p53, ras, and other reporter genes in humans. Molecular epidemiologic studies involving DNA adduct measurements have the potential to elucidate the role of DNA adduct formation in human cancer risk.

Structural modification of proteins to form carcinogen-protein adducts

In the same way that DNA can be modified by reactive chemical species, endogenous or exogenous carcinogens can become bound to proteins after direct interaction or metabolic activation. Typically, a reactive cation binds covalently at a nucleophilic amino acid. Alkylating agents most commonly attack the amino acid cysteine, however, aspartate, histidine, valine, tryptophan, glutamate, and lysine residues are also targets.

Because protein adducts are not repaired, protein adduct measurements are considered to reflect carcinogen dosimetry. Chemically-stable adducts are thought to provide a measure of dose integrated over the life time of a given protein. The blood proteins hemoglobin and serum albumin have been most studied as human biomarkers because they are readily accessible and have known rates of turnover. However, histone and collagen adducts have been explored as indicators of longer-term exposures.

Methods to measure carcinogen-protein adducts

The earliest animal model studies that examined hemoglobin or serum albumin adducts involved the use of a radiolabeled carcinogen. More recent approaches have included immunoassays, HPLC with fluorescence detection and various mass spectrometry approaches. These methods are particularly powerful because of the ability to determine the specific chemical structure of the purified protein adduct. In addition, sensitivity can be typically as low as ~0.1 fmole of adduct in mg quantities of protein. The strengths of this approach are the specificity of the methods and the availability of large quantities of sample material.

Significance of protein-adducts

The utility of proteins for human dosimetry in environmental and occupational chemical exposures was first demonstrated by the kinetic relationship between protein adduct persistence and protein lifetime. This important principle was established for hemoglobin modified by ethylene oxide or alkylating agents. It provided the basis for subsequent studies that investigated associations between carcinogen-protein adduct levels and carcinogen exposures. Protein adduct formation is a valuable surrogate for DNA adduct formation since many chemical carcinogens bind to both DNA and protein in blood with similar dose-response kinetics. In addition, blood proteins are available in large quantities, enhancing the feasibility of measuring carcinogen-protein adducts in human biomonitoring studies.

Many protein adduct studies have considered exposures to different chemicals including ethylene, methylmethane sulfonate, BP, aflatoxin B1, 2-amino-3-methylimidazo[4,5-f]quinoline, dimethylnitrosamine, ethylene and propylene oxide, NNK and styrene. However, hemoglobin adducts formed through the metabolic activation of aromatic amines have proven to be excellent indicators of tobacco smoking. Tobacco smokers are readily distinguished from non-smokers, and a dose response has been observed between smokers of black tobac-

co containing high levels of 4-ABP and blonde tobacco containing low levels of 4-ABP. Protein adducts have been measured for twenty aromatic amines contained in cigarette smoke. The 3-aminobiphenyl-hemoglobin is a unique marker for passive smoking because 3-aminobiphenyl is present in side-stream but not main stream tobacco smoke. Hydroxyethylvaline in hemoglobin is also a dosimeter of tobacco smoking, but it is less specific because ethylene oxide has other environmental origins in addition to tobacco smoke. Although questions remain concerning the relationship between protein adduct levels and disease risk, measurement of protein adducts has been, and will continue to be, a valuable tool in molecular epidemiology studies.

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Carcinogen Metabolism

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Synonyms

- → Detoxification

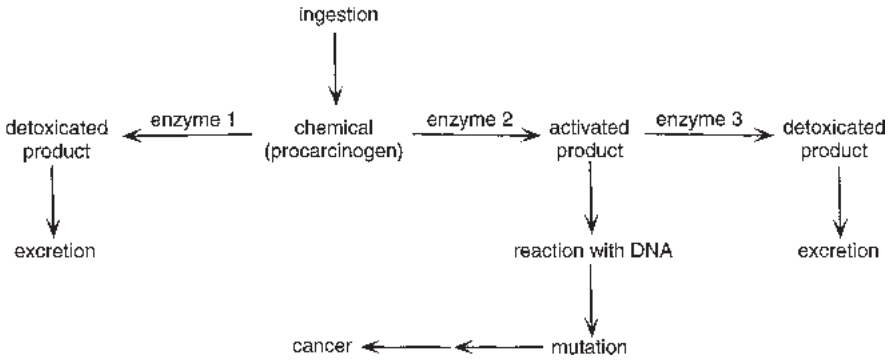
Definition

The transformation of chemicals is important in carcinogenesis both in terms of bioactivation as well as detoxication. Most chemical carcinogens need to be activated within the body. Such reactive forms can then cause biological damage (Fig. 1). As an example for competing processes urethane was chosen (Fig. 2). Exactly what proportion in human cancers are the result of chemical exposure is not clear. However, in most countries at least 1/3 of cancer cases are due to tobacco carcinogens. A significant number of cancer cases may be related to diet, although it is unknown exactly which chemicals in food cause or influence cancer. These days however, the number of cases due to industrial exposure seems to be very low.

Characteristics

History

In 1761, the London physician J. Hill made the observation that the use of snuff was associated with nasal cancers. More than hundred years later in 1895, Rehn and others, in Germany and Switzerland, reported a link of large-scale arylamine exposure of workers in the aniline dye industry to bladder cancer. In Japan, Yamagiwa and Ichikawa were in 1915 the first to demonstrate the formation of tumors in rabbits exposed to coal tar, a mixture of polycyclic hydrocarbons. The concept that metabolic processes are a necessity for the bioactivation of chemical carcinogens was primarily developed by J. A. and E. C. Miller at the University of Wisconsin in the early 1940's. Over the next few decades, they and others provided further insight, defining metabolically derived carcinogenic products that react with DNA ('ultimate carcinogens'). However, although the relationship between carcinogens and mutagenesis had been considered it was not clearly defined. It was only after B. N. Ames developed a (still widely used) bacterial mutation system in which rat liver extracts are able to transform carcinogens into mutagens that the correlation between carcinogenesis and mutagenesis became obvious. Advances in enzymology and re-



Carcinogen Metabolism. Fig. 1 – General paradigm for carcinogen metabolism, including both bioactivation and detoxication reactions.

combinant DNA technology made it possible to discern the role of individual human enzymes in various steps in carcinogen metabolism. Using inbred mouse strains and knockout mice it was possible to demonstrate the critical role of mouse orthologues in carcinogen activation.

Metabolism

Metabolism of carcinogens occurs in many tissues throughout the body. Many *in vitro* studies utilize liver tissue samples because many enzymes of interest are concentrated there. However, for tumors that originate elsewhere, extrahepatic sites are of greater interest. The question of which kind of tissue is most important, is related to the site of entry of a carcinogen as well as how much the activated form(s) of the carcinogen are able circulate within the body before reacting with the target tissue. Examples of important carcinogens and their metabolism are given below.

- Polycyclic aromatic hydrocarbons are systems of fused benzene rings that are found in carcinogenic soots, tars and tobacco smoke. A widely studied member of this class of compounds is benzo[*a*]pyrene. It is widely believed that the main metabolic pathway involves the oxidation of benzo[*a*]pyrene by cytochrome P450 (P450) to an epoxide. The hydrolysis of this epoxide to a dihydrodiol is followed by another oxidation by P450 that generates highly reactive diol epoxides. The latter can either react with DNA or are detoxicated by glutathione transferase.
- Aflatoxin B_1 is a mycotoxin and a prominent contributor to human → liver cancer. A critical feature of its metabolism is the formation of an epoxide by P450 enzymes. The epoxide (with a half life in water of $t_{1/2} = 1$ s) is able to react with DNA or can be conjugated with glutathione. P450 enzymes can also detoxicate aflatoxin B_1 by catalyzing several other oxidation steps (e.g. the oxidation to 3α- and 9α-hydroxylated products).
- Olefins (alkenes) can be oxidized to epoxides, as shown in the example of urethane (Fig. 2). A member of this group is vinyl chloride, a carcinogenic substance that was shown to cause a rare liver hemangiosarcoma in people working in the rubber industy.
- A further problematic group of substances are N-nitrosamines. They can result from some industrial settings but that are also produced endogenously from amines and nitrites in the acidic environment of the stomach. Sources are the so-called tobacco-specific nitrosamines as well as sodium nitrite that is used to preserve processed meats. Like in the examples stated above, P450 activates N-nitrosamines by oxidation. The formation of an alcohol on the adjacent carbon atom yields an unstable product that decomposes and alkylates DNA.

- Another group of chemicals of concern, when present in food or tobacco, are heterocyclic amines, substances that derive from creatinine and amino acids following pyrolysis. Amine activation involves its oxidation by a P450 enzyme to a hydroxylamine (-NHOH). An unstable compound (-NHOAc) is the result of the enzymatic transfer of an acetyl group. It ultimately breaks down to a nitrenium ion ($-NH^+$) that can react with DNA. Detoxication involves other P450 enzymes, glutathione transferases and UDP glucuronosyl transferases.

Mechanisms

Phase II reactions (including those catalyzed by the enzyme → N-acetyltransferase are usually involved in detoxication reactions; they can, however, be also part of bioactivation schemes. An example is the pesticide ethylene dibromide ($BrCH_2Cl_2Br$) and related compounds where the enzymatic conjugation of ethylene dibromide with the endogenous tripeptide glutathione yields a molecule (in this case glutathione-CH_2CH_2Br) that can react with DNA.

Cancers

Numerous studies support the important role of carcinogen metabolism in human cancers.

First, substances, such as aflatoxin B_1, whose metabolic products can cause cancers (see above) have been identified in foods. Second, it has been shown in animal models that either the absence or the induction of certain enzymes that are involved in carcinogen metabolism can have a dramatic effect on chemical-caused cancers. Third, humans are known to show great phenotypic variation in many enzymes involved in carcinogen metabolism. Dramatic effects on the metabolism of drugs have been demonstrated with these enzymes. Large inter-national and other inter-individual differences in cancer incidence, as well as the documented effects of diet on cancer, justify the considerable interest to study carcinogen metabolism, particularly in humans. Research in carcinogen metabolism and its applications can be divided into several areas. Investigating cancer cause and cancer etiology is aimed towards the understanding of basic chemistry, enzymology and physiology of metabolistic processes as well as how the chemicals react with DNA once they are activated. Molecular epidemiology utilizes information about carcinogen metabolism in order to establish their relevance in human cancer. A related topic is risk assessment, which uses the knowledge of carcinogen metabolism derived from animal bioassay studies and sometimes epidemiology, to determine critical exposure levels of evironmental carcinogens in humans.

Metabolic mechanisms play an important role in cancer safety assessment studies of prospective new drugs, including those used to treat cancer. Another important area is → chemoprevention where beneficial effects of certain chemicals are investigated, e.g. their ability to change the metabolism of carcinogens.

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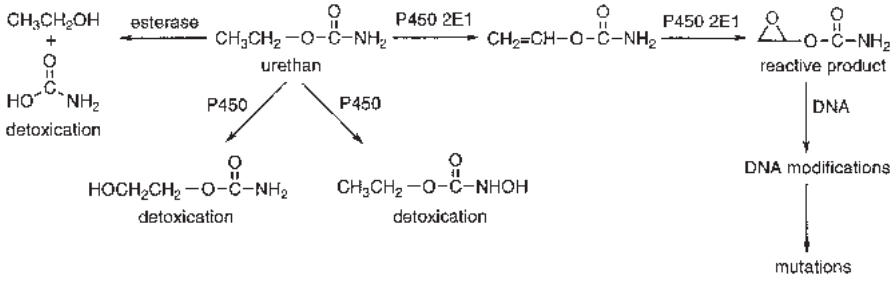

Carcinogen Metabolism. Fig. 2 – Metabolism of the carcinogen urethane (ethyl carbamate).

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Carcinogenesis

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Definition

Carcinogenesis is the process by which cancer develops in various tissues in the body.

Characteristics

In most cases carcinogenesis occurs via a stepwise process that can encompass a major fraction of the lifespan (→ multistep development). These progressive stages often include hyperplasia, dysplasia, metaplasia, benign tumors and then, eventually, malignant tumors. Malignant tumors can also undergo further progression to become more invasive and metastatic, autonomous of hormones and growth factors and resistant to chemotherapy or radiotherapy.

Causes

Known causes of carcinogenesis include various chemicals or mixture of chemicals present in several sources. This includes cigarette smoke, the diet, the workplace or the general environment, ultraviolet and ionizing radiation, specific viruses, bacteria and parasites and endogenous factors (oxidative DNA damage [→ oxidative DNA damage], DNA depurination, deamination). According to the International Agency for Research on Cancer (IARC) 69 agents, mixtures, and exposure circumstances are known to be carcinogenic to humans (group 1), 57 are probably carcinogenic (group 2A) and 215 are possibly carcinogenic to humans. Some of these agents, or their metabolites, form covalent → adducts to DNA and are → mutagenic. Others act at the epigenetic level by altering pathways of signal transduction and gene expression. These include tumor promoters, growth factors and specific hormones. Dietary factors also play an important role. Fruits and vegetables often have a protective effect. Excessive fat and/ or calories may enhance carcinogenesis in certain organs. Hereditary factors can also play an important role in cancer causation. Indeed, human cancers are often caused by complex interactions between these multiple factors. An example is the interaction between the naturally occurring carcinogen → aflatoxin and the chronic infection with hepatitis B virus in the causation of liver cancer in regions of China and Africa.

Molecular genetics

Recent studies indicate that the stepwise process of carcinogenesis reflects the progressive acquisition of activating mutations in dominant acting oncogenes and inactivating recessive mutations in tumor suppressor genes. It is also apparent that epigenetic abnormalities in the expression of these genes also play an important role in carcinogenesis. Thus far over 100 → oncogenes and at least 12 → tumor suppressor genes have been identified. Tumor progression is enhanced by genomic instability due to defects in DNA repair and other factors. The heterogeneous nature of human cancers appears to reflect heterogeneity in the genes that are mutated and/or abnormally expressed. Individual variations in susceptibility to carcinogenesis are influenced by hereditary variations in enzymes that either activate or inactivate potential carcinogens, variations in the efficiency of DNA repair and other factors yet to be determined. Age, gender and nutritional factors also influence individual susceptibility.

Clinical relevance

Prevention. Cancer is a major cause of death throughout the world. Therefore, the prevention of carcinogenesis is a major goal of medicine and public health. The carcinogenic process can be prevented by avoidance of exposure to various carcinogenic factors (i.e. cigarette

smoking, excessive sunlight, etc.), dietary changes, early detection of precursor lesions and chemoprevention.

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Carcinoid

Definition

→ Gastrinoma.

Carcinoid Syndrome

Definition

Carcinoid sydrome is associated with a variety of clinical symptoms including cutaneous flush, teleangiectasia, diarrhea, bronchial constriction, cardiac valvular lesions and right-sided endocardial fibrosis. These manifestations are mediated by serotonin (5-hydroxytryptamin) and other vasoactive amines that are released from neuroendocrine tumors (→ NETs) and may occur when the tumor has spread to the liver; → neuroendocrine tumors.

Carcinoma

Definition

A carcinoma is a malignant tumour of epithelial or other ectodermal origin.

Carcinoma *in situ*

Definition

Carcinoma *in situ* (CIS) is a lesion which exhibits the cytologic changes of invasive carcinoma but which is limited to the epithelium with no invasion of the basement membrane. Transitional cell CIS carries a high risk of eventual progression to muscle invasion.

CARD

Definition

→ Caspase recruitment domain.

Caretaker

Definition

Caretakers are a subgroup of tumour suppressor gene products whose major cellular function is in the mechanisms that preserve genetic stability. Inactivation of a caretaker gene typically induces an increase in the rate of genetic change, favouring the creation of somatic mutations in genes whose products regulate cell division, death or lifespan. Caretaker inactivation leads indirectly to neoplastic transformation. Caretaker gene products participate in DNA repair or the pathways that maintain chromosome stability. Examples include the breast cancer susceptibility genes → BRCA1 and → BRCA2, or the multiple genes mutated in different complementation groups of the disorder → Xeroderma pigmentosum. Caretaker genes,

such as many other genes, are often pleiotropic. They therefore can also have direct transforming ability; → gatekeeper.

Caspase

Definition

Caspases are protein degrading enzymes (proteases) that act as mediators of programmed cell death (→ apoptosis). Proteins within the large family of these cell-death proteases are all similar to each other. Caspases are highly conserved during evolution and can be found in humans as well as in insects and worms and are even found in lower multicellular organisms. More than a dozen caspases have been identified in humans. Usually caspases selectively cleave a restricted set of target proteins in the primary sequence at one position, or at a few positions at most. Cleavage always occurs behind an aspartate amino acid. The caspase-mediated cleavage of specific substrates supplies an explanation for several characteristic features of apoptosis. Cleavage of the nuclear lamins, for instance, is required for nuclear shrinking. Cleavage of cytoskeletal proteins causes the overall loss of cell shape. In healthy cells, caspases normally lie dormant. In response to diverse stimuli they become activated when cell death is required. Dormant caspases exist as precursor polypeptides or 'proenzymes' that are largely activated by proteolytic processing. This involves cleaving of proenzymes at specific points to generate the large and small subunits that associate to the active caspase enzyme. The proenzymes have low protease activity themselves and can therefore process each other when brought into vicinity. This process starts, when an external stimulus, a 'death ligand' binds to a receptor (such as → CD95/→ FAS/→ APO-1) on the cell surface. Ligand binding results in the aggregation of procaspase-8. The high density of caspase-8 proenzymes has the result that they mutually activate each other. Caspase-8 is an initiator caspase that can activate downstream procaspases, in particular procaspase-3, either by direct cleaving or indirectly by cleaving → BID and inducing cytochrome C release from mitochondria.

An alternative mechanism of caspase activation in response to death stimuli involves procaspase-9. In this case, the adaptor molecule → APAF-1 sequesters several procaspase-9 molecules that, within this complex (often referred to as apoptosome), are activated by a change in conformation, not by proteolysis. In response to that change, they can activate downstream caspases.

In short, initiator caspases become primarily activated by regulated protein-protein interaction, whereas downstream effector caspases are activated proteolytically. Besides caspase pathways, other death-inducing pathways must exist, since developmental apoptosis is functional in mice that are defective in regard to the caspase-8 and caspase-9 pathways.

Caspase 2

Synonyms

- NEDD2

Definition

Caspase 2 (CASP2) is an → apoptosis-related cysteine protease involved in the activation cascade of caspases responsible for apoptosis execution. It is a protein of 435 amino acids and 48 kD that is widely expressed in different tissues. Heterodimers are generated autocatalytically from a propeptide. The gene maps to 7q35.

Caspase 3

Definition

Caspase 3 is an → apoptosis-related cysteine protease, involved in the activation cascade of caspases that is responsible for apoptosis execution. At the onset it proteolytically cleaves poly(ADP-ribose) polymerase (→ Parp). It also

cleaves and activates caspase 6, caspase 7 and caspase 9. It is a cytoplasmic protein that is widely expressed and forms a heterodimer of 17 kD (p17) and 12 kD (p12). The gene maps to 4q33.

Caspase 4

Definition

Caspase 4 is an → apoptosis-related cysteine protease involved in the activation cascade of → caspases responsible for apoptosis execution. It is a protein of 377 amino acids and 43 kD that is widely expressed in different tissues and exists as a heterodimer. The gene maps to 11q22.

Caspase 5

Definition

Caspase 5 (CASP5) is an → apoptosis-related cysteine protease that promotes programmed cell death. It is involved in the activation cascade of caspases responsible for apoptosis execution, functioning by either activating proteins required for cell death or inactivating proteins necessary for cell survival. It is a protein of 418 amino acids and 47 kD, and the gene maps to 11q22.2-3.

Caspase 6

Definition

Caspase 6 is an → apoptosis-related cysteine protease involved in the activation cascade of → caspases responsible for apoptosis execution. It proteolytically cleaves poly(ADP-ribose) polymerase (Parp). It is a cytoplasmic protein of 293 amino acids and 33 kD and consists of a heterodimer of 18 kD (p18) and 11 kD (p11). The gene maps to 4q25.

Caspase 7

Definition

Caspase 7 is an → apoptosis-related cysteine protease, involved in the activation cascade of caspases. It proteolytically cleaves poly(-ADP-ribose) polymerase (Parp) and its overexpression promotes apoptosis. It is a widely expressed cytoplasmic protein of 303 amino acids and 34 kD and is a heterodimer of 20 kD (p20) and 11 kD (p11). The gene maps to 10q25.

Caspase 8

Definition

Caspase 8, also known as → FLICE, is an → apoptosis-related cysteine protease that contributes to apoptosis early in the cascade of caspase activation in → Fas receptor and → TNFR-1 pathways. Its binding to → FADD recruits it to either of the receptors. The resulting aggregate is referred to as 'death inducing signalling complex' (disc), which proteolytically activates Casp 8. The activated dimeric protease is then liberated from the disc and activates downstream apoptotic caspases, caspase 4, caspase 6, caspase 7, caspase 9 and caspase 10. Caspase 8 is a protein of 479 amino acids and 55kD and is a dimer of 18 kD (p18) and 10 kD (p10). The gene maps to 2q33.

Caspase 9

Definition

Caspase 9 is involved in the activation cascade of caspases in → apoptosis. The binding of caspase 9 to → APAF-1 leads to activation of the protease, which in turn cleaves and activates caspase 3 poly(ADP-ribose) polymerase (→ PARP). The protein is a heterodimer of 35 kD (p35) and 10 kD (p10) and is ubiquitously expressed.

Caspase 10

Definition

Caspases 10 is an → apoptosis-related cysteine protease, involved in the activation cascade of → caspases that are responsible for apoptosis execution. It is recruited to both → Fas and TNFR [→ TNFR-1]-1 receptors in a → FADD dependent manner, and cleaves and activates caspase 3, caspase 4, caspase 6, caspase 7, caspase 8 and caspase 9. It is a protein of 521 amino acids and 58 kD and is widely expressed in different tissues as a heterodimer of 23/17 kD (p23/17), due to alternative splicing, and 12 kD (p12). The gene maps to 2q33.

Caspase Recruitment Domain

Definition

The caspase recruitment domain is a motif commonly found in proteins involved in apoptotic signalling, including the → caspases and some → IAPs.

Catalytic triad

Definition

A catalytic triad is comprised of the three amino acid residues, asp, his and ser, which form the active site in serine-type proteinases.

Cathepsin B

Definition

Cathepsin B (Cpsb) is a thiol protease of 339 amino acids and 37 kD. The human CPSB gene locus maps at 8p22 and the mouse cpsb gene locus at chromosome 14 (28.00 cM). It is a lysosomal thiol protease with a specificity resembling that of papain and believed to participate in intracellular degradation and turnover of proteins, and also implicated in tumor invasion and metastasis. It consists of a heavy chain and a light chain cross-linked by a disulfide bond and belongs to the peptidase family C, also known as the papain family of thiol proteases.

Cathepsin D

Definition

Cathepsin D (Cpsd) is an aspartyl protease family member of 412 amino acids and 44 kD. The human CTSD gene locus maps at 11p15.5 and the mouse ctsd gene locus at chromosome 4. It is a lysosomal acid protease that is active in intracellular protein breakdown. It consists of a light chain and a heavy chain and belongs to peptidase family A1, also known as the eukaryotic aspartyl proteases family.

C-banding

Definition

C-banding is a technique for generating stained regions around centromeres of chromosomes.

C-bandless Chromosome

Definition

A C-bandless chromosome is a centric chromosome lacking the typical banding pattern after C-banding; chromosome arms show features of a → HSR.

CBF-B

Synonyms

- CBF-β

Definition

CCAAT-binding transcription factor subunit β; → NFYB.

CBFB

Synonyms

- CBF-β
- core-binding factor, β-subunit
- PEBP2B
- PEBP2β
- polyoma enhancer-binding protein, β-subunit

Definition

CBF binds to the core site 3′PyGPyGGT-3′ of a number of enhancers and promoters, including murine leukemia virus, polyomavirus enhancer, T-cell receptor enhancers; heteromeric factor consisting of an α- and β-subunit, the β-subunit acts to enhance the DNA binding of the α-subunit; protein 182 aa, 21 kd; gene maps to chromosome 16q22.1; the pericentric inversion of chromosome 16 [inv(16)(p13q22)] is a characteristic karyotypic abnormality associated with acute myeloid leukemia.

CBP/p300

Definition

cAMP-responsive element binding protein (CBP)/p300 is a co-activator for several transcription factors by bridging them to the basal transcriptional machinery and through their intrinic histone acetyltransferase activity.

CCND1

Definition

CCND1 (**cyclin D1**), also known as → *PRAD1*, is an oncogene localized to chromosomal band 11q13. It encodes the protein → cyclin D1, which functions as a cell-cycle regulator. Amplification of *CCND1* has been reported in several human tumors, including breast and head/neck carcinomas.

CCPB1

Definition

→ Granzyme B.

CD

Definition

Clusters of differentiation; → immunophenotype.

CD5 Positive Lymphomas

Definition

CD5 positive lymphomas are a subset of circulating, often autoreactive, B-lymphocytes defined by the expression of the surface membrane marker CD5.

CD15

Definition

CD15 is a carbohydrate antigen called the X hapten, found in several glycolipids and glycoproteins. The antigen is present on normal myeloid cells and a wide variety of epithelial cells, as well as their corresponding tumors.

CD20

Definition

CD20 is a membrane-embedded, non-glycosylated phosphoprotein that appears as the early pre-B cell matures into a late pre-B cell. The antigen is expressed on the surface of all mature B-lymphocytes, but is not present in secreting plasma cells.

CD30

Definition

CD30 is a member of the tumor necrosis factor/nerve growth factor receptor superfamily. It is an activation-associated antigen that is most often expressed on lymphoid cells, but also found on embryonal carcinoma cells.

CD44

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Synonyms

Cluster of differentiation 44 (CD44); hyaluronan receptor; homing receptor; ECMRIII; phagocytic glycoprotein-1 (pgp-1); H-CAM; $gp90^{Hermes}$.

Definition

CD44 is a type I transmembrane glycoprotein that exists in a large number of isoforms. The gene contains 20 exons within a region of approximately 60 kb on chromosome 11p13 in close proximity to the recombination-activating-genes Rag-1 and -2 (2).

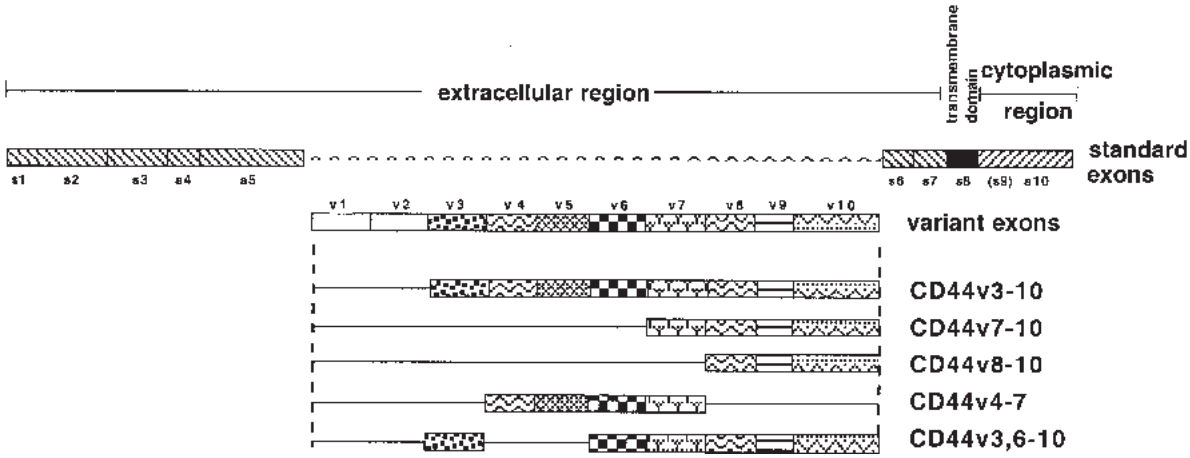
Characteristics

CD44 is the major receptor for → hyaluronic acid and other extracellular matrix molecules (fibronectin, laminin, collagen type IV, serglycine, osteopontin)(6). The standard molecule is heavily glycosylated by N- and O-linked residues and chondroitin sulfate side chains, while some of the variant isoforms carry heparan sulfate moieties, which present various growth factors and chemokines (local concentration and activation)(3). The matrix metalloproteinase-9 (MMP-9) can associate with CD44, inducing activation of latent transforming growth factor β (→ TGF-β) and hence promote invasion and → angiogenesis.

Upon cellular activation, CD44 localizes to plasma membrane microdomains (5) and associates with tyrosine kinases (lck and fyn), ezrin and via annexin II with the cytoskeleton. CD44 binding to hyaluronan induces migration.

Cellular and molecular regulation

The standard form of CD44 (CD44s) is expressed in almost all tissues and leukocytes, and is encoded by exons s1 - s10, yielding a product of 90 kD. The variant isoforms (CD44v) are generated by alternative splicing of the nuclear RNA between exons s5 and s6, and are encoded by exons v2 - v10 (exon v1 is silent in humans, but not in mice and rats)(2). Combinations of different variant exons with the standard backbone result in numerous variant isoforms, with masses of 100-250 kD. All the variant regions are located extracellularly and are highly hydrophilic (see Fig.). In contrast to the ubiquitous expression of CD44s, CD44v isoforms are expressed in a highly restricted manner in non-malignant tissues: in early embryogenesis, stem cells of epithelia and hemopoiesis, activated leukocytes and memory cells. However, in malignant tissues, CD44v are often upregulated, e.g. in carcinoma, various hematological malignancies and in autoimmune lesions (1, 3, 4, 7, 8).



CD44. Fig. – Organization of the exon structure of CD44. Within the framework of 10 standard exons (exons s1 to s10, while exon s9 is alternatively spliced and may in rare cases be expressed instead of exon s10), 10 alternatively, so-called variant exons (exons v1 to v10, although exon v1 is not expressed in humans due to a frameshift mutation resulting in a stop codon) are located. Predominantly, exons s1 to s10 (minus exon s9) are expressed, yielding CD44s. Much less frequent, during neoplastic transformation and leukocyte activation, the variant exons are expressed, yielding CD44v isoforms. The variant exons can be spliced into the framework sequence in any combination, either single or in multiples. The most frequently detected CD44v isoforms in human tumor tissue are shown.

Clinical Relevance

Originally identified by its metastasizing potential in rats, CD44v expression was identified in various human tumors and correlated with clinical relevance. Up-regulation of CD44v correlates with poor prognosis in (1, 3, 4, 7, 8):

- gastric carcinoma
- colorectal carcinoma
- non-small cell lung tumors
- hepatocellular carcinoma
- pancreatic cancer
- B-cell chronic lymphocytic leukemia
- multiple myeloma
- non-Hodgkin lymphoma
- acute myeloblastic leukemia.

Down-regulation of CD44v correlates with poor prognosis in oesophageal squamous cell carcinoma, bronchial carcinoid tumors, ovarian neoplasms, uterine cervical tumors, transitional cell bladder tumors, prostate cancers, while down-regulation of CD44s correlates with → amplification of → *MYCN* and is indicative of an unfavorable outcome in → neuroblastoma patients. In breast carcinoma no correlation between CD44v expression and survival was established.

Elevated serum levels of CD44v have prognostic value for gastric and colon carcinoma, non-Hodgkin lymphoma, which are indicative for a poor prognosis. CD44 is also strongly up-regulated in inflammatory lesions of patients with → autoimmune diseases.

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CD45

Definition

CD45, also known as leukocyte common antigen, is a family of protein tyrosine phosphatases expressed exclusively on the surface of almost all hematolymphoid cells and their progenitors.

CD57

Definition

CD57 is an antigen present on lymphocytes with natural killer and killer cell activity. The antigen is also found in subpopulations of T lymphocytes, some neural and neuroendocrine cells and a wide variety of neoplasms.

CD95

Definition

→ Fas.

CD99

Synonyms

- → MIC2

Definition

Antigen identified by monoclonal antibodies 12E7, F21 and O13. CD99 is the cluster definition for a transmembrane cell surface sialoglycoprotein previously named MIC2 or E2. It is present on almost every human tissue but most highly expressed on early hematopoietic precursor cells, some lymphomas and all Ewing sarcoma family tumors. CD99 is a glycoprotein (also designated p30/32 MIC2) with a molecular weight of approximately 30 kD that is located on the cell surface and probably involved in cell adhesion. Although immunohistochemical detection of membrane localized CD99 expression is a sensitive diagnostic marker for Ewing family tumors [→ Ewing sarcoma], it lacks specificity as many other tumors are also immunoreactive with anti-CD99 antibodies.

CDC2

Definition

Cell division control protein 2 homolog (Cdc2), also known as p34 protein kinase and cdk1 (cyclin-dependent kinase 1) is a serine/threonine kinase of 297 amino acids and 34 kD. The human *CDC2* gene locus maps to 10q21.1. Cdc2 plays a key role in the control of the eukaryotic cell cycle. It is required in higher cells for entry into S phase and mitosis. p34 is a component of the kinase complex that phosphorylates the repetitive carboxyl-terminus of RNA polymerase II. It forms a stable but non-covalent complex with a regulatory subunit and with a cyclin.

CDC7

Definition

Cdc7 is a protein kinase that promotes the initiation of → replication forks at licensed replication origins; → replication licensing system.

CDC25A

Definition

Cell division cycle 25A (CDC25A) is a M-phase inducer phosphatase 1 (ec 3.1.3.48) of 523 amino acids and 58 kD. The human CDC25A gene locus maps at 3p21 and the mouse cdc25a gene locus at chromosome 9 (62.00 cM). CDC25A functions as a dosage-dependent inducer in mitotic control. It is a tyrosine protein phosphatase required for progression of the cell cycle. It may directly dephosphorylate CDC2 and activate the CDC2 kinase activity, and also dephosphorylates p33(CDC2) in complex with Cyclin E.

CDC25C

Definition

Cell division cycle 25C is a nuclear protein of 473aa and 53kD that functions as a dosage-dependent inducer in mitotic control. It is a tyrosine protein phosphatase, required for progression of the cell cycle and is phosphorylated by → CHEK1. The gene maps to 5q31.

CDK

Definition

→ Cyclin-dependent kinase.

CDK4

Definition

Cyclin dependent kinase 4 (CDK4) forms a complex with D-type cyclins to promote progression through early G_1.

CDK Inhibitory Protein

Definition

CDK inhibitory protein, also known as cyclin-dependent kinase inhibitor (CKI), is a protein that inhibits the activity of cyclin/CDK complexes.

CDKN1B

Definition

→ Cyclin-dependent kinase inhibitor 1B.

CDKN2

Definition

→ CDKN2A.

CDKN2A

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Synonyms

- CDKN2
- CDK4I
- CMM2
- INK4A
- MTS1
- p16
- $p16^{INK4}$
- $p16^{INK4A}$
- $p16^{INK4a}$

Definition

Cyclin-dependent kinase inhibitor 2A gene (CDKN2A), the first identified → melanoma pre-

disposition gene, encodes the cyclin-dependent kinase inhibitor, p16.

Characteristics

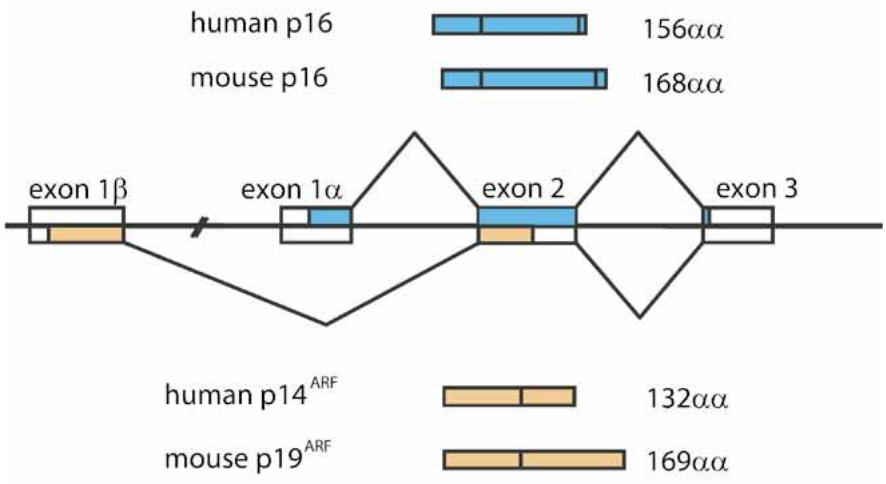
Identification of *CDKN2A*

The 9p21-22 chromosomal region was originally implicated in the development of melanomas through a combination of cytogenetic and loss of heterozygosity (LOH) studies. Subsequent linkage analysis in melanoma families indicated that this region harboured a melanoma predisposition locus. Homozygous deletions in cell lines derived from several different tumour types narrowed down the region significantly. This led to the isolation, by two independent groups, of the cell cycle regulatory gene encoding the cyclin-dependent kinase (CDK) inhibitor, p16, which had been previously identified in a yeast two-hybrid screen to identify proteins that bound to CDK4 (Fig. 1).

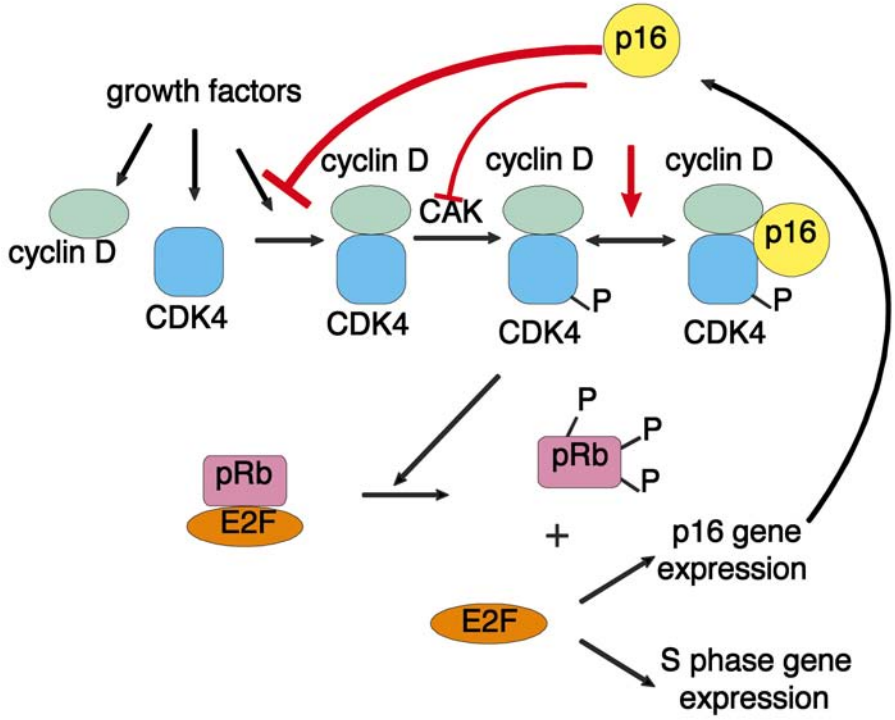
Gene structure of *CDKN2A*

In the original description of human p16 the initiating methionine was incorrectly identified. It was later found that the protein included eight additional amino acids at its amino terminus, although these residues are not present in murine p16. Three exons, spread over approximately 7.2 kb of genomic DNA, encode the 156 amino acid protein with predicted molecular weight of 16,533 Daltons, designated p16. The primary structural feature of p16 is the four tandem ankyrin-like repeats that comprise approximately 85% of the protein. This domain is believed to facilitate protein-protein interactions (Fig. 2).

The sizes of the translated regions encoded by exon 1a, exon 2 and exon 3 are 150 bp, 307 bp, and 11 bp, respectively. The *CDKN2A*-locus also has the capacity to encode two distinct transcripts from two different promoters. This is achieved by alternative splicing and the use of different reading frames. Each transcript has a specific 5′ exon, exon 1α (E1α) or exon 1β (E1β), which is spliced onto common second (E2) and third (E3) exons. The E1α-containing transcript encodes p16 and the E1β-containing transcript encodes a protein translated in an alternate reading frame initiated in E1β, designated $p19^{ARF}$ in mice and $p14^{ARF}$ in humans. In contrast to p16, where the murine and human genes share 85% amino acid homology, the alternative reading frame (ARF) proteins share only 59% amino acid homology. The different sizes of the encoded proteins are brought about by the earlier truncation of the ARF transcript in exon 2 in humans.



CDKN2A. Fig. 1 – Alternative transcripts and products encoded by the *CDKN2A* locus. The exons of *CDKN2A* are shown as boxes and identified as exons 1β, 1α, 2 and 3. Alternative splicing occurs as indicated to give rise to two transcripts, exons that splice to encode p16 are shown above and those that encode $p14^{ARF}$ are shown below. The sizes and composition of the respective mouse and human proteins are indicated.



CDKN2A. Fig. 2 – Schematic representation of the protein interactions in the cyclin D/CDK4/p16/pRb pathway. Through a complex system of signal transduction, growth factors lead to the assembly of cyclin D and CDK4. This complex is then activated through phosphorylation by the CDK-activating kinase (CAK), and cyclin D/CDK4 in turn phosphorylates pRb, leading to the release of transcription factors of the E2F family. These are then capable of transactivating the genes necessary for entry into S phase. P16 has been shown to inhibit this process in several ways; by binding to the complex and inhibiting the kinase activity of CDK4, inhibiting CAK dependent phosphorylation of CDK4, or inhibiting the assembly of the cyclin D/CDK4 complex, with the latter being the principal mechanism of inhibition *in vivo*. The scheme provided is necessarily simplistic, however, it appears that p16 may also inhibit the phosphorylation of pRb by indirectly inactivating other CDKs, e.g. CDK2, as a consequence of the redistribution of other CDK inhibitors, e.g. p27 and p21. There is also a feedback loop whereby the release of the E2F transcription factor results in the activation of p16 expression, although the absence of E2F binding sites in the *CDKN2A* promoter preclude direct transactivation by E2F. Aberration of this pathway through either; deletion or mutation of pRb, the binding of viral oncogenes to pRb, overexpression or activation of CDK4 or cyclin D, or deletion or mutation of *CDKN2A* all can result in constitutive transactivation of S phase genes by E2F transcription factors.

Tumour suppressor

CDKN2A is a tumour suppressor gene for multiple tumour types. The frequency of mutations at this locus in various cancers is rivalled only by mutations in → *TP53*. As with other classical tumour suppressor genes, both alleles need to be abrogated for tumorigenesis to occur. A wide variety of mechanisms of inactivation of *CDKN2A* have been documented, including intragenic mutation, homozygous deletion and transcriptional silencing through methylation of the promoter. Notably in melanomas, many of the intragenic mutations are C > T or tandem CC > TT transitions, implicating ultra-violet radiation (UVR) as the causal somatic mutagen.

Although *CDKN2A* is inactivated in the majority of melanoma cell lines examined, deletions and interstitial mutations of *CDKN2A* are much less common in uncultured melanoma tumours. Present studies indicate that only 5 – 10% of uncultured melanomas demonstrate mutations in *CDKN2A*, a surprisingly low figure given the obvious importance of *CDKN2A* in fa-

milial melanoma and the frequency of LOH seen at chromosome 9p21 in melanomas.

P16 is a CDK inhibitor

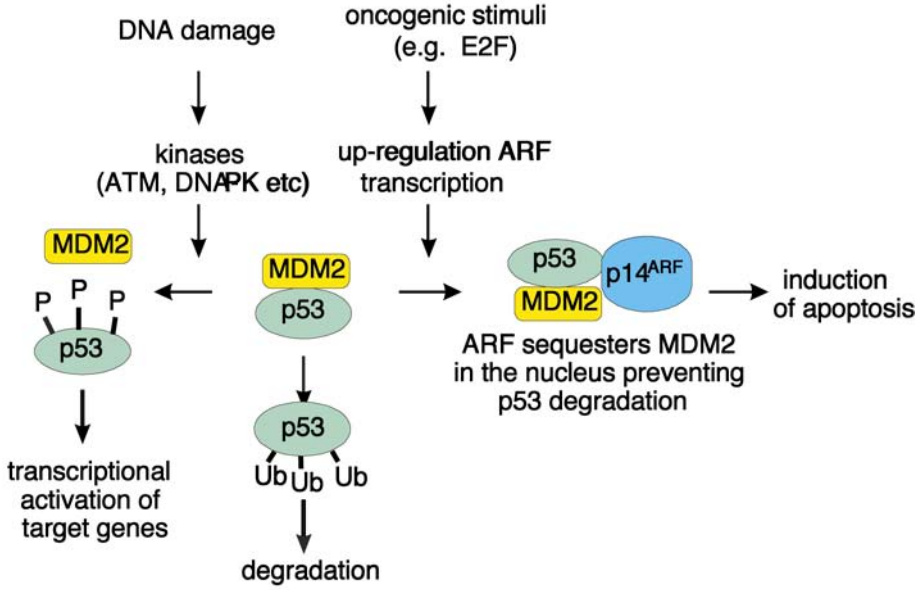
P16 is the archetype member of the INK4 (inhibitor of CDK4) family of CDK inhibitors, which is comprised of $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$ and $p19^{INK4D}$; encoded by *CDKN2A*, *CDKN2B*, *CDKN2C* and *CDKN2D*, respectively. Each of the proteins inhibits CDK4- or CDK6-mediated phosphorylation of the retinoblastoma susceptibility gene product, pRb, thereby providing a powerful negative signal, or 'brake', to progression through the cell cycle.

The cyclin D1/CDK4/p16/pRb signalling pathway is the major growth control pathway for entry into the cell cycle. For cells to progress through G1 into S phase they must pass the late G1 restriction point, which controls entry into S phase. For progression past this restriction point, cyclin D/CDK4 must phosphorylate the retinoblastoma protein pRb. During G0/G1 the Rb protein exists in a DNA-bound protein complex, where it is bound to the transactivation domain of E2F transcription factors, preventing transactivation of E2F target genes. The phosphorylation of pRb results in the dissociation of this protein complex and the release of E2F such that it can transactivate genes required for entry into S phase. Over-expression of p16, inhibits progression of cells through the G1 phase of the cell cycle by binding to CDK4/cyclin D complexes (or CDK6/cyclin D) and blocking the kinase activity of the holoenzyme. Given that p16 normally functions to inhibit CDK4, it is easy to understand how inactivation of this gene could result in uncontrolled cellular growth leading to cancer. In many tumour types, an inverse correlation between mutations of p16 and pRb has been observed. Since p16 lies upstream of pRb, inactivation of both proteins would be redundant.

Role of the alternative reading frame (ARF) product

The ARF protein also regulates the G1/S phase transition via a distinct pathway involving the tumour suppressor gene product p53, and MDM2, which function upstream of p21 (a cyclin-dependent kinase inhibitor closely related to p16) and the CDK2/cyclin E complex (Fig. 3). p53 is a transcription factor that plays a major role in monitoring the integrity of the genome, and can be activated to inhibit cell cycle progression or initiate apoptosis through two distinct pathways: (i) in response to a variety of cellular stresses including DNA damage and hypoxia, (ii) via overexpression of viral or cellular oncoproteins such as E1A and c-myc. In this way, cells prevent the repair of mutations in successive generations by inducing apoptosis in incipient cancer cells. ARF plays a crucial role in p53-induced apoptosis. Murine $p19^{ARF}$ is capable of inducing a p53-dependent G1 cell cycle arrest that is not mediated through the direct inhibition of known CDKs. Ectopic expression of ARF leads to stabilisation of p53 in multiple cell types, but unlike other known upstream effectors of p53 this activation is not through phosphorylation. Instead, ARF binds to MDM2 and blocks both MDM2-mediated p53 degradation and the transactivational silencing of p53. MDM2 continuously shuttles between the nucleus and the cytoplasm. This shuttling is essential for its ability to promote p53 degradation, indicating that MDM2 must export p53 from the nucleus to the cytoplasm to target p53 to the cytoplasmic proteosome. ARF activates p53 by binding to MDM2 in the nucleus and blocking the transport of the MDM2/p53 complex out of this organelle. Results obtained with murine and human ARF are somewhat different. In murine cells results indicate that $p19^{ARF}$ sequesters → MDM2 away from p53 into the nucleolus. In human cells $p14^{ARF}$ moves out from the nucleolus to form discrete nuclear bodies in conjunction with MDM2 and p53, thereby blocking their nuclear export and leading to p53 stabilisation. The discovery that ARF transcription is induced by the over-expression of a variety of cellular and viral oncoproteins including c-myc, E1A and → E2F has provided the link by which hyperproliferative signals result in p53-dependent → apoptosis.

To determine whether mutations in *CDKN2A* contribute to tumorigenesis via $p19^{ARF}$ in addition to p16, cDNAs carrying a



CDKN2A. Fig. 3 – Schematic representation of the role of ARF in p53 activation by DNA damage and oncogenic stimuli. ARF functions to sequester MDM2 in the nucleus preventing nucleo-cytoplasmic shuttling of the MDM2/p53 complex, however, the details have not yet been fully elucidated and results suggest the mechanism may differ between humans and mice.

variety of exon 2 mutations have been transfected into cell lines and cell cycle arrest monitored. These mutations have included several that are silent in p16 but caused missense mutations in $p19^{ARF}$, as well as several deletion mutants that removed either exon 1β or various portions of exon 2. Results indicate that the majority of $p19^{ARF}$ activity is encoded by the exon 1β sequences, as all missense mutations in exon 2 of $p19^{ARF}$ remained fully active in blocking cell cycle progression, and removal of exon 2 sequences only marginally reduced the ability to induce arrest. In contrast, deletion of exon 1β resulted in a transcript that was incapable of inhibiting cell cycle progression. Missense mutations in exon 2 of the human $p14^{ARF}$ transcript similarly did not reduce the growth suppressive function of $p14^{ARF}$. To date, only a handful of mutations in exon 1β have been found in tumours.

Senescence

p16 is not normally expressed at detectable levels in most cycling cells, however, *CDKN2A* mRNA and p16 protein accumulate in late-passage non-immortalised cells, implicating a role for p16 in cellular senescence. This is supported by studies revealing that loss of p16 expression is a critical event in immortalisation (the flip side to senescence) of a range of cell types. This conclusion was initially alluded to by finding that the frequency of deletions and intragenic mutations of *CDKN2A* in uncultured tumours was considerably lower than in immortalised cell lines.

Mouse models

The generation of a *Cdkn2a* 'knockout' mouse, carrying a germline homozygous deletion encompassing exons 2 and 3 of the gene, revealed that p16 and $p19^{ARF}$ (since both proteins are eliminated by deletion of exon 2) were not essential for viability or organomorphogenesis. However, the mice did demonstrate abnormal extramedullary haematopoiesis, suggesting that p16 or $p19^{ARF}$ may regulate the proliferation of some haematopoietic lineages. In addition, the mice developed spontaneous tumours at an early age, specifically fibrosarcomas and B cell lymphomas, and were highly sensitive to

carcinogens. In contrast to wildtype mouse embryonic fibroblasts (MEFs), cultured MEFs from *Cdkn2a* nullizygous mice (*Cdkn2a*$^{-/-}$) failed to undergo senescence crisis, and could be transformed by oncogenic *ras* alleles. Although *Cdkn2a*$^{-/-}$ mice did not develop melanomas, transformation of *Cdkn2a*$^{-/-}$ MEFs by activated ras prompted experiments to cross the *Cdkn2a*$^{-/-}$ mice with a previously generated transgenic mouse in which an activated *ras* allele was targeted exclusively to melanocytes under the control of the tyrosinase promoter. These mice spontaneous developed melanomas at high frequency and with short latency.

To determine whether p16 or p19^{ARF} was the principal mediator of the above effects, a mouse knockout was generated that carried a homozygous deletion of E1b, thereby only inactivating p19^{ARF}. Surprisingly, mice lacking p19^{ARF}, but retaining p16, spontaneously developed fibrosarcomas and lymphomas similar to the *Cdkn2a*$^{-/-}$ mice. In addition, MEFs derived from p19$^{ARF-/-}$ mice failed to undergo senescence crisis and can be transformed by activated ras. The similar findings in these two strains of knockout mice raises the issue of whether or not the loss of p16 alone is associated with murine tumorigenesis. Only the generation of a homozygous E1α knockout mouse will provide the answer.

Clinical aspects

- *CDKN2A mutations and melanoma.* Approximately half of all multiplex melanoma families show linkage to the *CDKN2A* gene, however, only 10 – 50% (depending on the population) of these 'linked' families carry germline mutations of *CDKN2A*. These findings suggest that melanoma predisposition in some of these families is caused by: (i) another gene in the vicinity of *CDKN2A*, (ii) mutations outside of the p16 coding region, (iii) another gene somewhere else in the genome, with linkage to this region occurring simply by chance. The most parsimonious explanation is that a combination of all these possibilities is likely. There is a strong trend towards increasing yield of *CDKN2A* mutations with increasing number of affected cases in families with melanoma. Globally, the frequency of mutations in 2-case families is low (~11%), and is lowest in regions of high UVR, e.g. Australia (1/65, 1.5%) and somewhat higher in regions with low incident UVR, e.g. Scotland (4/14, 29%). The population based frequency of *CDKN2A* mutations in melanoma cases is of the order of 0.2%, which translates to about 1:10,000 individuals in the general population. Disease associated mutations are distributed along the entire length of the p16 coding region and at least one mutation has been described in the promoter of the gene. Several mutations have been repeatedly reported, and where analysis has been performed these have invariably been shown to be due to common founders. To date there are no obvious phenotype-genotype correlations.
- *Penetrance.* There has not been a comprehensive study of the penetrance of *CDKN2A* mutations in melanoma families. However, anecdotal findings from a limited number of high-density melanoma kindreds, indicates that the lifetime risk of developing melanoma in germline mutation carriers from Australia is approximately 80 – 90%, whereas the figure is roughly half this in populations living at greater latitudes.
- *Multiple primary melanoma.* General characteristics of inherited susceptibility to many types of cancer are early age of onset and the development of multiple primary tumours. Hence the presence of multiple primary melanomas (MPM) in an individual may be a sign of them being a *CDKN2A* mutation carrier. This is the case for a small proportion (7/60, 12%) of MPM cases without a family history of the disease. In contrast, analysis of multiplex melanoma families in which there are one or more cases of MPM yields mutations in 11/34 (34%) 2-case melanoma families and 33/74 (45%) families with three or more affected members. Moreover, the proportion of *CDKN2A* mutations in sporadic MPM cases increases with increasing number of melanomas (2/38 (5%) of cases with two primary melanomas, compared to 3/10 (30%) cases with 3 or more primary tumours).

- *CDKN2A mutations and non-melanoma cancers.* Since *CDKN2A* is a tumour suppressor found to be inactivated in a wide range of different tumours, one might expect individuals carrying germline mutations of *CDKN2A* to be prone to cancers other than melanoma. However, to date convincing evidence for susceptibility to another tumour type has been shown only for pancreatic cancer.
- *CDKN2A and DNS.* Since the description of the 'B-K mole syndrome' much debate has ensued regarding the association between melanoma and the dysplastic naevus syndrome (DNS). Several authors have concluded that dysplastic naevi segregate independently of *CDKN2A* mutations, although individuals with high numbers of naevi in melanoma-prone families are three times more likely to be CDKN2A mutation carriers than those with a low number of naevi. Support for the notion that *CDKN2A* is naevogenic comes from a study of a large series of 12-year-old twins in which total naevus count was found to be tightly linked to *CDKN2A*.

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CDKNB1

Definition

→ Cyclin-dependent kinase inhibitor 1B.

CDNA

Definition

cDNA is single-stranded DNA that is complementary to an RNA, which is synthesized from it by reverse transcription *in vitro*; → complementary DNA.

CDNA Clone

Definition

A cDNA clone is a duplex DNA sequence generated after reverse transcription from an RNA template that is carried in a → cloning vector.

CEA

Definition

→ Carcinoembryonic antigen.

C/EBP

Definition

C/EBP is a family of CAATT-enhancer binding transcription factors.

CED-4

Synonyms

- CASP8AP2
- CASP8 associated protein 2
- FLASH
- proapoptotic protein

Definition

→ *Caenorhabditis elegans* death protein-4 is activated by → Bcl-2 and activates → caspases during → apoptosis with an N-terminal homology to the human homolog → APAF-1; APAF-1 maps to chromosome 6.

Cell Adhesion Molecules

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Definition

Cell → adhesion molecules are transmembrane or membrane-linked glycoproteins that mediate the connections between cells or the attachment of cells to substrate (such as stroma or basement membrane). Dynamic cell-cell and cell-substrate adhesion is a major morphogenetic factor in developing multicellular organisms. In adult animals, adhesive mechanisms underlie the maintenance of tissue architecture, allow the generation of force and movement, and guarantee the functionality of the organs (e.g. to create barriers in secreting organs, intestines and blood vessels) as well as the generation and maintenance of neuronal connections. Cell adhesion is also an integrated component of the immune system and wound healing. At the cellular level, cell adhesion molecules do not function just as molecular glue. Several signaling functions have been attributed to adhesion molecules, and cell adhesion is involved in processes such as → contact inhibition, growth and → apoptosis. Deficiencies in the function of cell adhesion molecules underlie a wide variety of human diseases including cancer. By their adhesive activities, adhesion molecules directly influence the invasive and metastatic behavior of tumor cells, and by their signaling function they can be involved in the initiation of tumorigenesis.

Characteristics

At the molecular level, cell adhesion is mediated by molecules that are exposed on the external surface of the cell and are somehow physically linked to the cell membrane. In essence, there are three possible mechanisms by which such membrane-attached adhesion molecules link cells to each other (Fig. 1a). First, molecules on one cell bind directly to similar molecules on the other cell (→ homophilic adhesion). Secondly, adhesion molecules on one cell bind to other adhesion receptors on the other cell (→ heterophilic adhesion). Finally, two different adhesion molecules on two cells may both bind to a shared secreted multivalent ligand in the extracellular space. Also, cell-cell adhesion between two identical cells is called → homotypic (cell) adhesion, while → heterotypic (cell) adhesion takes place between two different cell types. In the case of cell-substrate adhesion the adhesion molecules bind to the → extracellular matrix (ECM).

Cell adhesion molecules and the cytoskeleton

Adhesion molecules can be associated with the cell membrane either by a glycosylphosphatidyl-inositol (GPI) anchor or by a membrane-spanning region. In the latter case the cytoplasmic part of the molecule often associates indirectly with components of the → cytoskeleton (e.g. actin, intermediate filaments or submembranous cortex). This implies that adhesion molecules, which by themselves establish extracellular contacts, can be structurally integrated with the intracellular cytoskeleton, and they are often clustered in specific restricted areas in the membrane, the so-called → junctional complex (Fig. 1b). This combined behavior of linkage to the cytoskeleton and clustering, considerably strengthens the adhesive force of the adhesion molecules. In some cases, exposed adhesion molecules can be in a conformational configuration that does not support binding to its adhesion receptor. A signal within the cell can induce a conformational change that activates the adhesion molecule. These mechanisms of regulation allow for a dynamic process of cell adhesion that, amongst others, is required for morphogenesis during development and for efficient immunological defence.

Classification of cell adhesion molecules

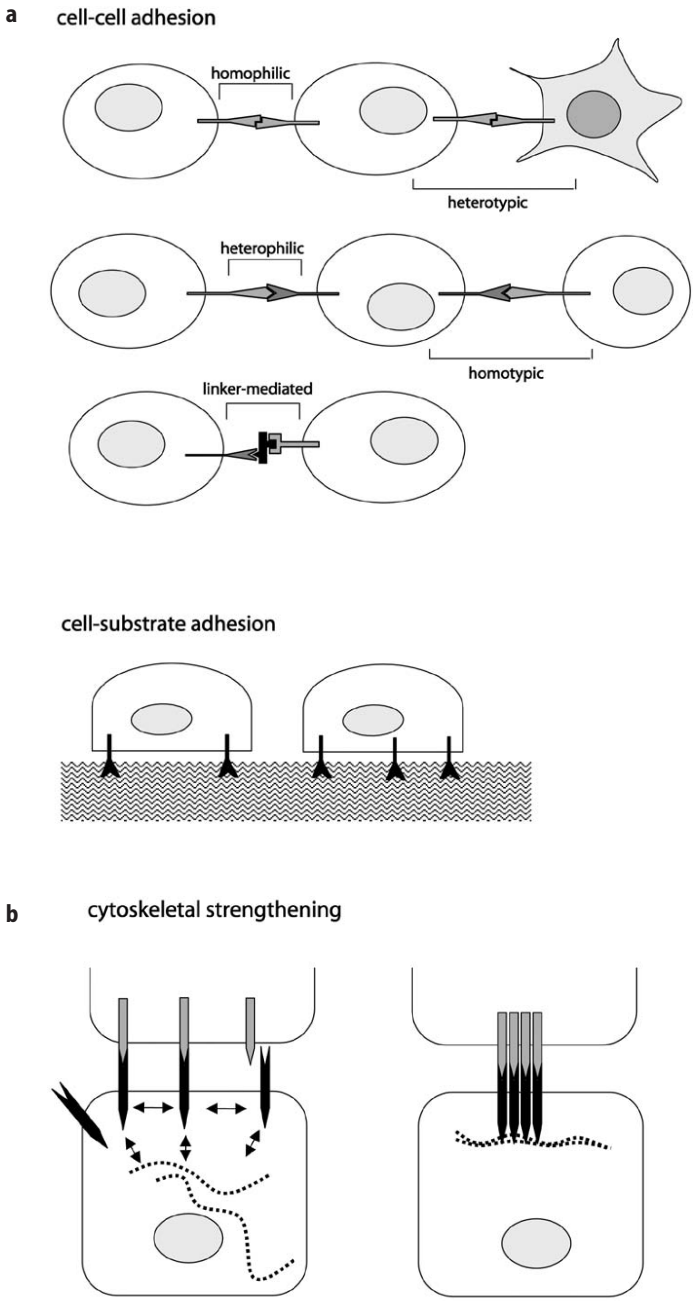
Based on their molecular structure and mode of interaction, 5 classes of adhesion molecules are generally distinguished; the → cadherins, → integrins, immunoglobulin (Ig) superfamily, selectins and proteoglycans (Fig. 2).

Cadherins

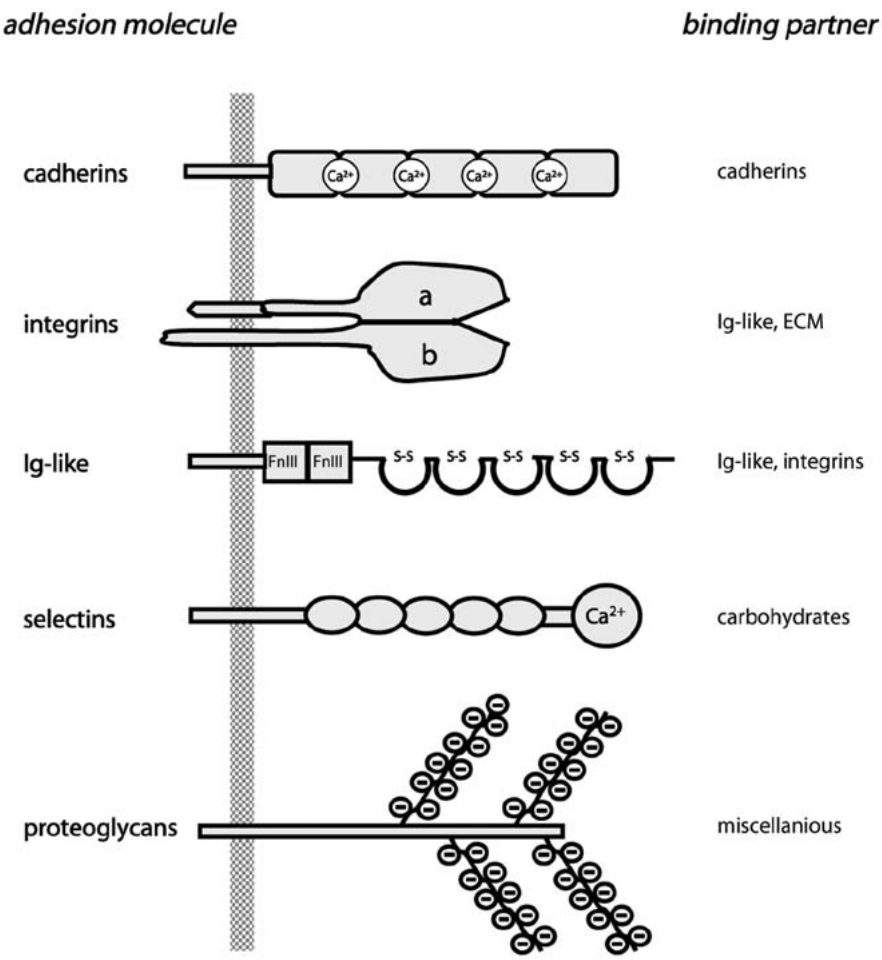
Cadherins and proto-cadherins form a large and diverse group of adhesion receptors. They are Ca^{2+}-dependent adhesion molecules, involved in a variety of adhesive interactions both in the embryo and the adult. Cadherins play a fundamental role in metazoan embryos, from the earliest gross morphogenetic events (e.g. separation of germ layers during gastrulation) to the most delicate tunings later in development (e.g. molecular wiring of the neural network). The extracellular part of vertebrate classical cadherins consists of a number of cadherin repeats whose conformation is highly dependent on the presence or absence of calcium ions. Homophilic interactions can only be realised in the presence of calcium, usually by the most distal cadherin repeat. Classical cadherins are generally exposed as homodimers, and their cytoplasmic domain is tightly associated with the actin cytoskeleton. Cadherins are the major adhesion molecules in tissues that are subject to high mechanical stress such as epithelia (→ E-cadherin) and endothelia (VE-cadherin). However, finer and more elegant intercellular interactions, such as synaptic contacts, also seem to involve cadherins.

Integrins

Integrins are another group of major players in the field of cell adhesion. They are involved in various processes such as morphogenesis and tissue integrity, homeostasis, immune response and inflammation. Integrins are a special class of adhesion molecules, not only because they mediate both cell-cell and cell-substrate interactions (with components in the ECM such as laminin, fibronectin and collagen) but also because they function as heterodimers



Cell Adhesion Molecules. Fig. 1 – Different modes of cell-cell and cell-substrate adhesion and the mechanism of cytoskeletal strengthening. a Three possible mechanisms by which cell adhesion molecules mediate intercellular adhesion. A cell surface molecule can bind to an identical molecule (→ homophilic adhesion) on the opposing cell or can interact with another adhesion receptor (→ heterophilic adhesion). Alternatively, cell adhesion receptors on two neighbouring cells can bind to the same multivalent, secreted ligand (linker-mediated adhesion). Intercellular adhesion can take place between identical cell types (→ homotypic adhesion) or between cells of different origin (→ heterotypic adhesion), independently of the involved adhesion molecules. Cell-substrate adhesion molecules attach cells to specific compounds of the → extracellular matrix. Cell-cell and cell-substrate adhesion can occur simultaneously. **b** Intercellular and cell-substrate adhesion can be strengthened by indirect intracellular linkage of the cytoplasmic tail of the adhesion molecules to the → cytoskeleton and by lateral clustering in the membrane.



Cell Adhesion Molecules. Fig. 2 – The five major classes of cell adhesion molecules and their binding partners. → Cadherins are Ca^{2+}-dependent adhesion molecules that consist of a varying number of cadherin repeats (5 in case of the classical cadherins). The conformation and activity of cadherins is highly dependent on the presence of Ca^{2+}-ions. In general, cadherin binding is homophilic. → Integrins are functional as heterodimers and consist of an α- and β-subunit. They interact with members of the immunoglobulin superfamily or with compounds of the → extracellular matrix (e.g. fibronectin, laminin). Members of the **immunoglobulin superfamily** (Ig-like proteins) are characterized by a various number of immunoglobulin-like domains (open circles). Membrane-proximal, fibronectin type III repeats are often observed (grey boxes). They can either bind to other members of the Ig-family (homophilic) or to integrins. **Selectins** contain an N-terminal Ca^{2+}-dependent → lectin domain (circle) that binds carbohydrates, a single EGF-like repeat (grey box) and a number of repeats that are related to those present in complement-binding proteins (ovals). → Proteoglycans are huge molecules that consist of a relatively small protein core to which long side chains of negatively charged glycosaminoglycans are covalently attached. They bind various molecules, including components of the extracellular matrix.

consisting of an α- and β-subunit. To date, at least 16 α-subunits and 8 β-subunits have been indentified. Of the theoretical 128 heterodimeric pairings, at least 21 are known to exist. While most integrin heterodimers bind to ECM components, some of them, more particularly those expressed on leukocytes, are heterophilic adhesion molecules binding to members of the Ig superfamily. The α-subunit mostly contains a ligand-binding domain and requires the binding of divalent cations (Mg^{2+}, Ca^{2+} and Mn^{2+}, depending on the integrin) for its function. In-

terestingly, integrins may be present on the cell-surface in a non-functional and a functional configuration. The cytoplasmic domain appears to be responsible for the conformational change that activates the integrin.

The Ig superfamily

Among the classes of adhesion molecules discussed here, the Ig superfamily is probably the most diverse. The main representatives are the neural cell adhesion molecules (NCAMs) and V(ascular)CAMs. As the name suggests, the members of this family all contain an extracellular domain consisting of different immunoglobulin-like domains. NCAMs sustain homophilic and heterophilic interactions that play a central role in regulation and organization of neural networks, specifically in neuron-target interactions and fasciculation. The basic extracellular structure consists of a number of Ig domains, which are responsible for homophilic interaction, followed by a discrete number of fibronectin type III repeats. This structure is linked to the membrane either by a GPI anchor or a transmembrane domain. The VCAM subgroup, including I(ntercellular)-CAMs and the mucosal vascular addressin adhesion molecule (MAdCAM), are involved in leukocyte trafficking (or homing) and extravasation. They consist of membrane-linked Ig domains that make heterophilic contacts with integrins. Other members of this family that are associated with cancer are carcinoembryonic antigen (CEA), 'deleted in colon cancer' (DCC) and platelet endothelial (PE)CAM-1.

Selectins

These types of adhesion molecules depend on carbohydrate structures for their adhesive interactions. Selectins have a C-type → lectin domain, that specifically binds to discrete carbohydrate structures present on cell-surface proteins. Intercellular interactions mediated by selectins are of particular interest in the immune system, where they play a fundamental role in trafficking and homing of leukocytes.

Proteoglycans

→ Proteoglycans are large extracellular proteins consisting of a relatively small protein core to which long chains of glycosaminoglycans are attached. Although poorly documented, proteoglycans may bind to each other or may be the attachment site for other adhesion molecules.

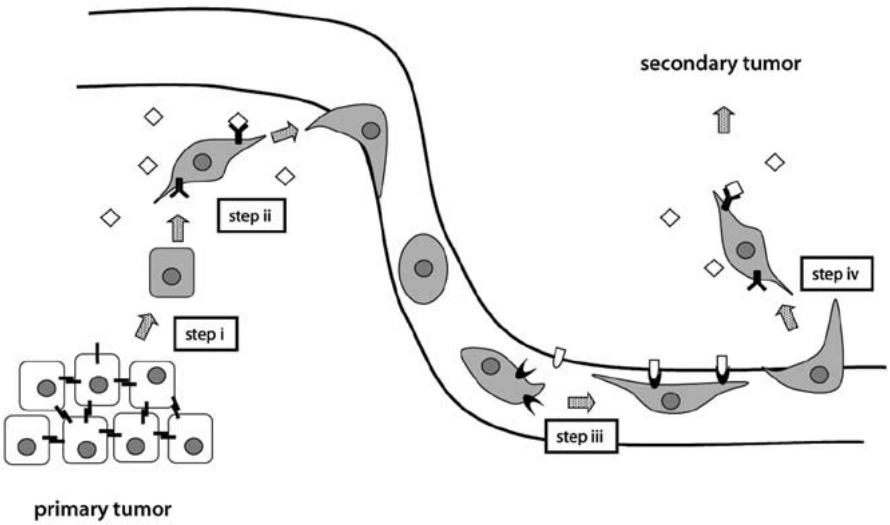
Bioactivity

The metastatic cascade

Cell adhesion molecules play an important role during the progression of tumors, more particularly in the metastatic cascade (Fig. 3). When a benign tumor becomes malignant, cells at the periphery of the tumor will lose cell-cell contact (step I) and invade the surrounding stroma (step II). Cells then extravasate and enter the vasculature or lymphatic system, where they are further transported. A fraction of the circulating tumor cells survives and is arrested at a distant site, attaches to the endothelium (step III) and extravasates through the blood vessel wall and into the surrounding tissue (step IV). Here the tumor cells grow, attract blood vessels and develop to a secondary tumor (→ metastasis).

Adhesive events in metastasis

All the classes of cell adhesion molecules play a role in the metastatic cascade. During the first step, tumor cells need to disrupt intercellular junctions in order to detach from the primary tumor. This step often involves suppression of cadherin function. The second step of migration through the stroma and into the blood vessels requires dynamic cell-substrate adhesion, mostly mediated by integrins. In the third step, where cells arrest in the circulation by aggregation with each other or attachment to platelets, leukocytes and endothelial cells, critical roles have been attributed to cell adhesion molecules of the Ig superfamily, selectins, integrins and specific membrane-associated carbohydrates. The fourth step is similar to step II and mostly involves integrins. Details on the adhesive events associated with metastasis are outlined below.



Cell Adhesion Molecules. Fig. 3 – Cell adhesion processes involved in the metastatic cascade. A subset of cells (grey) growing in a primary tumor will reduce cell-cell contacts (**Step I**) and migrate in the surrounding stroma by increasing specific cell-substrate adhesion (**Step II**). These invasive tumor cells can extravasate into the circulation and, at distant sites, attach to the endothelial blood vessel wall through specific cell-cell interactions (**Step III**). Once these cells have extravasated through the vessel wall they use cell-substrate adhesion molecules to invade the surrounding stroma (**Step IV**). See text for details.

1. In benign epithelial tumors, cells maintain firm intercellular adhesive contacts, mostly by formation of a junctional complex (including tight junctions, adherens junctions and desmosomes). Establishment and maintenance of such a strong junctional complex requires expression and function of cadherins (more particularly E-cadherin). Loss of E-cadherin expression or function appears to be a hallmark of progression of a benign epithelial tumor (adenoma) to a malignant one (carcinoma). Epithelial tumor cells often acquire invasive properties by mutational inactivation of E-cadherin or one of its cytoplasmic binding partners (catenins). It is important to keep in mind that cadherin-mediated adhesion is a dynamic process and that E-cadherin can be temporarily inactivated at the functional level, for example by phosphorylation or other posttranslational modifications.
2. Dynamic cell-substrate adhesion is a critical factor in the migration of invasive tumor cells into the surrounding stroma. Integrins are instrumental in this process. Several studies have correlated the migratory behavior of tumor cells either with an increased or decreased expression of particular integrins. This apparent paradox may be explained by the fact that firm but temporary cell-substrate contacts are required for cells to migrate on a substrate. In order to crawl directionally through the stroma, a cell needs to 'grab' the ECM, release after pulling itself forward and then has to establish the next contact. Both inhibiting adhesion and preventing release of the substrate contacts 'locks' the cell in its position and prevents migration. It should be remembered that integrins may exist in two functional states and that signals passed through the cytoplasm determine whether membrane-exposed integrins are functional or not.
3. In the third step of the metastatic cascade, cell-cell interactions are again the most determining. Homotypic interactions between circulating tumor cells promote formation of aggregates that are preferentially retained in the capillary network. PECAM-1 is a cell adhesion molecule potentially involved in this process. It should be pointed out that (re)expression of the invasion-suppressor mole-

cule E-cadherin would actually promote metastasis formation. Besides these homotypic interactions, heterotypic interactions are also of major importance in the metastatic process. Tumor cells can attach to the blood-vessel wall either directly or indirectly through platelets and leukocytes. The adhesion molecules involved in this process are similar to those involved in the 'multistep adhesion cascade' observed during homing and extravasation of leukocytes or trafficking of lymphocytes. Cell adhesion events include interactions of tumor-associated lectins with selectins expressed on platelets, leukocytes and endothelium (P-, L- and E-selectins, respectively). These adhesion molecules are also involved in the initial transient low-affinity interactions ('rolling') of circulating leukocytes (and probably tumor cells) with the endothelium. Other and more stringent heterotypic heterophilic interactions in this metastatic stage include the binding of integrins on tumor cells to ICAMs expressed on the surface of the endothelial cells.

4. The fourth step in the metastatic cascade is extravasation and invasion at a distant site. This process is very similar to step 2 and similar adhesion molecules are likely to be involved.

Other cancer-related functions of cell adhesion molecules

Recently, it has become clear that some cell adhesion molecules are involved in signaling processes that are relevant to cancer. Germline mutations in E-cadherin predispose patients to the development of diffuse gastric carcinomas, and in lobular breast carcinoma E-cadherin seems to act as a tumor suppressor. Interestingly, β-catenin, a protein cytoplasmically linked to cadherins, has oncogenic properties that are counteracted by the adenomatous polyposis coli (→ APC) gene product. Signaling by integrins can also be an important factor that prevents cells from undergoing apoptosis, which might be critical when tumor cells are traveling in the circulation. Interdisciplinary research has revealed new unexpected functions for known cell adhesion molecules. The suspected tumor suppressor DCC, a member of the Ig superfamily of adhesion molecules, turns out to be the receptor for netrin-1, an axonal chemoattractant crucial in neuronal development. Similar surprising developments are to be expected, especially in view of the increasing impact of gene-knockout technologies.

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Cell Cycle

Definition

Cell cycle, more correctly known as cell division cycle, is a highly ordered series of stages through which a cell progresses in order to duplicate its chromosomes and divide. It is typically divided into four phases:

- G_1 phase (Gap 1)
- S phase (DNA synthesis)
- G_2 phase (Gap 2)
- M phase (mitosis).

Cell-cycle Checkpoint

Definition

The cell-cycle checkpoint is a mechanism for stopping progression through the cell cycle when a key event, such as DNA replication, is not completed or when the genome is damaged. It is a restriction point during the cell cycle in which a cell monitors if preceding events required for cell division have been correctly com-

pleted. It is a regulatory mechanisms that monitors the progression of the cell cycle, so that one phase is not started before another has finished. The activation of checkpoints, for example by damaged DNA, arrests cell cycle progression.

Cell-cycle Targets for Cancer Therapy

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Definition

Knowledge of the molecular mechanisms governing the mammalian cell cycle and their dysfunction in cancer cells has grown considerably in the last decade. It is now clear that the cell utilises two distinct kinds of regulatory mechanisms to control cell-cycle progression: while progression past the restriction point in late G_1 is solely governed by extracellular signals, checkpoints sense cellular damage or dysfunctions that are not compatible with a proper cell division, such as DNA damage. The detailed knowledge of the underlying molecular mechanisms, pathways and molecules provides the basis for a new approach to cancer therapy.

Characteristics

Cell-cycle progression in mammalian cells is controlled through fundamentally different regulatory pathways. Progression through G_1 across the restriction point (R-point) is controlled by external signals that are transmitted, for example, by mitogens or through cell adhesion processes. Beyond this point, cell-cycle progression is governed by a genetic program that is largely independent of extracellular signals but regulated by internally controlled checkpoints. These checkpoints ensure proper DNA replication, DNA integrity, progression through G_2 and mitosis. A central role in cell-cycle progression is exerted by the cyclin-dependent kinases (CDKs), which are composed of a regulatory cyclin subunit (e.g., cyclin A, B, D or E) and a catalytic kinase subunit (e.g., CDK1, 2, 4 or 6). The activity of CDKs is controlled by phosphorylation, phase-specific expression and proteolysis as well as the association with CDK inhibitors (CDIs) belonging to the INK4 (p15, → p16, p18, p19) or KIP (p27, p57)/CIP (p21) families.

Restriction point control

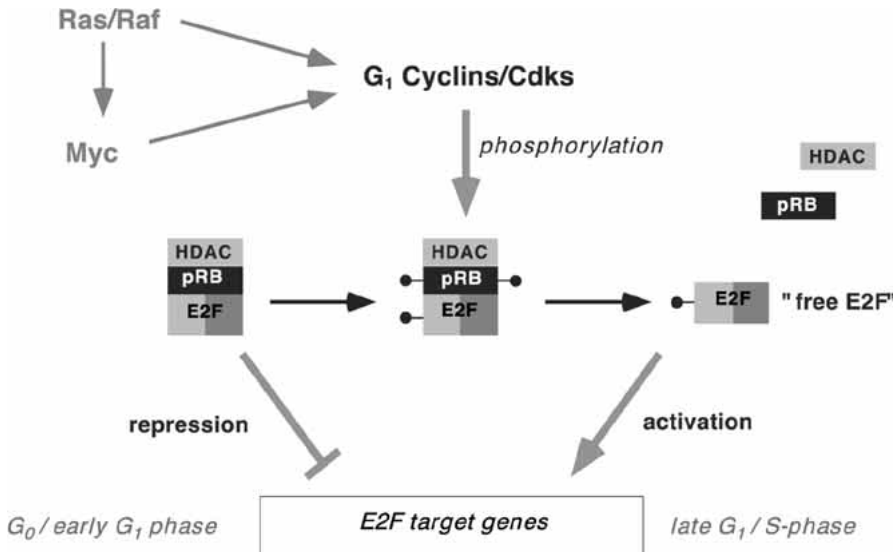
The G_1 CDK-cyclin complexes regulate progression across the → restriction point through phosphorylation of the → retinoblastoma protein Rb and its kins p107 and p130. In early-mid G_1 the transcription factor → E2F is found in complexes with Rb and histone deacetylase (HDAC). These complexes actively repress transcription via E2F binding sites in the respective target genes. The phosphorylation of the E2F-Rb-HDAC complexes by → cyclin D-CDK4/6 and cyclin E-CDK in mid-late G_1 leads to the disruption of these complexes and the generation of transcriptionally active 'free' E2F, which results in the induction of numerous E2F target genes (Fig. 1). The relevance of R-point control for tumorigenesis is emphasized by the fact that the INK4-cyclin D-CDK4-Rb pathway is defective in the vast majority of human tumors due to genetic alteration of its components (Fig. 2). Therefore, this pathway is of major interest with respect to therapeutic intervention.

Checkpoint control

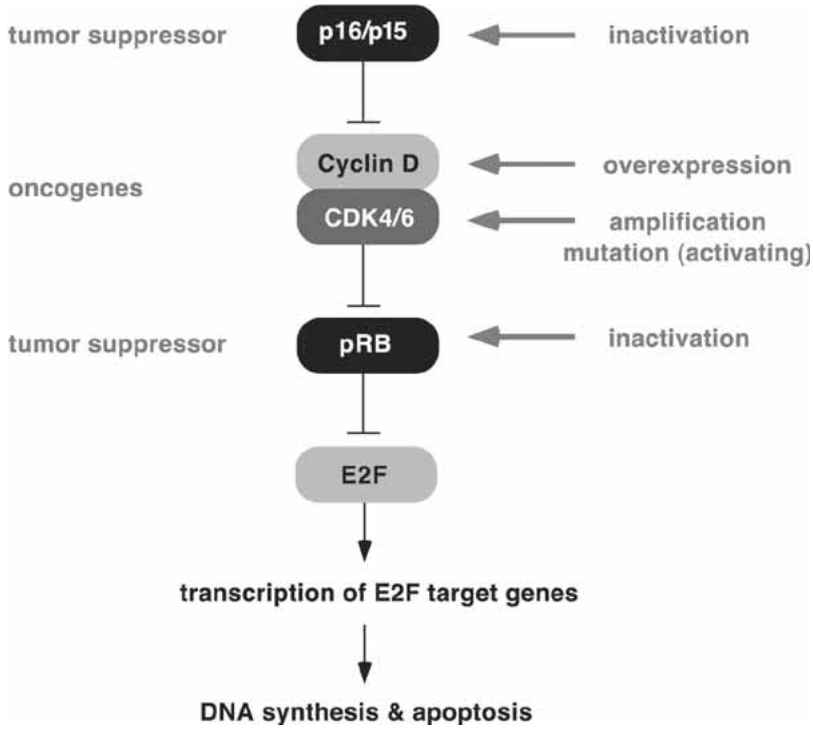
A major role in → checkpoint control is exerted by the p53 tumor suppressor pathway. In response to DNA damage (or other insults to the cell) p53 induces a number of genes that either invoke cell-cycle arrest (such as the CDI p21/CIP) or trigger apoptosis (Fig. 3). The activity and the steady-state level of p53 is regulated by MDM-2, a oncoprotein that associates with p53, inhibits its transcriptional activity and targets p53 for degradation by the proteasome. MDM-2 itself is targeted for proteolysis by the tumor suppressor p14ARF (or p19ARF in mice). The importance of this pathway is demonstrated by the fact that each of its

components can be a target for genetic alterations in human tumors, and that a defective p53 pathway is found in more than 50% of all human malignancies. This emphasizes the relevance of p53 for the development of new anticancer therapies.

Another checkpoint activated in G_2 in response to DNA damage is governed by the checkpoint kinase-1 (CHK1; Fig. 4). →CHK1 phosphorylates the CDC2 (CDK1) phosphatase CDC25C, which results in the association of CDC25C with a p53-induced specific isoform



Cell-cycle Targets for Cancer Therapy. Fig. 1 – The E2F pathway and its regulation by Rb and G_1 CDKs.



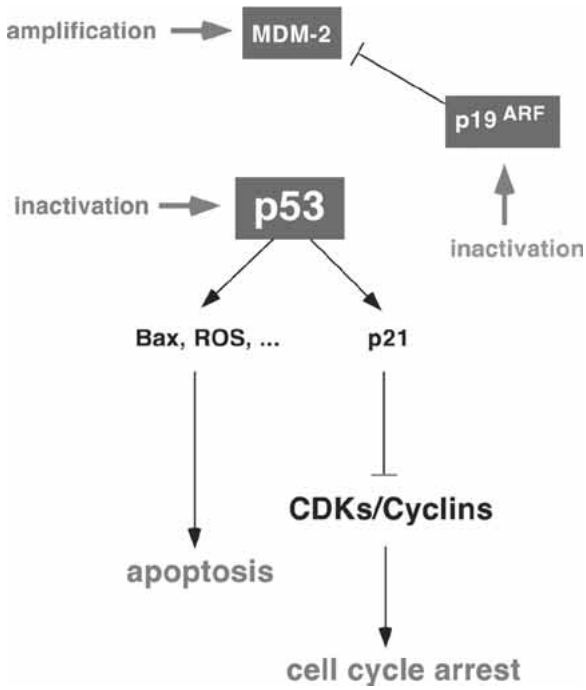
Cell-cycle Targets for Cancer Therapy. Fig. 2 – Deregulation of E2F activity in cancer cells through impairment of the INK4-cyclin D-CDK 4-8211;Rb pathway.

of 14-3-3. This renders CDC25C inactive, so that the cyclin B-CDC2 complex remains in its phosphorylated inactive form. As a consequence, progression into mitosis is prevented and DNA repair can occur. Since many anti-cancer drugs exert their function through DNA damage, this checkpoint may have a negative impact on their efficacy. An analogous checkpoint operating in G_1 has recently been identified. This checkpoint is activated when the CDK2 phosphatase CDC25B is targeted for degradation in response to DNA-damage that will leave the cyclin E kinase in an inactive (phosphorylated) state. As a consequence, cell-cycle progression into S-phase is prevented.

Clinical relevance

Cancer is clearly a proliferative disease resulting from deregulated cell-cycle progression. The inhibition of specific proteins driving the cell cycle is therefore an obvious strategy for the rational discovery of new anti-cancer drugs. In this context it is of particular interest that the interference with coordinated cell-cycle progression can result in apoptosis of tumor cells. This is exemplified by the observation that the deregulated expression of proteins, such as c-→ Myc or E2F-1, in conjunction with a non-physiological cell-cycle block is incompatible with cell survival. It has also been shown that the direct inhibition of CDKs, for example by CDIs or through antisense mechanisms, can trigger programmed cell death in tumor cells. These and other findings have laid the foundation for the definition of a new class of anti-tumor agents that function through a direct inhibition of proteins driving the cell cycle.

One of the prototypes of this class of compounds is the synthetic flavone Flavopiridol. Flavopiridol is a general inhibitor of CDKs, induces cell-cycle arrest and apoptosis, and is not influenced by many of the genetic alterations conferring resistance on human tumor cells. Accordingly, Flavopiridol has shown promising tumor responses in preclinical models and is currently undergoing clinical trials. Numerous other chemical CDK inhibitors have recently been identified and are currently being evaluated for their anti-tumor properties. It can be anticipated that CDK-inhibiting drugs will constitute a new class of powerful chemotherapeutics.



Cell-cycle Targets for Cancer Therapy. Fig. 3 – Loss of p53 function in human cancer cells.

Other interesting targets for therapeutic intervention are the proteins governing checkpoint control, for instance in response to DNA damage. As outlined above, checkpoint control can invoke a transient cell cycle block, but can also trigger apoptosis. Both types of checkpoints are of relevance to tumor therapy. While the functionality of an apoptosis-inducing mechanism in response to drug- or radiation-induced cellular damage is desirable, checkpoint control leading to cell-cycle arrest is counterproductive for any therapy that relies on cell proliferation, such as radiation or conventional therapy.

The p53 checkpoint is lost in many tumor cells, and thus the ability to undergo apoptosis in response to chemo- or radiotherapy. The restoration of this checkpoint could therefore sensitize many tumor cells to conventional therapies. Strategies along these lines involve the development of compounds that can reactivate mutant p53 or inhibit MDM-2, or the use of gene therapeutic approaches for the reintroduction of functional p53 genes.

On the other hand, other drug-based strategies aim to improve the efficacy of existing therapies that rely on DNA-damage, such as radiation or DNA-damaging chemotherapy. A prime candidate in this context is the kinase CHK1 that regulates the G_2 checkpoint (Fig. 4). First results obtained with an inhibitor of the G_2 checkpoint, UCN-01, suggest that this may indeed be the case.

Numerous other mechanisms controlling cell-cycle progression have been discovered, and approaches for therapeutic intervention are being developed, pointing to the great potential of targeting the cell cycle for the development of new anti-cancer drugs. It can be anticipated that this new class of anti-cancer drugs will lead to a clear advance in clinical oncology.

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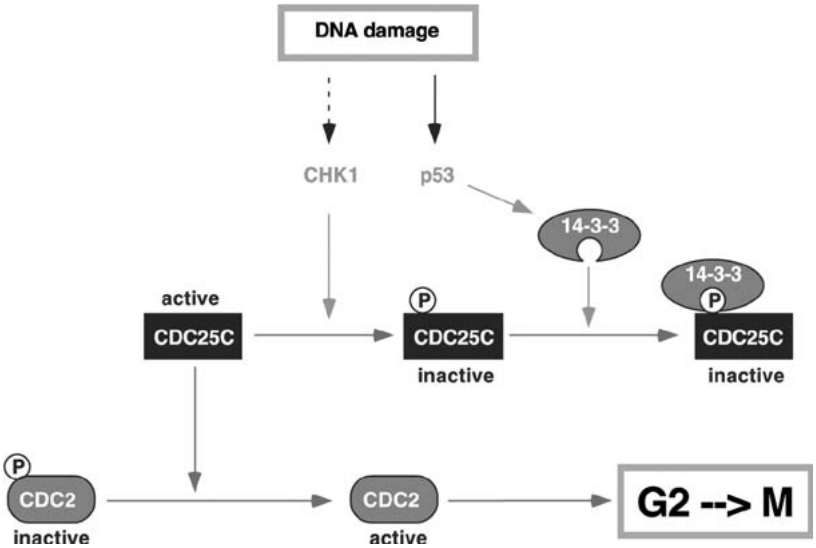

Cell-cycle Targets for Cancer Therapy. Fig. 4 – Regulation of the G_2 checkpoint.

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Cell Line

Definition

The term cell line is not always used consistently, but usually refers to immortalized cells. Cells with a limited proliferative capacity ('mortal' cells, especially normal cells) are referred to as a cell strain.

CentiMorgans

Definition

CentiMorgans is the measurement between markers on a genetic map; two markers are said to be 1 cM apart if they are separated by recombination 1% of the time, roughly equal to a distance of 1 million bp.

Central Neurofibromatosis

Definition

→ Neurofibromatosis 2.

Centric Fusion

Definition

→ Robertsonian translocation.

Centromere

Definition

A centromere is a constricted region of a chromosome that includes the site of attachment to the mitotic or meiotic spindle. It divides the chromosome into a short 'p' and a long 'q' arm.

Cervical Cancers

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Definition

The regions of the uterus are the corpus and the cervix. Cancer originating from the cervix is defined as cancer of the cervix. When cancers are simultaneously detected in the cervix and corpus, squamous cell carcinoma is designated as a cancer of the cervix and adenocarcinoma is designated as a cancer of the corpus. When cancer occupies both the cervix and vagina without the junctional area (the fornix), the cancer extending to the exocervix is recognized as a cancer of the cervix. Thus, cervical cancer is defined apart from cancer of the uterine corpus (cancer of the uterine endometrium) and cancer of the vagina.

Characteristics

The main gynecological cancers originate from the cervix, endometrium and ovary. Among them, cervical cancer is the most common malignancy in women.

Main risk factors are:

- young age at first intercourse, especially shortly after the menarche,
- high number of sexual partners,
- high number of sexual partners of the partner,
- high number of children,
- excessive douching.

Smoking appears to increase the incidence of squamous cell carcinoma, but not of adenocarcinoma or adenosquamous carcinoma. Immunosuppression by smoke-derived nicotine and its metabolite cotinine in the cervical mucus may enhance the effects of sexually transmitted disease (→ STD) including → human papillomavirus (HPV) infection. Most epidemiological risk factors for cervical cancer are associated with STDs. HPV induces a STD, human venereal condyloma, which is associated with cervical, vaginal and vulvar dysplasia, and invasive carcinomas. HPV particles and DNA, especially HPV-16, HPV-18, and HPV-33, are detected in cervical and vulvar dysplasia and in invasive carcinomas. Additionally, it has been demonstrated that HPV transforms human cell lines. HPV infection of the cervix is a main etiology of cervical cancer.

Symptoms

Main symptoms of cervical cancer are:

- vaginal bleeding, which may be recognized as postmenopausal bleeding, irregular menses or postcoital bleeding;
- abnormal vaginal (watery, purulent or mucoid) discharge.

In advanced cases, corresponding local symptoms occur. A → Pap smear even in unsymptomatic cases is useful for the early detection of cervical dysplasia and cancers. Among women over the age of 18 who have had sexual intercourse, high-risk women should be screened at least yearly.

Pathology

Histopathological types in cervical cancers are mainly squamous cell carcinoma and adenocarcinoma, which account for about 90% of all cervical cancers (adenosquamous carcinoma, glassy cell carcinoma, adenoid cystic carcinoma, adenoid basal carcinoma, carcinoid, small cell carcinoma and undifferentiated carcinoma also occur). Squamous cell carcinomas are keratinizing or nonkeratinizing in most cases and may be verrucous, condylomatous, papillary or lymphoepithelioma-like carcinomas in a few cases. Adenocarcinomas are classified into mucinous, endometrioid, clear cell, serous and mesonephric adenocarcinomas; mucinous adenocarcinomas are subclassified with endocervical type into adenoma malignum and villoglandular papillary adenocarcinoma, and intestinal type adenocarcinoma.

Staging

Clinical staging represents the degree of advancement of the tumor, and is defined by the → FIGO classification established in 1994 and by the TNM classification of malignant tumors set by the → UICC in 1997 as follows (classified by FIGO [→ TNM]):

- Stage 0 (Tis): carcinoma *in situ* (preinvasive carcinoma).
- Stage I (T1): cervical carcinoma confined to the uterus.
- Stage II (T2): tumor invades beyond the uterus but not to the pelvic wall or to the lower third of the vagina.
- Stage III (T3): tumor extends to the pelvic wall and/or involves the lower third of the vagina and/or causes hydronephrosis or non-functioning kidney.
- Stage IVA (T4): tumor invades the mucosa of the bladder or rectum and/or extends beyond the true pelvis.
- Stage IVA (M1): distant metastasis.

Stage IA (T1a) has been further classified by microinvasive depth and width into stage IA1 (T1a1) (depth of stromal invasion $\leq$3 mm, horizontal spread $\leq$7 mm) and stage IA2 (T1a2) (depth of stromal invasion $>$3 mm, $\leq$5 mm; horizontal spread $\leq$7 mm). Stage IB (T1b) has been further classified by tumor size into stage IB1 (T1b1) (greatest dimension $\leq$4 cm) and stage IB2 (T1b2) (greatest dimension $>$4 cm). In cases staged IA2 (T1a2) or less advanced, colposcopically directed biopsy in the transformation zone of the cervix, endocervical curettage or cervical conization are required.

Prognosis

Unfavorable prognostic factors include younger age, advanced clinical stage, certain histopathological types, vessel permeation, large tumor volume, parametrium involvement and lymph node metastasis. Nodal metastasis is an especially critical prognostic factor after curative resection. For many years there was no prognostic indicator for lymph node-positive cervical cancer patients. However, recently it was demonstrated that platelet-derived endothelial cell growth factor → PD-ECGF contributes to the advancement of metastatic lesions and that the PD-ECGF level in metastatic lesions is a prognostic indicator. Furthermore, serum PD-ECGF level reflects the status of advancement of cervical cancers and is recognized as a novel tumor marker for both squamous cell carcinoma and adenocarcinoma of the cervix, while the tumor marker → SCC is well known only as an indicator for squamous cell carcinoma of the cervix.

Therapy

The treatment for cervical cancer consists mainly of surgery and radiation. Chemotherapy is proceeded in combination with surgery and/or radiation for advanced cases, and immunotherapy is an adjuvant treatment for surgery, radiation and chemotherapy. The standard treatment for carcinoma *in situ* is → cervical conization or total → hysterectomy. The standard treatment for microinvasive carcinoma stage IA (T1a) is modified radical hysterectomy regardless of regional lymphadenectomy. The standard surgical treatment for invasive carcinoma is radical hysterectomy with regional lymphadenectomy. In more advanced cases, extended radical hysterectomy or pelvic exenteration is appropriate. After surgery external irradiation is followed in some cases. The standard radiotherapy without surgery for invasive carcinoma is intra-cavitary and/or external irradiation. Recently, → neoadjuvant therapy (chemotherapy) has been tried in order to make surgery more successful, and concurrent radiochemotherapy has been tested for the purpose of enhancing the effect of radiation.

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Cervical Conization

Definition

Cervical conization is the conic resection of the cervix for diagnosis and treatment of the early stage of → cervical cancers.

CGL-1

Definition

→ Granzyme B.

Chaperone

Definition

A chaperone is a protein that recognises other proteins in a certain conformation (usually unfolded) and supports conformational changes (usually folding). It is needed for the assembly or proper folding of another protein, but which is not itself a component of the target complex.

Checkpoint

Definition

Checkpoints represent intrinsic mechanisms that are activated when cell-cycle progression

would be detrimental to the cell, as in case of DNA-damage, incomplete DNA synthesis, metabolic dysfunctions or mitotic spindle damage. In such cases, cell-cycle progression is transiently halted until the respective problem is fixed, for instance by the DNA repair machinery. Major checkpoints are governed by the tumor suppressor gene product → p53 or the check point kinases → CHK1 and CHK2.

Checkpoint Kinases

Definition

Checkpoint kinases are a group of at least four protein kinases (→ ATM, → ATR, → CHK1 and → CHK2) and their relatives, which play an important role in the mechanisms that sense and signal DNA damage, culminating in the activation of cell-cycle checkpoints and DNA repair.

CHEK1

Definition

CHEK1 (checkpoint kinase 1), also known as → CHK1 is a serine/threonine protein kinase of 476aa and 54kD that is involved in cell cycle arrest following DNA damage (→ checkpoint). It is a ubiquitously expressed nuclear protein that binds and phosphorylates → cdc25 (a-c). The gene maps to 11q22-23.

Chemokines

Definition

Chemokines are a sub-family of → cytokines that are involved in cell recruitment.

Chemoprevention

Definition

Chemoprevention is the use of pharmacological or natural agents that inhibit the development of invasive cancer either by blocking DNA damage, such as from → DNA adducts, that initiates carcinogenesis or by arresting or reversing the progression of premalignant cells in which such damage has already occurred. This is also one of the final expected benefits of a healthy diet. Thus, dietary prevention can be considered to be a complex form of chemoprevention; → cancer chemoprevention.

Chemotaxis

Definition

Chemotaxis is the migration of cells in response to a soluble gradient of chemo-attractants.

Chemotherapy

Definition

Chemotherapy is a kind of therapy that uses chemical compounds to fight neoplastic disease.

Chemotherapy of Cancer, progress and perspectives

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Definition

Chemotherapy is defined as the use of chemical agents for treatment. Chemotherapy as used for cancer generally refers to small molecules that

damage proliferating cells. It represents systemic treatment in contrast to radiotherapy and surgery that represent local treatment. Classes of systemic agents may also include hormones, cytokines and vaccines.

Characteristics

The challenge

Cancer is the most feared, morbid and mortal of diseases. In the United States five million people contract cancer per year, of whom one-third, or almost half a million citizens will die of their disease. Most cancers start in a specific location (e.g. breast, lung) and spread to regional lymph nodes; in breast cancer spread is to the armpit and subsequent dissemination by the bloodstream to distant organs. For cancers that are diagnosed before such dissemination, local treatment with surgery and/or radiotherapy may be curative. Most patients who die of cancer die because of disseminated metastatic tumor. These are either clinically present at the time of diagnosis or occur months to years after diagnosis because of microscopic clinically undetectable cancer that only becomes clinically evident following local treatment. Cancer chemotherapy along with hormone therapy and immunotherapy is designed to treat and ideally eradicate metastatic cancer.

The agents

Most of the currently effective chemotherapeutic agents were discovered by serendipity and/or empiricism (by trial and error). For example, the first effective agent, nitrogen mustard, was a derivative of chemical warfare studies conducted in World War I. Among the side effects of mustard gas was the suppression of normal bone marrow. Because of this, it was given to mice bearing a tumor derived from the bone marrow, i.e. leukemia, and found to be effective. Subsequent clinical trials affirmed this effectiveness. Analogs were synthesized and mustard-like compounds, termed alkylating agents, are effective in many forms of cancer.

The antimetabolites are compounds that are similar to normal metabolites, such that they enter the same metabolic system but because of slight differences, inhibit or antagonize that system. For example, white cells consume high quantities of the vitamin folic acid, and this is particularly true of cancerous white cells, that is leukemic cells. Slight chemical modifications of folic acid have lead to the antifol class of compounds, and these have been found to be active in patients with leukemias, as well as in many solid tumor patients.

Two important classes of compounds are

1. the anthracyclines and
2. the platinum analogs

both of which were discovered by serendipity, and developed largely through screening methods. All of the above compounds target DNA and therefore cell proliferation. Another class of compounds target the cytoskeleton of the tumor cell. These are derived from fungi and plants, and include the vinca alkaloids and the recently discovered → taxol.

These examples of currently used agents, while varyingly effective against human cancers (see below), have a significant limitation in the area of specificity. They attack not only the tumor but also certain rapidly growing normal tissues such as the bone marrow and bowel, and hence produce dose-limiting toxicity relating to depression of the marrow (infection and bleeding), nausea and vomiting and ulceration of the gastrointestinal tract.

The use of high dose combination chemotherapy with stem cell rescue for patients with breast cancer was the subject of considerable enthusiasm during the 1980s and early 1990s. However, in the late 1990s and particularly since the American Society of clinical Oncology (ASCO) reports in 1999 have been considered to be largely ineffective. This paper considers some of the reasons for this change and particularly on the basis of preclinical and clinical models, considers current and future directions. The intensification regimen may produce resistance that could compromise the important intensification component. Microenvironmental and clinical trials of adjuvant che-

motherapy strongly indicate that one cycle of intensification is not enough and that two and perhaps three will be required. The components of the intensification regimen are reviewed with respect to dose response and with respect to mechanisms of resistance, cross resistance and potential additive or synergistic effects.

To reiterate, the major limitation of classical cancer chemotherapy is the lack of specificity for the tumor as compared to normal tissue. This limitation is being addressed by basic science particularly relating to molecular biology, a summary of which follows:

Cancer is a genetic disease of somatic cells, following a series of mutations or genetic events incident to lifestyles such as smoking. (→ tobacco carcinogenesis) and genetic susceptibility, a sufficient number of events occurs such that a cell becomes transformed into a cancer cell. The vast majority of cancers therefore derive from a single cell. However, the process that produces cancer also results in a marked increase in genetic instability such that daughter cells are variable. This variation permits selection of those daughter variants that have a survival advantage, such as resistance to certain drugs, a higher proliferative thrust or a greater capacity to invade and metastasize. This clonal evolution to heterogeneity is adverse. However, it does lead to events that are unique to the cancer cell, that is, they are not present in normal cells in the same person. For example, chronic myelogenous leukemia is due to white cells being driven to cancer behavior by a product of the fusion of two genes. By advanced pharmacologic techniques including designer drug synthesis and high throughput screening, an agent termed STI571 was developed that inhibits the action of the fusion gene protein product. STI571 has been found to be capable of producing complete regression of leukemia in the majority of patients, and in contrast to essentially all chemotherapy, is nontoxic. There are a number of molecular targets in other tumors that have been identified, and academia and the pharmaceutical industry have given major priority to the development of agents capable of selectively attacking and inhibiting such molecular targets. It is this process more than any other that leads to optimism on the part of cancer investigators concerning the future of cancer treatment.

Clinical strategies

Although there are numerous different types of cancer, they often share many biological and molecular processes, an important feature to keep in mind.

In the late 1950's a series of integrated clinical trials were conducted. As a result, the cure rate for childhood leukemia increased from 0% to 70% and many scientific principles of cancer therapy were established. These were (in chronological order):

1. The application of quantitative clinical trials, involving comparisons and randomizations.
2. The use of agents in an appropriate combination can increase the complete remission rate from zero to more than 90%.
3. The generation of complete remission as the most powerful discriminant for survival.
4. The identification of active agents in an experimental model with the duration of complete remission (DCR) being central parameter of response.
5. The use of the DCR model to develop and evaluate optimal doses, schedules, and combinations.
6. It was observed that meningeal leukemia occurred with increasing frequency in patients with prolonged complete remission. Pharmacological and clinical trial studies found this be due to the failure of standard antileukemia agents to cross the blood/brain barrier. The introduction of intrathecal chemotherapy and radiotherapy to the brain, markedly reduced this type of complication.
7. The importance of supportive and symptomatic care; for example, platelet transfusions, antibiotics and anti-emitics, markedly reduced the morbidity and mortality of chemotherapy.

The result of these advances was an increase in the cure rate for childhood leukemia from 0% in 1955, to 35% by 1970, up to 80% within the last 15 years. This experience with childhood

leukemia had a profound effect on the field of cancer chemotherapy in general. Most importantly, it established the position that it can be done, that is, systemic cancer could be cured by systemic therapy.

Most patients who die of cancer die because of disseminated metastatic tumor. Today, in major centers and cooperative groups the cure rate of childhood leukemia is close to 80%. It was hoped that the solid tumors would follow on closely behind the leukemias, but they have in the main proven more difficult to treat. This is unfortunate as adult solid tumors such as breast, bowel and lung cancer constitute 80% of all cancer. A major strategy has been to use agents in combination. This is because solid tumor cells are heterogeneous and thus they have multiple targets. The second rationale for combination chemotherapy is that it works, with essentially all highly effective and certainly curative cancer chemotherapy involving combinations. The best of combinations produce partial responses in 30% to 50% of patients with, for example, metastatic cancer of bowel and lung. Complete tumor regression rarely occurs.

It has long been known that in experimental tumors, for example in mice, tumor burden is critical to chemotherapeutic effect. Thus, chemotherapy that has a minor effect on palpable tumor, is often curative of the same tumor in microscopic form. This led to the strategy known as adjuvant chemotherapy. Here patients known to be of high risk of having micrometastatic cancer at the time of initial treatment, are given chemotherapy immediately following surgery and/or radiotherapy. This increases the cure rate some 20% for breast and large bowel cancers. In a more recent strategy (termed neoadjuvant chemotherapy), chemotherapy is given prior to surgery. This moves chemotherapy still further forward in the disease. It provides shrinkage of the primary tumor and thus facilitating the use and effectiveness of local treatment. It may decrease the need for radical surgery in certain tumors such as head, neck and bladder cancer. Finally, combination chemotherapy can be given concurrently with radiotherapy as initial treatment. In addition to the above advantages, local control may be superior with many chemotherapeutic agents since some of them, particularly the platinum analogs and fluoriuracil, are highly radiosensitizing.

In addition to combination chemotherapy, dose is a significant factor in cancer chemotherapy. The dose of certain chemotherapeutic agents, particularly the alkylating agents, can be substantially increased if one protects the bone marrow. Protection is provided by harvesting marrow stem cells before the high dose chemotherapy and returning the marrow to the patient following chemotherapy. Such peripheral blood stem cell rescue has been effective in the leukemias and lymphomas, and is under study often in combination with some of the above-mentioned strategies in selected solid tumors.

Supportive care. Bone marrow or peripheral blood stem cell transplantation is one form of supportive care. The first major advance in supportive care involved platelet transfusions for the treatment and prevention of thrombocytopenic hemorrhage, starting in the late 1950s and early 1960s. The nausea and vomiting associated with cancer, and some forms of cancer treatment, has been markedly reduced by the development of anti-emetics. Pain control has markedly improved and radical surgery has been reduced by neoadjuvant approaches.

Long term effects of cancer treatment. Perhaps the most worrisome long-term effect has been the development of second cancers, particularly leukemia and leukemia-like illnesses. There is a latent period of 5-10 years for most of these secondary cancers, and for solid tumors it is even longer. Clinical treatment, environmental and genetic factors, and *in vitro* and *in vivo* laboratory models are being developed to study these events. The alkylating agents and X-ray are the chief offenders. → Hodgkin disease was found to be curable by strategies similar to that of acute lymphocytic leukemia, but there was a cumulative long-term risk of secondary cancer. With this knowledge and the development of newer active agents for Hodgkin's disease, the combination regimen that included alkylating agents has been modified without loss of effectiveness, but with major diminution in secondary cancers.

Conclusions. With a marked increase in support for cancer research including both basic and clinical and the extraordinary increase in molecular sophistication of such research, it is expected that major progress in the curative treatment of most, if not all cancers, will be achieved in the next decade. It is the clinical scientist who must translate this progress in basic research to the clinic. This ultimate challenge would require the most sophisticated of treatment methodology and must always be conducted in a setting where the primary beneficiary of such research is the patient.

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Chimaerins

Definition

α and β chimaerins resemble chimeras between the regulatory domain of PKC and the break point cluster region (→ BCR), involved in the translocation of → Philadelphia chromosome in chronic myelogenous leukemia.

CHK1

Definition

CHK1 (ckeckpoint kinase 1), also known as → CHEK1, is a protein kinase that controls a checkpoint in G2. CHK1 is activated by DNA damage and phosphorylates the protein phosphatase CDC25C. This prevents the CDC25C-mediated activation of the CDC2-cyclin B complex and thus M-phase entry; → cell-cycle targets for cancer therapy.

CHK2

Definition

The *CHK2* (checkpoint kinase 2) gene encodes the human homolog of the yeast Cds1 (for checking DNA synthesis 1) and Rad53 G_2 checkpoint kinases, whose activation in response to DNA damage prevents cellular entry into mitosis. The gene maps to 22q12 and heterozygous germ line mutations occur in → Li-Fraumeni syndrome.

Cholangiocarcinoma

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Synonyms

- bile duct carcinoma
- Klatskin tumor
- cholangiocellular carcinoma

Definition

An adenocarcinoma arising from the epithelium of the intrahepatic or extrahepatic bile ducts. The term 'cholangiocarcinoma' distinguishes this tumor of the biliary tract from the more common gallbladder carcinoma. In Japan, 'cholangiocarcinoma' refers most typically to intrahepatic carcinomas, developing proximal to the bifurcation of the right and left hepatic ducts.

- 95% of bile duct tumors are adenocarcinomas,
- the remainder are a mix of squamous, carcinoid, sarcomatous and mixed cholangiocellular/hepatocellular tumors.

Here, cholangiocarcinoma is defined as the adenocarcinoma cell type of either intrahepatic or extrahepatic bile duct origin.

Characteristics

Etiology

Cholangiocarcinomas represent 10% of all primary liver tumors. Cholangiocarcinomas occur with an approximate 1:100,000 incidence in the Western world but are more common in Southeast Asia and Japan. There is a slight male preponderance, with tumors occurring most frequently in the seventh decade of life. More than 90% occur sporadically in an otherwise normal liver. Risk factors for the development of cholangiocarcinoma include → primary sclerosing cholangitis (PSC), congenital dilatation of the biliary tree (i.e. Caroli's disease), → hepatolithiasis and chronic infestation of the biliary tree by the parasitic → liver flukes *Opisthorchis viverrini* and *Clonorchis sinensis*. Infection with these parasites is endemic in some parts of Southeast Asia, and in part may contribute to the higher incidence of cholangiocarcinoma in this region. These conditions all have in common chronic inflammation of the biliary tree or bile stagnancy leading to the development of cholangiocarcinoma. A few associations with environmental factors have been postulated. Epidemiological studies have shown high levels of dietary nitrosamines to be a risk factor for cholangiocarcinoma. A past history of receiving thorium dioxide, an α-particle emitting radiocontrast agent, has been linked to later cholangiocarcinoma development. Some studies have shown smoking and alcohol use to be risk factors for the development of cholangiocarcinoma in patients with primary sclerosing cholangitis, however, subsequent studies have shown no correlation.

The mechanistic link between inflammation and the development and progression of cholangiocarcinoma is of interest. Several inflammatory mediators have been shown to stimulate cholangiocyte proliferation, inhibit DNA repair or block → apoptosis - all factors important in carcinogenesis. Indeed, interleukin-6 (IL-6) is a potent cholangiocyte mitogen and drives proliferation of this cell type via the MAP-kinase pathway. Proinflammatory cytokines induce cholangiocyte DNA damage and inhibit DNA repair by a nitric oxide-dependent mechanism. In addition, immunohistochemical studies of human cholangiocarcinoma specimens have shown the ubiquitous presence of → inducible nitric oxide synthase (iNOS). Thus IL-6 and iNOS with nitric oxide generation in chronically inflamed tissues likely contributes to the initiation and progression of cholangiocarcinoma.

Genetic changes

Numerous chromosomal and genetic abnormalities have been catalogued in cholangiocarcinomas but vary between geographic regions, ethnic populations and predisposing conditions. Chromosomal loss at 9p21 and inactivation of tumor suppressor gene p16 has been detected in PSC-associated cholangiocarcinoma. → Telomerase activity has been observed in the majority of malignant and dysplastic but not normal biliary epithelial cells. Mutations in p53 and K-ras are among the most frequent in both sporadic and PSC-associated cholangiocarcinoma. Detection of K-ras mutations in blood, bile, cytological specimens or stool may be useful in detecting cholangiocarcinoma at an early stage. Mutations in → *MET* and → *ERBB2* have also been observed. Over-expression of the anti-apoptotic protein → Bcl-2 has been seen in cell lines of human cholangiocarcinoma. Nonetheless, there is no clear relationship between any of these abnormalities and histologic grade or prognosis, making judgments as to the existence of any causal relationship speculative at best.

Diagnosis

The great majority of patients present with jaundice alone. Others will present with abdominal pain, weight loss or cholangitis. Laboratory evaluation will show elevated bilirubin and alkaline phosphatase levels consistent with obstructive cholestasis. Serum tumor markers are also sensitive and specific for cholangiocarcinoma. A serum → carbohydrate antigen

19-9 (CA 19-9) level (although not specific only for bile duct carcinoma) greater than 100 units per milliliter is suggestive of the diagnosis. Imaging studies may show a tumor mass but in most cases the finding of dilated bile ducts leads to the performance of cholangiography. At this time, an intraductal mass or diffuse stricture may be seen. These strictures can mimic many benign conditions. Brush cytology or needle biopsy is performed for histologic confirmation, but histologic confirmation is often difficult due to the desmoplastic nature of these cancers.

Histologically, cholangiocarcinomas are usually nodular or diffuse and infiltrating in their gross appearance. Less than 10% have a papillary appearance that is associated with a slightly more favorable prognosis. Often it is difficult to distinguish benign schirrous reactions from malignant processes. Small collections of tumor cells complete with glandular elements may be scattered amongst a matrix of collagen and fibroblasts and have an outwardly benign appearance. Special stains for mucin and → carcinoembryonic antigen (CEA) often aid the pathologist in making this distinction. Although the majority of cholangiocarcinomas produce mucin, a small percentage of tumors may produce vast quantities of mucin and are designated as the 'mucinous' subtype, which portends an adverse prognosis.

Classification

Tumors are also classified according to the site of origin. Two-thirds of cholangiocarcinomas occur in or near the large ducts of the hilum of the liver. One quarter occur in the distal common bile duct, and the remainder originate in the intrahepatic bile ducts. Prognosis correlates strongly to the American Joint Committee Classification staging (TNM) for cancer. The site of origin has important implications as to the stage at presentation, type of resection planned and likelihood of cure after surgical resection. If resectable, intrahepatic (sometimes called peripheral) cholangiocarcinomas are found to have the highest cure rates. Although more than 50% of perihilar cholangiocarcinomas are resectable at discovery, only 15%-20% of patients are long-term survivors. Distal bile duct tumors have the highest resectability rates and have an intermediate, albeit still poor, long-term survival. Treatment failure in all cases is most often due to failure to achieve tumor-free margins, loco-regional recurrence and peritoneal metastasis. Distant metastases are less common.

Therapy

No type of cholangiocarcinoma is sensitive to chemotherapy alone. Adjunctive radiation therapy combined with radiosensitizing 5-fluorouracil based regimens can be palliative and prolong median and long-term survival in patients who are not cured by surgery. These measures are generally regarded to be of modest benefit. Intensification with intraoperative radiation or intraluminal → brachytherapy may marginally improve survival. → Photodynamic therapy with biliary stenting may provide longer relief from obstruction and liver failure than conventional biliary stenting alone. Rarely, selected patients are candidates for orthotopic liver transplantation. Death in all cases other than transplantation is usually due to liver failure secondary to obstruction or septic cholangitis.

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Cholangiocellular Carcinoma

Definition

Cholangiocellular carcinoma is an adenocarcinoma arising from the small, intrahepatic bile ducts of the liver. Although occasionally still used, it has largely been replaced by the more common nominatives intrahepatic or peripheral → cholangiocarcinoma.

Cholera Toxin

Definition

Cholera toxin is an exotoxin from *Vibrio cholerae* which ADP-ribosylates the α-subunit of → G-protein G_s at arg201 leading to the constitutive activation of the α-subunit by blockade of its GTPase activity. Modification of $G\alpha_s$ in epithelial cells of the gut leads to an increase in cAMP levels that causes watery diarrhea.

Chromatids

Definition

Chromatids are the copies of a chromosome produced by replication. The term is usually used to describe chromosomes in the period before they separate at the subsequent cell division.

Chromatin

Definition

Chromatin is the complex of DNA and protein in the nucleus of the interphase cell. It was originally recognized by its reaction with stains specific for DNA. Modern approaches of → interphase cytogenetics can visualize chromosomes at interphase.

Chromophore-assisted Laser Inactivation

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Synonyms

- CALI

Definition

Technology to address protein function *in situ*. CALI uses → laser light of 620 nm, targeted *via* specific Malachite Green-labeled non-function-blocking antibodies, which generate short-lived protein-damaging free radicals (Fig. 1). This wavelength is not absorbed by cells, such that nonspecific light damage does not occur. The short lifetime of the free radicals generated restricts the damage largely to the bound antigen (~15 Å) such that even neighboring proteins are not significantly affected. Micro-CALI focuses the laser light through microscope optics such that proteins within a 10 micron spot may be inactivated.

Description

The advent of complete genomic information will herald a new revolution in molecular biology to develop a mechanistic understanding of how proteins function together in the living cell. This increased understanding will provide insight into cancer (as well as other diseases) and potentially will help define protein targets for drug discovery. Target validation of proteins of disease relevance (the site of most drugs) is the limiting first step in obtaining drugs of clinical value. In particular, identifying proteins that have essential roles in cancer-relevant cellular processes remains a major challenge. There is a current lack of technology that addresses protein function directly and rapidly, as most functional inactivation approaches target genes or mRNAs. One useful tool to address this is Chromophore-Assisted Laser Inactiva-

tion (CALI). CALI uses targeted laser light to inactivate proteins of interest via a dye-labeled antibody that by itself does not block function. CALI provides a high degree of temporal and spatial resolution to acutely perturb protein function *in situ*.

Advantages

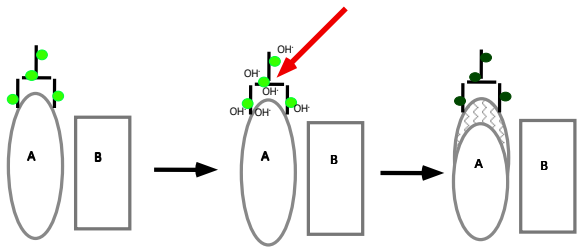
The major advantages of CALI compared to other functional inactivation approaches are its unprecedented temporal and spatial resolution. The range of area for CALI-based inactivation is localized to regions within the beam, between microns to millimeters depending on the focus of the laser. Inactivation occurs acutely upon initiating laser irradiation. Because the loss of protein function is acute and transient, CALI does not appear to be subject to genetic compensation that is occasionally observed in chronic deletion strategies such as gene knockouts in mice. CALI is particularly useful for systems lacking genetic methods. For example, CALI may be used for human tissue culture cells that are disease-relevant. As such, it is not necessary to extrapolate target validation from model systems. Many proteins of interest to cancer research have essential roles in early development and are thus difficult to address by gene knockout. CALI may be used to study the roles of these essential gene products in cellular processes after development is completed. The coupling of micro-CALI with real time imaging has been used for studying dynamic cellular processes. The application of micro-CALI on part of a single cell generates a transient asymmetry of function across a cell, and this has been particularly useful in addressing proteins required for cell motility and migration. The capacity and relative ease of multiplex approaches for CALI (compared to gene knockouts) may lend itself well to whole → proteome approaches and → high throughput screens.

Limitations

As with any technology, CALI has its limitations, and a clear understanding of these is required for its judicious application. Inactivation is dependent on quality, specificity and site of antibody binding and also on the susceptibility of the targeted protein to free radical damage. It should be noted that only the protein (not the gene) is inactivated and hence recovery is dependent on *de novo* synthesis. This usually allows a loss of function of hours to perhaps a day. The loss of function may not be complete so that activity is 'knocked down' as opposed to 'knocked out'. As such, residual activity may obscure a potential phenotype. In general, a negative result is difficult to interpret for CALI as with most other inactivation approaches.

Current applications

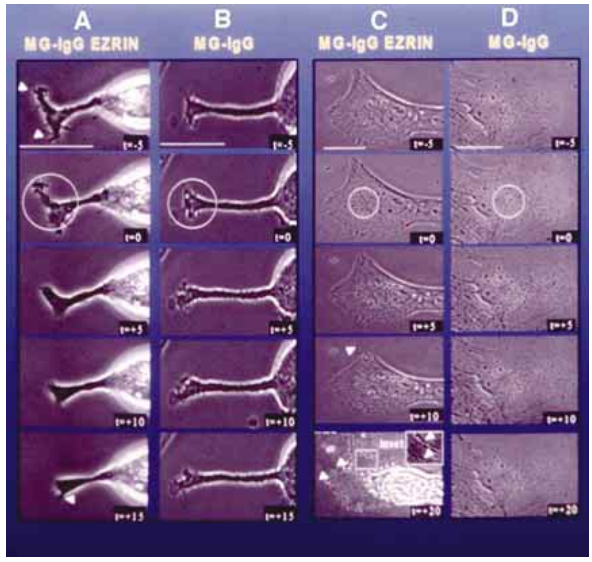
CALI has been used for over 50 proteins and been successful in approximately 90% of these cases. CALI has precisely mimicked *Drosophila* genetic loss of function mutations in several direct comparisons. The proteins studied in many



Chromophore-assisted Laser Inactivation. Fig. 1 – The principle of CALI. Specific proteins (A) in cells are bound by an antibody and labeled with the chromophore Malachite Green (MG). Irradiation with pulsed laser light of 620 nm (red arrow) generates short-lived hydroxyl radicals. These radicals selectively inactivate the bound protein by oxidative damage because their half-maximal radius is about 15 Å due to their short life time in cells. Neighboring proteins (B) are not significantly affected.

cell and animal systems span a diverse array that include membrane receptors, cytoskeletal proteins, signal transduction molecules and transcription factors. The understanding of how proteins function in the nerve growth cone has been a major area of study that has utilised CALI. The functional roles of proteins such as NCAM, L1, calcineurin, talin, vinculin, myosin V and Ib, radixin and tau have been recently addressed. For these studies, methods for introducing antibodies into living cells have been optimized including electroporation, trituration and microinjection.

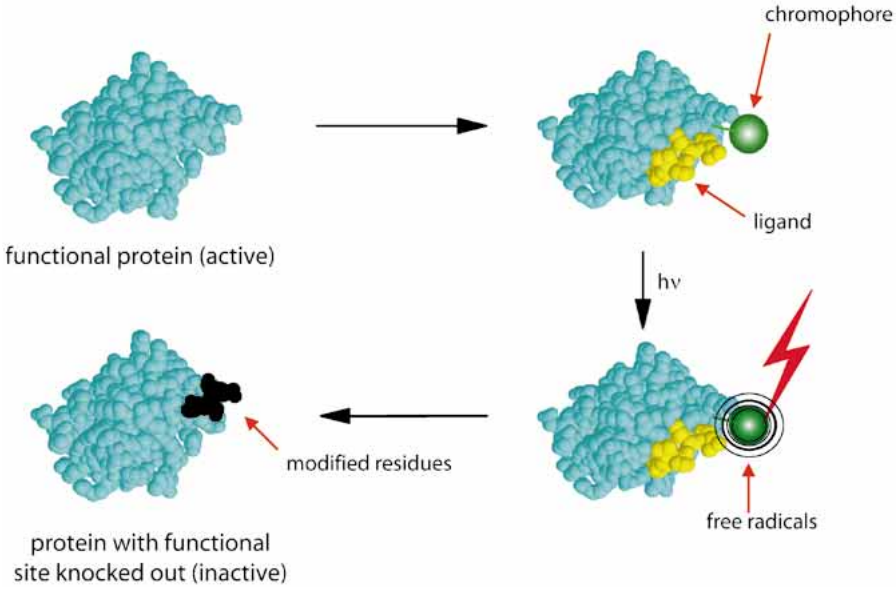
Of particular relevance to cancer research is the application of CALI to proteins that have roles in cancer cell migration. One example of the application of CALI to a protein of cancer relevance is the prototypic ERM-family [→ ERM proteins] member, ezrin. Ezrin is an actin-associated protein that shows increased expression and phosphorylation upon fos-mediated transformation of fibroblasts that is correlated with a change in cell shape (from flat to rounded) and motility (from lamellipodial to pseudopodial). CALI of ezrin in transformed fibroblasts causes a decrease in membrane ruffling and pseudopodial retraction. CALI of ezrin in normal fibroblasts causes a marked collapse of the leading edge lamellipodia (Fig. 2). These studies implicate ezrin in cell shape and motility and suggest that ezrin has a critical role in the shape and motility changes associated with oncogenic transformation. A second protein of interest is the tumor supressor → hamartin. Hamartin binds to ERM proteins and its function is regulated by the small GTPase, Rho. CALI was used to show a role for hamartin in cell adhesion and suggests it might be involved in a rate-limiting step in tumor formation.



Chromophore-assisted Laser Inactivation. Fig. 2 – Micro-CALI of Ezrin affects fibroblast shape and motility. Fibroblasts transformed with v-fos, change their shape and motile behavior and show an increase in the expression and phosphorylation of the actin-associated ERM protein, Ezrin. We applied micro-CALI of Ezrin within the circled areas to v-fos-transformed firbroblasts (A and B) and normal fibroblasts (C and D). Micro-CALI of Ezrin in v-fos transformed cells caused a loss of membrane ruffling (arrowheads) and pseudopodial retraction (A) while laser irradiation of cells injected with Malachite Green-labeled non-immune IgG had no effect on motility (B). Micro-CALI of Ezrin in normal fibroblasts caused a marked collapse of the leading edge (C) with filaments remaining attached to the substratum (arrowheads in inset of panel labeled t = +20). Irradiation of cells, injected with Malachite Green-labeled non-immune IgG had no effect on cell shape (D). Scale bars = 10μm; time is in minutes.

Future applications

CALI is currently being combined with advances in dynamic imaging to visualize subcellular changes in response to the loss of function of specific proteins. We view that a major application of CALI for cancer research will be in target validation. As CALI lends itself well to combinatorial approaches and high throughput methods, it may be a powerful tool in addressing function in a proteome wide manner. A new use for CALI is in refining drug discovery screens to direct them against binders of a single domain on the target protein. CALI causes localized oxidative damage to modify residues of the protein near the antibody-binding site. By combining CALI with high resolution mass spectrometry to map those sites of damage, it may be possible to correlate loss of function with particular domains on a protein (Fig. 3).



Chromophore-assisted Laser Inactivation. Fig. 3 – Principle of Xplore. Xplore uses CALI and high resolution mass spectroscopy to map regions of functional importance on proteins. After CALI inactivation, sites of oxidative damage are mapped by high resolution mass spectroscopy, providing a correlation of a protein function with specific domains of the targeted protein.

Conclusions

CALI is a means for the inactivation of specific proteins *in situ* with a high degree of spatial and temporal resolution. CALI converts a binding reagent (such as an antibody) into an functional inhibitor. A large number of studies has demonstrated the potential of CALI in addressing cellular processes. It has recently been employed to address cellular mechanisms of cancer, and we believe that this technology is poised to contribute significantly to target validation and drug discovery for cancer-relevant processes.

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Chromosomal Instability

Definition

Chromosomal instability refers to abnormalities in chromosome number and/or structure which are often a feature of cancer cells. A number of genetic diseases, including Bloom syndrome, → Fanconi anemia and → Ataxia telangiectasia, have been identified in which chromosomal instability is associated with cancer predisposition.

Chromosomal Translocation

Definition

Chromosomal translocation is a genetic alteration in which pieces of two chromsomes are reciprocally exchanged. Such translocations are seen in several human cancers and often occur at specific sites, leading to the disruption of genes found at the chromosomal breakpoints.

Chromosome

Definition

A chromosome is a discrete unit of the genome carrying many genes, which is visible as a morphological entity only during cell division. Each chromosome consists of a very long molecule of duplex DNA and an approximately equal mass of proteins. Modern techniques of → interphase cytogenetics can visualize chromosomes at interphase. Chromosomes are classified according to size, the location of the centromere and the banding pattern along each arm. The autosomes are numbered from 1 to 22 in descending order of length. The sex chromosomes are referred to as X and Y. Both the long and short chromosome arms divided by the → centromere consist of one or more regions.

Chromosome Abnormality

Definition

A chromosome abnormality include changes that can be either structural, implying that the banding pattern or size of a chromosome is altered, or numerical, which means additional or missing whole chromosomes. Loss or gain of whole chromosomes are indicated by a plus '+' or minus '-' sign before a chromosome number.

Chromosome Band

Definition

A chromosome band is a chromosomal area distinguishable from adjacent segments by its lighter or darker staining intensity. Bands are numbered consecutively from the centromere outward along each chromosome arm. Each band can be individually designated by first listing the chromosome number, then the chromosome arm, the region, and band number with the region. For example, 12q13 means chromosome 12, the long arm, region 1, band 3.

Chromosome Condensation

Definition

Chromosome changes occur throughout the cell cycle, however, chromosome condensation is first visible at the → G_2/M transition. Human naked DNA must be compacted approximately 10,000 fold to allow it to resolve into pairs of sister chromatids that can be separated from one another during the middle stage of mitosis. The binding of multisubunit phoshoprotein complexes, known as condensins, tightly regulates chromosome condensation. Topoisomerase [→ topoisomerase enzymes as drug targets] II, an enzyme that regulates DNA topology, by breaking one DNA duplex and passing another through the gap, is required for complete chromosome condensation. Inhibitors of this enzyme stop complete chromosome condensation.

Chromosome Walking

Definition

Chromosome walking describes the sequential isolation of clones carrying overlapping sequences of DNA, allowing large regions of

the chromosome to be spanned. Walking is often performed in order to identify and clone a particular locus of interest.

Chronic Lymphocytic Leukemia (B-CLL)

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Definition

Adult form of leukemia with systemic accumulation of mature B-lymphocytes, derived from a subset of CD5-positive B-lymphocytes. The NCI-sponsored working group guidelines (1) for the diagnosis of chronic lymphocytic leukemia (B-CLL) require:

- Absolute lymphocytosis with more than $5x10^9$ mature lymphocytes/ml of peripheral blood.
- Less than 55% and/or $15x10^9$/ml of prolymphocytes (larger atypical cells).
- Monoclonality with regard to the expression of either κ or λ surface immunoglobulin.
- Co-expression of the surface membrane markers CD19, CD20, CD5 and CD23.
- More than 30% mature lymphocytes in the bone marrow.

These criteria specifically exclude related B-cell diseases of the immunopoiesis, i.e. the other CD5-positive B-cell malignancy Mantle Cell Lymphoma (MCL), and the CD5-negative Pro-Lymphocytic Leukemia (B-PLL), Hairy Cell Leukemia (HCL), Splenic Lymphoma with circulating Villous Lymphocytes (SLVL), Marginal Zone B-cell Lymphoma (MZL) and Waldenström's macroglobulinemia as well as other B-cell non-Hodgkin lymphomas [→ malignant lymphoma, hallmarks and concepts] in leukemic phase and → chronic T-lymphocytic leukemias.

Characteristics

B-CLL is the most common form of leukemia in the western hemisphere and it is rarely seen in Asian countries. The incidence increases with age and is in excess of 20 cases per 100,000 persons over 60 years of age. Although the disease is exceedingly rare in childhood, it is no longer unusual to find sporadic cases among younger adults.

The prognosis of B-CLL is highly variable. Some patients have an aggressive disease with a survival of 2 years or less, others have a benign indolent form in which the diagnosis of B-CLL does not affect their life expectancy. A number of variables have been tested for their ability to predict outcome. The most important markers of a poor prognosis are advanced clinical stage and short lymphocyte doubling time. The clinical stages can be defined by the Binet and Rai staging systems and summarized as follows:

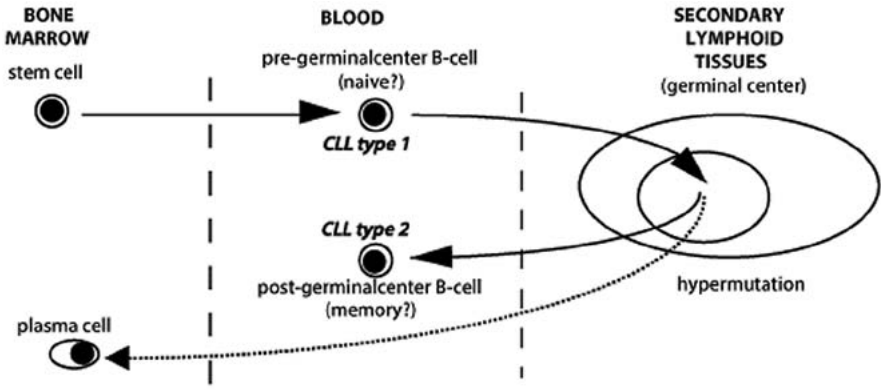
- Stage A: Lymphnode or organ enlargement involving no more than two of the regions: 1) head and neck, 2) axillae, 3) groin, 4) spleen or 5) liver.
- Stage B: Three or more areas of nodal or organ enlargement.
- Stage C: All cases with anemia (Hb<10 g/dl (6.2 mmol/l)) or thrombocytopenia (less than $100x10^9$ platelets/l).

Within each stage, the absence of somatic hypermutations in the immunoglobulin genes appears to be a very strong predictor of poor outcome (4, 5). Somatic hypermutations result from insertion of random nucleotides in the immunoglobulin genes during immune responses in secondary lymphoid tissues, and is defined as a nucleotide homology to the closest related Ig-germline sequence that is less than 98%. Since these mutations are not ongoing within the B-CLL clone, the 1:1 distribution of B-CLL cases with and without somatic hypermutations strongly suggests that B-CLL in fact is at least two different diseases: one is an aggressive disease of pre-germinal center (antigen naive) B-cells with a very poor prognosis, the other is an often indolent disease

of post-germinal center (antigen experienced) B-cells with a good prognosis (Fig.).

The clinical manifestations of B-CLL consists of the triad lymphocyte accumulation, immunodeficiency and autoimmune phenomena. Sometimes enormous numbers of lymphocytes infiltrate primarily the blood, bone marrow, lymphatic tissues, liver and spleen, but B-CLL infiltration can be found in any organ system including the skin and central nervous system. The symptoms are those generally associated with malignant disease including weakness, fatigue, weight loss, night sweats and fever of unknown origin. Over time many, if not all, patients develop an immunodeficiency mainly characterised by the loss of normal B-cell function, with a failure to raise antibody responses following vacination and development of decreased titers of specific antibodies, if not ouvert hypogammaglobulinemia. Many patients therefore suffer from reccurrent infections, in particular infections of the respiratory tract caused by polysaccharide encapsuled bacteria. Autoimmune phenomena are common, including Coombs-positive hemolytic anemia and other autoimmune cytopenias.

At present B-CLL must still be regarded as an incurable disease. The goal of the treatment is to obtain as strong and durable a response as possible, while keeping the toxicity of the treatment at an minimum. In general, patients in stage A should not be treated unless they demonstrate disease progression and short lymphocyte doubling time. At the other end of the spectrum is younger stage C cases, with short lymphocyte doubling time and (perhaps) absence of somatic hypermutations, in whom experimental treatment within clinical trials including high-dose chemotherapy and bone marrow transplantation should be considered. There is no consensus concerning the primary regimen in therapy-naive patients, but it should include either an alkylating agent (chlorambucil or cyclophosphamide) or a purine analogue (fludarabine or 2-chloro-deoxyadenosine). The combined usage of alkylating agents and purine



Chronic Lymphocytic Leukemia (B-CLL). Fig. – Genotypic classification of B-CLL. During B-cell development, the immunoglobulin genes undergo two rounds of modifications. The first modification occurs in the bone marrow during antigen-independent lymphopoiesis. Here V-D- and J-gene segments are rearranged to form a gene encoding the Ig heavy chain. During this rearrangement random nucleotides are inserted, which makes the rearrangement unique to the individual B-cell clone. Similar rearrangements take place in the genes encoding the Ig light chains, before the mature naive B-cell leaves the bone marrow to circulate in the bloodstream. The second round of Ig gene modifications, somatic hypermutations, occurs during antigen-dependent immunopoiesis in secondary lymphoid tissues. Following exposure to antigen, the B-cell acts in concert with T-cells and antigen-presenting cells to form a germinal center reaction. In the latter, random nucleotides are inserted in the Ig gene sequences encoding the hypervariable regions of the antibody. Consequently, the B-cell aquires a higher affinity for the initiating antigen. Only cells with highest affinity are allowed to clonally expand, to leave the germinal center and to differentiate into memory B-cells or plasma cells. Recent data suggest the existence of two types of B-CLL, based on the presence or absence of somatic hypermutations: an aggressive unmutated pre-germinal center form and an indolent mutated post-germinalcenter form.

analogues may make subsequent autologous stem cell harvest difficult or impossible, but may on the other hand represent an option for patients refractory to both drugs as single agents. If additional factors such as treatment with alkylating agents or purine analogues contribute to the immunodeficiency by induction of neutropenia or low CD4 T-cell counts, the spectrum of opportunistic infections becomes wider with risk of life-threatening septicemia, CNS-infections and other infections associated with decreased CD4 cell function. This disease and/or treatment related immunodeficiency is the major cause of morbidity and mortality in B-CLL, and in selected patients may require prophylactic antibiotics, growth factor therapy and intravenous immunoglobulin replacement therapy. Steroids should be reserved for the treatment of autoimmune phenomena.

The molecular and cellular characteristics of the B-CLL cell depends on whether the cell is described *in vivo* or following culture *in vitro*. Inside the patient, the B-CLL cell is a long-lived CD19/CD5/CD23 triple positive B-lymphocyte arrested in early G1-phase of the cell cycle. However, once isolated from the patient, the B-CLL cell will spontaneously initiate the process of → apoptosis. The cell death can be delayed, but not completely prevented, if the the cultures are supplemented with a number of biological substances including cytokines, thioredoxin and accessory cells such as endothelial and bone marrow stromal cells. Although these substances in various combinations may induce some DNA synthesis in primary B-CLL cultures, B-CLL cells are notoriously reluctant to proliferate *in vitro*. Unlike many other cancers, attempts to establish B-CLL cell lines that retain their key B-CLL characteristics have not been succesful, nor has a generally accepted *in vivo* model of B-CLL been established. Given these characteristics and the apoptosis resistant phenotype of B-CLL cells *in vivo*, the major pathogenetic drive leading to *in vivo* accumulation is believed to be apoptosis resistance, although a (small) degree of proliferation must occur *in vivo* in order for the cell counts to increase. It is likely, but not proven, that this apoptosis resistant phenotype may be the result of specific interactions between B-CLL cells and other cells *in vivo*, probably through a number of surface membrane receptors known to be able to delay programmed cell death *in vitro*.

The etiology of B-CLL is unknown, and unlike other forms of leukemia B-CLL is not associated with ionizing radiation or exposure to DNA-damaging drugs. Also unlike other forms of leukemia, the genetic lesions found in B-CLL are not → balanced translocations, but unbalanced structural chromosomal abnormalities with gain or loss of genetic material within the leukemic clone. The most common chromosomal aberrations are deletions at 13q14 (not involving the *retinoblastoma* gene [→ retinoblastoma, cancer genetics]), trisomy 12 (→ *p27*, → *MDM2*, → *Cyclin D2* and → *CDK4*), deletions at 11q22-23 (involving the *ATM* gene) and deletions at 17p (involving the *p53* gene). Bcl-2 overexpression have been noted in B-CLL, but this is not the result of a balanced translocation as in → follicullar lymphoma, and it is not clear that the expression level of this antiapoptotic protein is different in B-CLL cells compared to normal CD5-positive B-lymphocytes. Thus, it has not been possible to identify a specific gene for which the loss or overexpression is consistently associated with the development of B-CLL.

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Chronic Myeloid Leukemia

Definition

Chronic myeloid leukemia (CML) is a chronic form of leukemia hallmarked by a proliferation of white blood cells; → BCR-ABL.

Chronic Rejection

Definition

Chronic rejection is the severe damage and inflammation to engrafted organs, which usually occurs several months to several years after transplantation and is caused by humoral and cellular responses to donor endothelium; → graft acceptance and rejection.

CHRPE

Definition

Congenital hypertrophy of the retinal pigment epithelia (CHRPE) are pigmented lesions of the retinal epithelium. These patches result from an increased content of melanin granules within enlarged retinal pigment epithelial cells. The majority of FAP [→ APC gene in Familial Adenomatous Polyposis] patients (75 – 80%) have CHRPEs.

CHUK

Synonyms

- IKK1 (inhibitor of κ light polypeptide gene enhancer in B cells, kinase 1);
- IKK-α (inhibitor of κ light polypeptide gene enhancer in B cells, kinase α);
- NFKBIKA (inhibitor of κ light polypeptide gene enhancer in B cells, kinase α);
- IKBKA (inhibitor of κ light polypeptide gene enhancer in B cells, kinase α).

Definition

Conserved helix-loop-helix ubiquitous kinase. The gene maps to chromosome 10q24-25. See → IκB kinases.

CIP/KIP Family

Definition

Cip/Kip family is one of two groups of cyclin-dependent kinase (→ CDK) inhibitors (→ CKIs). The three known members of the Cip/Kip family are p21Waf1/Cip1, p27Kip1 and p57Kip2.

Cirrhosis

Definition

→ Liver cirrhosis.

CKI

Definition

→ Cyclin-dependent kinase (CDK) inhibitor.

c-KIT

Definition

→ KIT/stem cell factor receptor.

Class Switching

Definition

Class switching is a process by which the rearranged variable region from an immunoglobulin heavy chain gene is brought into the vicinity of a constant region gene other than IgM or IgD. Typically the intervening DNA between the variable region and the downstream constant region is lost during this recombination; → B-cell tumours.

Clathrin

Definition

Clathrin is the major structural component of coated pits constituted by a larger, ~ 190 kD protein, the clathrin heavy chain, which is complex to a smaller (~ 25 kD) subunit, the clathrin light chain. Heavy-light chain dimers can further assemble into complexes called triskelions, which represent the building blocks of the polygonal array that constitutes the organizing scaffold of a pit; → endocytosis.

Clathrin-mediated Endocytosis

Definition

→ Endocytosis.

Clone

Definition

A clone describes a large number of cells or molecules that are identical, with a single ancestral cell or molecule.

Cloning Vector

Definition

A cloning vector is a DNA molecule with a replication origin originating from a virus, plasmid, cosmid, phage, bacteria or yeast into which a foreign DNA fragment is integrated and then introduced into host cells, where it can be reproduced in large quantities (cloned).

CM

Definition

→ C-bandless chromosome.

CML

Definition

→ Chronic myeloid leukemia.

CMM2

Definition

Cutaneous melanotic melanoma 2, → CDKN2A.

CMV

Definition

→ Cytomegalovirus.

C-MYB

Definition

c-myb is a member of → MYB family of oncogenes.

C-MYC

Definition

→ MYC.

CNS Tumors

Definition

Central nervous system (CNS) tumors; → brain tumors.

COCA2

Synonyms

- → *MLH1*
- *mutL (E.coli) homolog 1*

Definition

Colorectal cancer, hereditary nonpolyposis, type 2; germ line mutations are associated with familial hereditary nonpolyposis colon cancer (→ HNPCC, → Lynch syndrome). HNPCC is one of the most common genetic diseases in the Western world and accounts for 15% of all colon cancers. It is often divided into two subgroups:

Type I: hereditary predisposition to colorectal cancer. Onset at young age and carcinoma observed in the proximal colon.

Type II: increased risk to develop cancers in certain tissues such as the uterus, ovary, breast, stomach, small intestine, skin and larynx, in addition to the colon.

Cockayne Syndrome

Definition

Cockayne syndrome is a genetic syndrome associated with a defect in transcription-coupled DNA repair (→ TCR); → xeroderma pigmentosum.

Codominant Alleles

Definition

Codominant alleles occur where both alleles contribute to the phenotype; neither one is dominant over the other.

Codon

Definition

A codon is a triplet of nucleotides that encodes an amino acid or represents a termination signal.

Coiled-coil Domain

Definition

A coiled coil domain is a heptad repeat of amino acids (a b c d e f g), with hydrophobic (H) residues at positions a and d and polar (P) residues elsewhere. Coiling results from two right-handed helices around one another with a slight left-handed superhelical twist. Coiled-coil sequences adopt dimeric, trimeric and antiparallel tetrameric conformations. A coiled coil is a quaternary protein structure of two or three α-helices that permits the highly coordinated dimerization and heteromer formation of proteins.

Colon Cancer

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Synonyms

- cancer of the large intestine
- malignant neoplastic changes of the colon

Definition

Colon cancer refers to malignant neoplasia of the large intestine. The demarcation line to the more distal rectal cancer is defined as being proximal to 16 cm of the anocutaneous line.

Characteristics

Due to the slow development of precursor lesions in the form of adenomatous polyps or dysplastic lesions, no other tumor offers as many possibilities and as much time for preventive measures. Diagnosis of colon cancer in early stages highly increases the probability of curative resection. A five-year survival rate of patients diagnosed with stage one colon cancer (limited to the bowel wall) is 90%, which is decreased to 35 – 60% in patients with a positive nodal status (stage III) and drops to less than 10% in the metastatic disease (stage IV) (1,2,3).

Screening strategies for the average risk population

Early colon cancer detection programs have been suggested to asymptomatic population of a certain age. The World Health Organisation (WHO), the American Cancer Society and the Agency for Health Care Policy and Research (AHCPR) recommended an annual fecal blood test (FOBT) and a five-yearly sigmoidoscopy for the asymptomatic population older than 50 (4,5). In Germany this proposal has been extended to annual FOBT and rectal-digital examination beginning at the age of 45. In 1994 however, this was followed by only 44,1% of women and 14,4% of men. To date it has not been demonstrated that the rectal digital examination by itself is an efficient means for the early detection of rectal cancer. It therefore seems unreasonable to replace the sigmoidoscopic examination by the rectal digital examination, although it is an essential part of every physical examination in patients older than 50.

- *FOBT (fecal occult blood test):* Three large randomised studies carried out in USA, Denmark and Great Britain in a period of 8 to 13 years, have demonstrated the benefits of FOBT in early colon cancer detection and in the reduction of mortality by 15 – 33% (6,7). Although FOBT is more specific without rehydration, best results were achieved when the test was carried out once a year and included rehydration. FOBT is an adequate screening modality for early cancer detection, reducing mortality rates as well as treatment costs. It is in itself, however, not the appropriate means in cancer prevention since it implies the removal of neoplastic changes even before the event of malignant transformation.
- *Sigmoidoscopy:* Periodic sigmoidoscopy from age 50 onwards, reduces the mortality of rectosigmoidal cancers by 60% (8,9,10) and usually a control interval of five years is sufficient (11). The risk of developing colon cancer proximal to the splenic flexure, however, remains unaffected. Compared to FOBT alone, the combination of the two procedures increases the cancer-preventive effect by a factor of 2,2 (12).
- *Colonoscopy:* In the age group of 55 to 64 year old asymptomatic persons, the combination of FOBT and sigmoidoscopy result will, in the case of positive test results, lead to the recommendation of performing colonoscopy. The question may therefore be raised, if a base-line colonoscopy is a suitable altemative for this age-group. In approximately one third of all patients, polyps will be detected (and removed) such that this strategy would imply a true cancer-prevention. On the other hand, 70% of persons

with a negative colonoscopy would not require any further screening modalities for a period of five years (13).

- *Double contrast barium enema:* This radiologic examination cannot replace colonoscopy since the sensitivity is significantly lower (83 versus 95%). The probability of overlooking a small cancer is increased four-fold compared to the endoscopic procedure (14). Small polyps, however, are also frequently not recognized during the endoscopic examination. In a prospective study, every fourth adenoma under the size of 5 mm was overlooked. The detection of adenomas larger than 1 cm in diameter was reproducible in 94% of the cases (15). The reliability of colonoscopy depends much on the experience of the person performing the examination (16).

a) Preoperative diagnosis of colon cancer
Required examinations:

- History (including family history).
- Physical examination (including rectal-digital examination).
- Colonoscopy with biopsy or double-contrast barium enema with subsequent biopsy of a pathological alteration. If the barium enema does not show a pathological lesion, it may entirely replace endoscopy.
- If a stenosis cannot be surpassed preoperatively, colonoscopic examination in the first three months after operation is warranted.
- Ultrasound sonography of the abdomen.
- Radiologic thorax examination.
- Tumormarker CEA (carcino embryonal antigen).
- MRI (as an altemative or extended examination).
- CT scan of the thorax if in doubt about lung metastases.
- In case of sigmoid cancers: urine sedimentation, CT scan. If ultrasound examination suggests infiltration of the urinary tract or if red blood cells are demonstrated in the urine sedimentation, cystoscopy is recommended to investigate bladder infiltration. Gynaecological examination, if infiltration of the uterus or the ovaries is suspected.

b) Preoperative (neoadjuvant) therapy
To date no benefits have been shown in using neodajuvant therapy in colon cancer.

c) Surgical therapy (with the aim to cure)
Surgery aims at the curative resection of the tumor-bearing segment of the colon together with the regional lymph nodes. In addition, the (partial) resection of adjacent organs, if these are infiltrated by tumor (multivisceral resection) may be necessary. Colon cancers usually have a circular growth pattern. In order to remove the intramural tumor cell spread, a minimal margin of 2 cm suffices. A regional lymphnode involvement is more widespread. Lymphnodes show a tangential metastatic involvement (up to 10 cm away from the macroscopic tumor), their preferred distribution being towards the center.

- *Cancers of the cecum and ascending colon:* Generally, a right-sided hemicolectomy is the treatment of choice in such patients, including the radicular removal of the lymphnodes of the right colic artery and the ileocolic vessels. The large omentum of the colon is removed together with the colon segment. If the dissection of the gastrocolic ligament is considered, one might be confronted with contrasting opinions regarding the right gastroepiploic artery. Some authors recommend the preservation of the vessel, others do not.
- *Cancers of the right flexure and the proximal transverse colon:* As a rule of thumb, extended right-sided hemicolectomy is warranted if the right colic artery is dissected at its origin, out of the superior mesenteric artery. The distal resection lies close to the splenic flexure, allowing circulation. If the blood supply of the distal transverse segment appears unsufficient, the additional resection of this segment becomes necessary. The large omentum is completely removed, together with the gastroepiploic ligament and the right gastroepiploic vessels (resection of potentially involved Iymphnodes above the pancreas).
- *Cancers of the transverse colon:* Cancers in the mid-transverse colon are treated with

an entire resection of the segment, including the flexures. The omentum as well as the gastroepiploic ligament and arcade are removed together with the colonic specimen. If the cancers are close to the flexures, hemicolectomy extended to the right and the left is the procedure of choice.

- *Tumors of the left colonic flexure:* Suggested is an extended left-sided hemicolectomy, together with the removal of the lymphnodes of the medial colic vessel and the inferior mesenteric vessels. Equally radical is the central ligature of the left colic artery at its origin, leaving the central part of the inferior mesenteric vessels intact. Under these circumstances the superior rectal vessels remain unaffected, such that the circulation in the remaining sigmoid colon is not impaired. Depending on the exact tumor localisation and blood supply, the right colonic flexure may be preserved. Lymphnodes along the central portion of the superior mesenteric vessels should always be removed for diagnostic evaluation.
- *Tumors of the descending colon and proximal sigmoid colon:* Generally, left-sided hemicolectomy is recommended, with radicular ligation of the inferior mesenteric vessel. The distal margin of resection lies in the upper part of the rectum and usually the left flexure has to be removed. In order to obtain a tension-free anastomosis, sometimes the medial colic artery has to be sacrificed.
- *Tumors of the middle and distant sigmoid colon:* In this case, radicular segmental sigmoid resection is the preferred option. The inferior mesenteric artery is ligated either centrally or distal, relative to the origin of the left colic artery. The inferior mesenteric vein should be ligated at the lower edge of the pancreas.

- *Further constellations influencing surgical strategy.*

  Multivisceral resections:
  If adjacent structures are inherent to the tumor, these should, in addition to the lymphnode resection, be resected 'en bloc'. In contrast, biopsies to confirm tumor infiltration of adjacent organs are to be avoided since cell dissemination might be initiated.
  Distant metastases:
  The resection of synchronous or metachronous metastases of the liver, lung etc. is indicated only, if this resection has curative intent and complies with the oncological principles. If the metastases are unresectable, 'palliative measures' apply.
  Multiple colonic primaries:
  The extent of resectional surgery depends on additional lymphnode dissections recommended for each tumor. As a result colectomy with ileorectal anastomosis may be indicated.
  Synchronous occurrence of colonic polyps:
  Adenomas that are not removable endoscopically should be resected during colon cancer surgery. In this case a margin of 2 cm applies. Extended resection to the segmental lymphnode is not necessary.
  Cancer diagnosis in endoscopically removed polyps:
  If, unexpectedly, the histological examination reveals malignancy, oncological resection of the colonic segment is indicated. This may be neglected only in the case of a polyp with tumor-free stem which is confined to the submucosa with 'low risk' (pT1, G1 – 2, no lymphvessel involvement).
  Segmental resection:
  In patients with metastatic disease, radical resection of the colonic segment with lymph node removal may not be indicated. Very poor physical condition or the high age of some patients may justify colonic surgery which does not follow oncological principles.
  Emergency operation:
  Nevertheless, high-urgency surgery, unavoidable due to bowel obstruction, tumor or colon perforation should comply with oncological principles.
  Laparoscopic surgery:
  To date, no data are available that document the operative outcome in patients that underwent laparoscopic colon cancer surgery. Future consideration of ongoing studies with long follow-up periods is therefore necessary for the optimal treatment of colon

cancer. Nevertheless, there are no objections to carry out laparoscopic colon cancer surgery in a palliative setting.

Ulcerative colitis and familial adenomatous polyposis (FAP [→ APC gene in Familial Adenomatous Polyposis]):

Cancers of this type require proctocolectomy, if possible continence-preserving. Especially in an early stage, cancer in the proximal two thirds of the rectum is not a contraindication for ileoanal pouch surgery.

→ HNPCC (hereditary nonpolyposis colorectal surgery):

In the event of this autosomal-dominant syndrome, many authors suggest extended cancer surgery in the form of prophylactic bowel removal (colectomy and ileorectal anastomosis or restorative proctocolectomy). Occurrance of metachronous colorectal cancer and the observation of so-called interval cancers is significant. However, the benefit of prophylactic colon removal (without the evidence of neoplasia) remains uncertain, especially if considering a reduced penetrance of approximately 80%.

d) Intra- and postoperative histopathological diagnosis

Due to technical complications the immediate pathological classification of a tumor/polyp in a frozen section is not an option. Pathological evaluation after oncological surgery is, however, of prognostic significance in the locoregional resection (R-classification), the depth of invasion (pT classification) and the grading and lymphnodal status (pN classification) and forms the basis in the decision process concerning → adjuvant therapy. The total number of resected lymph nodes and metastatic lymphnode occurrence is therefore of essential relevance. Perforation of the tumor during surgery is of prognostic significance and must be documented.

→ Microsatellite tumor instability is of special relevance in the setting of HNPCC, and of increasing interest since response rates to adjuvant therapy have shown that stable and instable tumors differ in their biological response to chemotherapeutic agents. Although the natural course of unstable tumors is more benign, than the natural course of stable tumors, the biological response to conventional chemotherapy in stable tumors is better.

Classification of colorectal cancers:

There are two classifications that are used separately, the Dukes and the TNM (*t*umor, lymph*n*odes, *m*etastases) classification.

The Dukes-Classification (Table 1) is preferred in the US and UK and describes the following stages:

Colon Cancer. Table 1 – Dukes-Classification.

Dukes A	growth limited to wall, nodes negative
Dukes B	growth beyond muscularis propia, nodes negative
Dukes C1	nodes positive and apical negative
Dukes C2	apical node positiv
Dukes D	growth beyond originating organ

The TNM-staging (suggested by the Union internationale contre le cancer, UICC) (Table 2) is preferred in European countries and distinguishes between the stages listed below. T stands for the expansion of the primary tumor; N for the lack or the presence of metastases of the lymphnodes; M for the lack or the presence of distant metastases. Numbers indicate the extent of malignant processes; p, postoperative.

e) Adjuvant therapy (16).

- In order to recommend adjuvant therapy, complete removal of all regional and metastatic lesions (R0 resection) in addition to the tumor removal is necessary. Recommending adjuvant therapy is based on the pathohistological classification of the tumor, specifically of the pN status. In order to define the lymphnode status, a minimum of twelve regional lymphnodes should be examined. Immunocytological studies of isolated tumor cells in either bone marrow aspiration biopsies or in the peritoneal fluid should not, at least at this point, be referred to in the decision for or against adjuvant therapy since the impact of 'minimal residual disease' remains to be established.

Colon Cancer. Table 2 – TNM-staging.

pT1	local invasion of submucosa
pT2	local invasion of the muscularis propia
pT3	local invasion beyond the muscularis propia
pT4	tumor cells have reached peritoneal surface or invaded adjacent organs
pN0	no lymphnodes affected by metastases
pN1	one to three lymphnodes affected by metastases
pN2	four or more lymphnodes affected by metastases
pM0	no distant metastasis
pM1	distant metastasis

- Patients with early-stage colorectal cancer (stage I or II) and patients after R0 resection of distant metastases should receive adjuvant therapy in the setting of controlled studies only.
- The benefits of adjuvant therapy in UICC stage III cancers (all pT stages, pN1-2, M0) remain to be established. A quality-controlled surgical treatment with and without adjuvant therapy is currently under evaluation. Outside these studies, adjuvant therapy is recommended for stage III cancers
- Adjuvant chemotherapy for stage III colon cancers: One year administration of 5-FU (fluouracil) and levamisol proved to be as effective as a 6-monthly administration of 5-FU and folinic acid.
  Although there is variation between different adjuvant protocols, general contraindications for adjuvant therapy are listed below:
  - general physical condition under the score of 2 (WHO)
  - uncontrolled infection
  - liver cirrhosis
  - severe coronary heart disease, cardiac insufficiency (NYHA III and IV)
  - preterminal and terminal renal insufficiency
  - limited bone marrow function
  - unavailability for regular control check-ups.

To date there appears to be no benefit in the administration of monoclonal antibody treatment (17 1A) in addition to conventional chemotherapy.

f) Follow-up

Due to the low rate recurrence rate, no major prognostic advantage from follow-ups is expected for patients with early cancer (UICC I) and R0 resection. The advice to perform two colonoscopies, two and five years post colon cancer surgery, is aimed towards an early identification of second primaries. An intensified surveillance of individual cases is justified in circumstances that lead to suspect a higher recurrence rate, i.e. tumor perforation, G3 and G4 tumors or histologically verified pericolic vessel infiltration. After palliative tumor resection (R2) a symptomatic follow-up is recommended.

Following R0 resections of tumor stages II and III, the main benefits of follow-up strategies can be expected, if the general physical condition of the patient does not object to recurrent surgical intervention. Two specific follow-ups are recommended within five years of primary surgery (year 2 and year 5) and include physical examination, CEA-level, abdominal ultrasound, X-ray of the thorax and colonoscopy. Intensified follow-up is recommended for patients with an increased hereditary risk.

Cellular and Molecular Features

Colorectal tumors provide an excellent system in which to search for and study genetic alterations involved in the development of neoplasia. It appears that most if not all malignant colorectal tumors arise from preexisting benign adenomas. These precursor lesions can be removed and studied at various stages of development.- Colorectal tumors develop as a result of onco-

gene mutations in combination with the mutated tumor suppressor genes, the latter being predominant.

Human colorectal tumors, including very small adenomas, have a monoclonal composition. Adenomas therefore arise from a single or a small number of cells which initiate the process of neoplasia by clonal expansion. Genetic alterations within the majority of neoplastic cells studied so far, suggest an impaired regulation of cell growth that enables those cells to become the predominant cell type, eventually constituing the neoplasm.

The development process in patients with sporadic cancer (as opposed to → familial cancer) occurs over a period of decades. The series of genetic alterations involves oncogenes such as *Ras* as well as tumor suppressor genes (particularly those on chromosome 5q, 17p and 18q). In general, the three stages are represented by increasing tumor size, dysplasia and villous content. The mutation of the *Ras* gene (usually K-*Ras*), appears to occur within a single cell of a pre-existing small adenoma followed by clonal expansion which produces a larger and more dysplastic tumor. Deletions of chromosome 17p and 18q generally arise at a later stage of tumorigenesis, than deletions of chromosome 5q or *Ras* gene mutations. In this → multistep development the total number of genetic alterations rather than their order of occurrence, determines the biological properties of neoplasia.

Tumors continue to progress once cancers have formed and the cumulative loss of tumor suppressor genes on different chromosomes correlates with the ability of the tumor to metastasize and to cause death.

Recent investigations have shown that approximately 25% of randomly selected colorectal cancers are unstable, a phenomenon used as an independant prognostic factor in colorectal cancer. In addition, the loss of heterozygosity (LOH) at a chromosome 8p marker (termed allelic imbalance) has, as recently reported, been related with a poor patient outcome. It may therefore be expected, that the molecular characterization of colorectal tumors will increasingly affect the individual risk assessment and the suitable intervention strategies.

The identification and characterization of molecular mechanisms underlying tumor development and tumor growth offer new opportunities in cancer treatment. An intricate genetic scenario is responsible for a complex human neoplastic condition. The relevance of individual steps within, might open doors for therapeuticals that specifically target essential, although malfunctioning, check-points.

Perspective

In developing countries, especially Asia, incidences of colon cancer are rapidly rising. In the United States and in Germany, approximately 130.000 and 50.000 patients, respectively, are diagnosed with colorectal cancer every year (16). Colorectal cancer comes second in the group of tumor-related deaths. The lifetime risk of developing colorectal cancer in Germany is 4 – 6%; with the majority of these cancers ocurring in people aged 50 and above. Gaining new insight into the molecular pathogenesis of colorectal cancer will allow progress in many facets of disease control. They include the identification of genetically predisposed groups for targeted surveillance and/or chemo-prevention, prognosis for patients with established cancer, predictions of treatment efficacy and the development of novel treatment strategies.

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Colony Hybridization

Definition

Colony hybridization is a technique using *in situ* hybridization to identify bacteria carrying chimeric vectors whose inserted DNA is homologous with a particular sequence.

Comet Assay

Definition

The comet assay, also called the 'single cell gel assay', is one of the fastest-applied techniques not only in the cancer world but in other areas as well with far reaching implications. It is the only technique to monitor DNA damage and repair at the level of single cells. It can be used to analyse genotoxic effects where there are low cell numbers, such as those available from biopsies of human tumor target tissues. The original form of the assay was first described by Swedish researchers in 1984. The alkaline conditions used makes the comet assay highly sensitive.

In brief, the cells are embedded in agarose and spread on glass microscope slides. The slides are then placed in electrophoresis buffer of required pH, the DNA allowed to unwind and then the electrophoresis is switched on for few minutes and the DNA allowed to migrate. After the electrophoresis, the cells are scored for DNA migration under a microscope. The cells with DNA damage, present as comets with long tails of DNA migrating from the center of the cells, hence the name 'comet assay'. The comet assay is also a useful biomarker technique and can be used in human studies on dietary chemoprevention. Papers of historical interest as well as review papers are listed on the 'comet assay interest group website' (http://www.geocities.com/cometassay/); illustrations in → biomarkers.

Complementary DNA

Definition

Complementary DNA, also known as copy DNA and cDNA, is double-stranded DNA copied from RNA by RNA-directed DNA polymerase (→ reverse transcriptase); reverse transcriptase is the key enzyme in the life cycle of RNA viruses and is widely used in gene technology.

Complementation Groups

Definition

Complementation groups are subgroups of DNA repair-deficiency syndromes, each representing a separate (defective) DNA repair gene; → xeroderma pigmentosum.

Computed Tomography

Definition

Computed tomography (CT) is X-ray information coupled with data processing used for cross-sectional imaging of the human body.

Confounder

Definition

A confounder is an independent factor for cancer risk; → adducts to DNA. To identify a causal relationship between exposure to any agent (chemical, physical) and disease, a possible effect of other factors (confounders) must be excluded. In epidemiological studies addressing risk factors and lung cancer, cigarette smoking is a typical confounder. If cigarette smoking is not considered, the significance of exposure to another environmental agents could vastly be overestimated as a disease cause.

Congenital Hypertrophy of the Retinal Pigment Epithelia

Definition

Congenital hypertrophy of the retinal pigment (→ CHRPE).

Connexins

Definition

Connexins are a class of cell surface proteins organized in special adhesion structures (gap junctions) that are permeable to small macromolecules.

Consensus Interferon-α

Definition

Consensus interferon-α is a synthetic interferon-α that was originally derived by assigning the most common observed amino acid of the known species at each site.

Constant Regions

Definition

The constant regions of immunoglobulins are coded by C genes and are the parts of the chain that vary least; those of heavy chains identify the type of immunoglobulin.

Constitutive Genes

Definition

Constitutive genes are expressed as a function of the interaction of RNA polymerase with the promoter, without additional regulation. They are sometimes also called 'household' genes in the context of describing functions expressed in all cells at a low level.

Constitutive Heterochromatin

Definition

Constitutive heterochromatin describes the inert state of permanently nonexpressed sequences, usually repetitive satellite DNA.

Contact Inhibition

Definition

Contact inhibition occurs when cells, grown in monolayer under tissue culture conditions, arrest growth when they contact each other and reach confluency. Under these circumstances, cancer cells usually continue growth and pile up on top of one another.

Continent Urinary Diversion

Definition

Continent urinary diversion is when a segment of small or large bowel is removed from continuity from the remainder of the bowel and then fashioned into a urinary reservoir. It provides continent storage of urine and is emptied *via* intermittent catheterization of a cutaneous stoma or *via* the native urethra.

Cosmid

Definition

A cosmid is an artificially constructed cloning vector. It contains the *cos* gene of bacteriophage λ, which allows the package of DNA to generate phages and to infect *E. coli*, permitting cloning of DNA fragments up to 45 kb, larger than those possible using plasmid vectors.

Cot

Definition

A physical parameter describing the reassociation kinetics of denatured DNA *in vitro*. Parameters controlling reassociation are the DNA concentration at the beginning of the reaction (Co) and the time (t) of incubation. Reassociation of complementary sequences occurs by base pairing, reversing the process of denaturation. The reassociation kinetics reflect the variety of sequences that are present. The reassociation reaction can therefore be used to quantify represented sequence components within a heterogeneous collection of DNA. The degree of DNA reassociation is usually displayed as a Cot curve. Denatured DNA of eukaryotic organisms that is allowed to reassociate, usually has different sequence components. They can be identified by their Cot curve since each give rise to different Cot curves. This is possible because highly repeated sequences that are repeated very often, reassociate rapidly (due to their high concentration in the mixture of DNA sequences); sequences that occur with average frequency, reassociate at an intermediate rate of speed, while single copy sequences reassociate only slowly. The amount of individual components can then be calculated from the reassociation rate. Moreover, differential reassociation rates can be used to fractionate sequence components according to their frequency, i.e. repetitive sequences can be separated from single copy sequences.

$Cot_{1/2}$ is required to half complete the reassociation reaction; it is directly proportional to the unique length of reassociating DNA.

$Cot_{1/2}$

Definition

$Cot_{1/2}$ is the → Cot required to proceed to half completion of the reaction; it is directly proportional to the unique length of reassociating DNA.

Cotransfection

Definition

Cotransfection is the simultaneous transfection of two markers (different DNA preparations, different DNA clones etc.) into cells.

Covalent Adducts

Definition

Covalent adducts is the formation of a chemical bond between a chemical carcinogen or its derivatives and DNA bases; → adducts to DNA.

Cowden Syndrome

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Definition

Cowden syndrome (CS, OMIM#158350), along with Bannayan-Riley-Ruvalcaba syndrome (BRR, OMIM#153480), Peutz-Jeghers syndrome (PJS, #175200) and juvenile polyposis syndrome (JPS, OMIM#174900), is a member of a group of rare autosomally dominant inherited conditions classified as the hamartoma syndromes. CS, alternatively referred to as multiple hamartoma syndrome, takes its name from the proposita of the first family described. It is characterised by an increased risk of developing breast and thyroid cancer along with the presence of hamartomas (benign, tumour-like overgrowths of tissue with a developmentally disorganised structure) in multiple organ systems. The susceptibility gene for both CS and BRR is the tumour suppressor → *PTEN* (alternatively named *MMAC1* or *TEP1*).

Characteristics

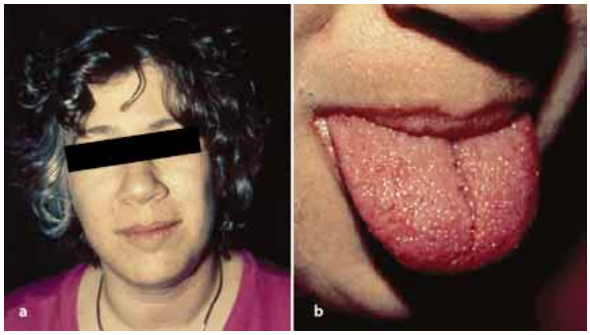
CS displays variable expressivity within families, however usually presents in the third decade. CS hamartomas are present in tissues derived from all 3 germ cell layers, specifically the breast, thyroid, skin, central nervous system and gastrointestinal tract. Ninety-nine percent of CS patients display the hallmark CS hamartoma known as trichilemmomas, benign tumours of the hair follicle infundibulum, in addition to mucocutaneous papules. Seventy percent of female CS patients develop breast fibroadenomas, 40 – 60% have thyroid adenomas, whilst gastrointestinal polyps occur in 35 – 40% of CS patients. Breast cancer develops in 25 – 50% of female CS patients and on occasion also in males, whilst thyroid cancer develops in 3 – 10% of all affected individuals. Disease of the central nervous system, most often benign but in some cases malignant, occurs in 40% of cases. Lhermitte-Duclos disease, a condition of dysplastic gangliocytoma of the cerebellum manifesting as seizures, tremours and poor co-ordination, has been reported in conjunction with CS [1]. Megencephaly or macrocephaly occur in approximately 38% of CS patients. Other abnormalities including those of the genitourinary tract may also be present. Given the subtle and poorly recognised physical findings in individuals with CS, it is thought that this condition may often be underdiagnosed. The International CS Consortium has developed diagnostic criteria to aid in the identification of this syndrome [2].

CS shows partial clinical overlap with BRR as patients with either syndrome may develop intestinal hamartomatous polyps, macrocephaly (in nearly 100% of cases of BRR) and lipomas (occurring frequently in BRR but in a minority of patients with CS). Other features of BRR include very early age of onset, pigmented macules of the glans penis ('speckled penis') in males, haemangiomas, mild mental retardation and developmental delay. Both the unique and overlapping clinical features of CS and BRR are described in the table. A number of anecdotal cases of malignancy affecting the thyroid and brain have been reported in BRR, however whilst malignancy is well described in CS, it

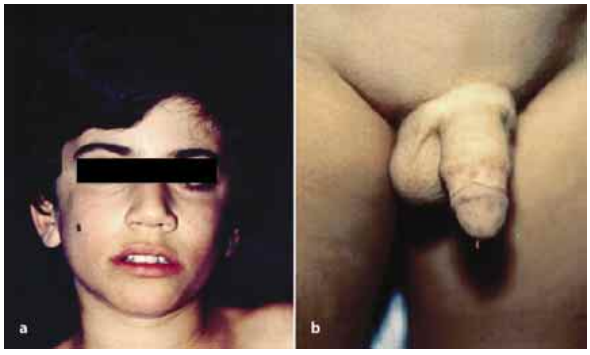
is not part of the classic BRR phenotype. In addition, a number of families have been reported in which both CS and BRR are present. In these families, CS is generally present in the parental generation, whilst BRR appears in the younger generation, suggesting a form of anticipation (Fig.s 1 & 2) [3,4].

Molecular features

PTEN, the first protein tyrosine phosphatase shown to function as a tumour suppressor, is the susceptibility gene for both CS and BRR [5, 6]. A processed pseudogene is located on chromosome band 9p21, missing the initiating methionine present in *PTEN* but sharing greater than 98% homology with the *PTEN* coding region.



Cowden Syndrome. Fig. 1 – Female with Cowden Syndrome. a Macrocephaly (head circumference of $59^1/_2$ cm, greater than the 97^{th} percentile). **b** Multiple small papules of the tongue and mouth.



Cowden Syndrome. Fig. 2 – Male with Bannayan-Riley-Ruvalcaba Syndrome (son of female in Fig. 1). **a** Macrocephaly (head circumference of 59 cm, greater than the 97^{th} percentile). **b** Multiple hyperpigmented macules of the penis.

PTEN contains 9 exons encoding a dual-specificity phosphatase mapped to 10q23.3, with homology to the cytoskeletal proteins tensin and auxillin. Residues 122-132 located in exon 5 encode the classic phosphatase core motif (I/V)HC*XX*G*XX*R(S/T)G. The COOH-terminus contains 3 potential tyrosine phosphorylation sites at residues 240, 315 and 336, as well as 2 potential serine phosphorylation sites at residues 335 and 338. It also contains a potential PDZ binding domain encoded by the last 4 amino acids (ITKV) that may have a role in its subcellular localisation and/or substrate interactions.

PTEN has been shown to reduce tyrosine phosphorylation of focal adhesion kinase (FAK) *in vitro* suggesting a role in cell migration and invasion. However, the major endogenous substrate of PTEN would seem to be phosphatidylinositol 3,4,5-triphosphate [Ptd-Ins(3,4,5)P_3], a phospholipid in the phosphotidylinositol 3-kinase (PI-3 kinase) pathway and an important second messenger in cell growth regulation. In this pathway growth factors such as insulin, platelet derived growth factor and fibroblast growth factor stimulate the enzyme PI-3 kinase to phosphorylate Ptd-Ins(4,5)P_2 to produce Ptd-Ins(3,4,5)P_3. PTEN is postulated to act as a 3-phosphatase to dephosphorylate Ptd-Ins(3,4,5)P_3 to Ptd-Ins(4,5)P_2. When PTEN is mutant, such as in CS and BRR, Ptd-Ins(3,4,5)P_3 accumulates and activates protein kinase B (PKB)/AKT to function as an oncogene, thus causing the tumourigenic state. AKT is a serine-threonine kinase and a known cell survival (anti-apoptotic) factor. Thus, apoptosis is a likely mechanism for PTEN-induced growth suppression. However, PTEN is also able to cause cell cycle arrest in cells in the G1 phase, possibly *via* modulation of levels of RB phosphorylation.

Elucidation of the crystal structure of PTEN has revealed a wider and deeper phosphatase active site than is usually described in other dual-specificity phosphatases that allows the accomodation of Ptd-Ins (3,4,5)P_3 [7]. Further, the make up of the residues in this pocket cause it to have a positive charge consistent with the negative charge of Ptd-Ins (3,4,5)P_3 and with the preference displayed by PTEN for highly

Cowden Syndrome. Table – Clinical features seen in Cowden Syndrome and Bannayan-Riley-Ruvalcaba Syndrome. *common in BRR, occasionally seen in CS; #reported in CS, occasionally seen in BRR.

	Cowden Syndrome	Bannayan-Riley-Ruvalcaba
CNS	Lhermitte-Duclos disease	developmental delay*, seizures, myopathy
endocrine	multinodular goitre, adenoma, thyroid anomalies, thyroiditis, hypothyroidism	Hashimoto's thyroiditis*, diabetes mellitus
growth disturbances:		
generalized	macrocephaly	macrosomia at birth, enlarged penis and testes, localised overgrowth, macrocephaly
skin	facial trichilemmoma#, acral keratoses, haemangioma, mucosal lesions, papillomatous papules#, hypertrichosis, vitiligo, pseudoacanthosis nigricans, skin cancers (basal cell, squamous cell and melanoma)	penile lentigines, acanthosis nigricans, verruca vulgaris type facial changes, tongue polyps, Café au lait spots, angiokeratoma, lipoma/lipomatosis*
gastrointestinal	polyps in entire gastrointestinal tract, gastrointestinal cancers	polyps in distal gastrointestinal tract
breast	fibrocystic breast disease, adenocarcinoma	—
other benign tumor	uterine leiomyoma, ovarian cysts, fibroma, meningioma, glioma, neuroma	meningioma, angiolipoma, haemangioma* (especially intracerebral and bony) lymphangioma
other malignant tumors	thyroid (non-medullary)#, cervix, uterus, bladder, liver, renal, acute myelogenous leukemia, non-Hodgkin lymphoma, liposarcoma, trichilemmomal carcinoma, renal cell carcinoma	

acidic polypeptide substrates. One particular germline mutant found only in CS, G129E, has been shown to have normal phosphatase activity against non-phospholipid substrates *in vitro* and in cell lines but has no phosphatase activity against Ptd-Ins(3,4,5)P_3. It is believed that mutation of this residue reduces the size of the active pocket so that it can no longer accommodate the phospholipid substrate Ptd-Ins(3,4,5)P_3. However, catalysis of the smaller substrates of phospho-tyrosine, serine and threonine are not disrupted [7]. Furthermore, a C2 domain is present in the C-terminal domain and associates over an extensive interface with the phosphatase domain creating interdomain hydrogen bonds between conserved residues. This interphase region provides strong evidence that the C2 domain not only functions to recruit substrate, but also optimally positions it available to the phosphatase catalytic domain. Germline mutations of conserved residues involved in the creation of this interphase, including serine at position 170, have been reported in BRR [6]. Thus, there is strong evidence that the lipid phosphatase activity of PTEN is essential for its tumour suppressor activity.

Clinical aspects

PTEN is mutated in the germline of up to 80% of patients with CS and up to 60% of patients with BRR [4,8]. Mutations are scattered largely along the entire gene with the exception of exon 1, including point mutations, insertions, deletions, deletion-insertions and splice site mutations. In BRR alone, gross hemizygous deletions and also a balanced translocation likely affecting the *PTEN* gene have been reported [4]. Further, loss of the wild type allele has been identified in hamartomas from a subset of CS individuals with *PTEN* mutation, providing additional evidence that PTEN is functioning as a classic tumour suppressor according to Knudson's two-mutation model [9]. However, there are many cases where loss of the wild type allele is not observed in affected CS tissue [9]. As is suggested by one of the *Pten*+/- mouse models, *PTEN* haploinsufficiency may be all that is required for the presence of the characteristic developmental defects and tumour formation seen in CS and BRR [10]. It was first thought that none of the 3 *Pten*+/- mouse models described developed the classic benign and malignant tumours of CS and BRR, although the presence of colonic microscopic hamartomatous polyps not dissimilar to what is seen in CS and BRR was reported. However, a recent study of *Pten*+/- mice older than 6 months reported the development of a range of tumours more similar to the spectrum of tumours observed in CS patients, specifically breast tumours in 50% of females, 100% of females with endometrial hyperplasia and a high incidence of endometrial cancer, prostate and adrenal neoplasia and tumours of the gastrointestinal tract [11].

PTEN germline mutations in CS and BRR have been found to cluster in exons 5, 7 and 8, with the great majority occurring in exon 5. This may be a function of the fact that exon 5 is the largest exon of this gene, constituting 20% of the coding region, but this exon also contains the protein tyrosine phosphatase (PTPase) core motif. Of note in CS, most mutations that occur in the core motif are non-truncating, suggesting the importance of this functional domain.

Identical mutations, including Q110X, R130X, R233X and R335X have been reported in both CS and BRR, making the presence of other genetic and/or epigenetic factors such as modifier loci highly likely in the determination of phenotype. Furthermore, a number of families have been reported with CS diagnosed in the older generation and BRR present in the younger generation suggesting some form of anticipation that is currently not well understood [3]. From this, it could be concluded that BRR and CS are different presentations of a single syndrome with broad clinical expression. In fact, it has been suggested that *PTEN* mutation positive CS and BRR patients should be clincally grouped as a single entity and classified as the 'PTEN hamartoma-tumour syndrome' (PHTS) [4].

DNA based predictive testing programs can now be incorporated as part of the clinical management of CS and BRR individuals. At the level of clinical management, cancer surveillance coupled with genetic counselling becomes important for CS and BRR patients as well as their first degree relatives.

Preliminary genotype-phenotype correlations have been reported for both CS and BRR and a number of trends observed. Firstly in a study of BRR and CS/BRR overlap families, the correlation of a germline *PTEN* mutation with the presence of lipomas and also with any cancer or breast fibroadenoma was determined [4]. In CS families a number of correlations were observed including an association between the presence of a *PTEN* mutation and breast involvement, as well as the presence of a missense mutation and the involvement of all 5 organ systems (ie. breast, thyroid, gastrointestinal tract, central nervous system and skin). It is possible that this latter trend may in fact be a positional effect given that the majority of missense mutations occur in the PTPase core motif [8]. One study states that the presence of a *PTEN* mutation in either CS alone, BRR alone or CS/BRR overlap families predisposes individuals to the presence of tumours, whether they be benign such as the lipomas seen predominantly in BRR, or malignant such as the breast, thyroid and uterine carcinomas seen in CS or CS/BRR overlap fa-

milies [4]. Confirmation of these preliminary findings requires analysis of a larger number of families before they can be directly transferred to the clinic.

In addition to being mutated in the germline of patients with CS and BRR, *PTEN* has been described as '...the most highly mutated tumour-suppressor gene in the post-p53 era...' [12]. It is mutated in a spectrum of human malignancies including glioblastoma (where *PTEN* mutation would seem to be a late event in tumour progression), endometrial hyperplasias (likely an early event) and carcinomas, prostate cancer and malignant melanoma and less commonly in thyroid neoplasias, breast and colon cancer. Thus, it is likely that syndromic hamartomas and cancers in CS and BRR develop on a background created by loss of the tumour suppressor function of *PTEN*. Furthermore, *PTEN* is a highly significant gene in the development of a wide range of sporadic human cancers.

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COX-2

Definition

Cyclooxygenase-2 (Cox-2), also known as prostaglandin-endoperoxide synthase 2 (Ptgs2) (ec 1.14.99.1), belongs to the prostaglandin G/H synthase family. The human PTGS2 or COX2 locus maps at 1q25.2-q25.3 and the mouse ptgs gene locus at chromosome 1 (76.20 cM). Cox-2 catalyses the first step in the formation of prostaglandins and thromboxanes and may have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity. It is a membrane-associated protein of 604 amino acids and 68 kD, and is induced by cytokines and mitogens. It is the target of non-steroidal anti-inflammatory drugs such as aspirin.

See also cyclooxygenase-2 in colorectal cancer [→ cyclooxygenase-2 in colorectal cancer].

CpG Islands

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Synonyms

- *Hpa*II tiny fragments (HTF) islands

Definition

CpG islands are short stretches of DNA sequences with an unusually high GC content and a higher frequency of CpG dinucleotides compared to the rest of the genome. Together CpG islands account for about 1-2% of the genome and their location is mainly in the 5′ regulatory regions of all housekeeping genes as well as up to 40% tissue specifically expressed genes.

Characteristics

With the rapid accumulation of sequencing data it became obvious that the distribution of the four bases, adenine (A), cytosine (C), guanine (G), and thymine (T), in the genomic sequence is not even. Normal DNA has an average GC content of 40% and an AT content of 60%. Early work by Bird et al. in 1985 (3) identified stretches of genomic sequence characterized by an unusual high number of *Hpa*II restriction sites (restriction site: C^CGG). These sequences were initially called '*Hpa*II tiny fragments (HTF) islands'. Careful inspection of those sequences indicated that the ratio of CpG dinucleotides is higher than in the rest of the genome. Normal DNA sequence contains only 25% of the CpG dinucleotides expected from the base composition. These stretches of DNA sequence, with a high GC content and a frequency of CpG dinucleotides that is close to the expected value, are now called CpG islands.

The following three criteria, established by Gardiner-Garden and Frommer in 1987 (4), are commonly used to define CpG islands: First, the sequence is longer than 200 basepairs but can be up to several kilo basepairs in size. Second, the GC content is above 50% while the rest of the genome is at about 40%. Third, the CpG ratio (observed/expected) is above 0.6 while the rest of the genome is 0.2.

The human genome contains about 45,000 CpG islands and the estimated number in the mouse genome is about 37,000 (1). The majority of these sequences are located in the 5′ region (promoter and or exon 1) of all housekeeping genes and a large number of tissue specifically regulated genes. However, CpG islands in the 3′ end of genes or in intronic sequences have been found. The preferential location of CpG islands in 5′ regions of genes can be used for the identification of novel genes. Rare cutting restriction enzymes with GC rich recognition sequences such as *Not*I (GC^GGCCGC), *Asc*I (GG^CGCGCC), *Bss*HII (G^CGCGC) and *Eag*I (C^GGCCG) can be used for the restriction mapping of large genomic clones. Clusters of those restriction enzyme cutting sites would indicate the presence of a

CpG island. It is unknown why the mouse genome has fewer CpG islands than the human genome, when the estimated number of genes in both genomes is expected to be very similar. One possible explanation is that the rate of CpG dinucleotide loss due to deamination (see below) is higher in the mouse than it is in the human genome. The location of CpG islands is in the early replicating, less condensed and GC-rich R-bands of chromosomes.

Preservation of CpG islands

The origin of CpG islands in the vertebrate genomes is closely associated with DNA → methylation and a process called deamination. DNA methylation in the vertebrate genomes is found mainly in CpG dinucleotides. Those CpG dinucleotides that are located within CpG islands are usually unmethylated. However, CpG dinucleotides located outside of CpG islands are methylated at the 5′ position of the cytosine. Methylation of CpG dinucleotides makes these sites vulnerable to spontaneous deamination leading to a transition of the 5-methyl-cytosine to thymine, a process that is believed to be the cause for depletion of CpG dinucleotides from the genome.

Clinical relevance

Although CpG islands are usually unmethylated there are a few important exceptions. Methylation in CpG islands has been correlated with the transcriptional silencing of the adjacent genes. The detailed molecular process controlling this inactivation, however, is not known. Protein complexes, containing the methyl CpG-binding protein MeCP2, Sin3 and histone deacetylases as major components, are able to bind to methylated promoters and induce the deacetylation of histones, which mediates the formation of transcription-repressing chromatin. In *in vitro* experiments, re-expression could be achieved by adding trichostatin A (TSA), a specific inhibitor of histone deacetylases. Two general methylation events in CpG islands can be distinguished: First, the developmentally regulated process of CpG island methylation found in the inactive X-chromosomes, in promoter regions of genes that are regulated in a tissue specific manner and in imprinted genes. Second, the aberrant CpG island methylation in cancer.

Normal, developmentally regulated, CpG island methylation

- Most CpG islands in the inactive X-chromosome of females are densely methylated. This process of X-chromosome inactivation is linked to the transcriptional silencing of genes on the inactive X-chromosome (*phosphoglycerate kinase 1* (*PGK1*), *glucose 6-phosphate dehydrogenase* (*G6PD*) or *androgen receptor* (*AR*). Exceptions are found in a few number of CpG islands in genes that escape X inactivation (e.g. *STS*, *ZFX* or *UBE1*).
- Some CpG islands become methylated in other normal developmental processes including cell differentiation and aging. The result of this methylation is the selective inactivation of genes in specific tissues or at certain developmental stages (e.g. *estrogen receptor*).
- Genes that are expressed from either the paternal or the maternal allele are called imprinted genes. These genes are found to have CpG island methylation of one allele. While methylation usually occurs in the inactive allele, CpG island methylation was found in some instances in the active allele. This feature of allele specific methylation in a CpG island was used as a tag for the identification of novel imprinted genes in the mouse using the → restriction landmark genomic scanning (RLGS) technique for a genome wide scan for patterns of allele specific methylation.

Aberrant CpG island methylation in cancer

- Hypermethylation of CpG islands in various cancers has been observed and is correlated with the transcriptional inactivation of tumor suppressor genes and other cancer related genes (2). It was shown that methylation in a CpG island can serve as one of the two 'hits' needed for the inactivation of a tumor suppressor gene. While CpG island

methylation in some tumors is restricted to a small number of CpG islands, other tumors show a methylation phenotype with up to 10% methylated CpG islands (4). A subset of CpG islands is methylated in a tumor type specific matter, while other CpG islands can be methylated in different tumor types. It was also shown that many of the genes associated with methylated CpG islands could be reactivated in cell lines by experimental demethylation using 5′-aza-2′deoxycytidine.

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CPT-11

Definition

→ Camptothecin.

CRE

Definition

The cyclic AMP responsive element (CRE) was first identified in the somatostatin promoter. Its 8 bp palindromic sequence -TGACGTCA- is conserved in other genes regulated by cAMP, such as *junB*, *zif268*, *nur77*, and provides the binding site for the CRE binding protein → CREB.

CREB

Definition

Cyclic AMP response element binding protein (CREB) is a 43 kD basic leucine zipper transcription factor that binds the CRE (cyclic AMP response element) consensus motif TGACGTCA. CREB was originally isolated as the protein that bound the CRE in the somatostatin gene. CREB was first shown to be activated by elevated levels of cAMP which results in its phosphorylation on serine-133 by PKA. CREB phosphorylation can be achieved by signalling cascades (e.g. the → MAP kinase signalling cascade), eventually triggering transcription of the somatostatin gene as well as other genes that contain the CRE sequence in their promoter.

CRKL

Definition

CRKL is a 36-39 kD adaptor protein, originally cloned in proximity to the BCR gene on chromosome 22, which has a key regulatory role in hematopoietic cells. CRKL is also the most prominent substrate of the → BCR-ABL1 oncoprotein that causes human → chronic myeloid leukemias (CML).

Cross Linker

Definition

Cross linkers are a class of compounds, also known as 'bifunctional alkylating agents', which are characterized by their capacity to make a covalent molecular cross link between different molecules. When these molecules are the complementary strands of a DNA double helix the result is a DNA interstrand. However, additional intermolecular covalent connections can be generated, such as intra-strand DNA, DNA-protein and protein-protein cross links.

Crossing-over

Definition

Crossing-over is the reciprocal exchange of material between chromosomes that occurs during meiosis and is responsible for genetic recombination.

CRP-ductin

Definition

CRP-ductin is the mouse homologue of → DMBT1 and is most prevalent in the gastrointestinal tract and in pancreatic and hepatic ducts; → muclin; → vomeroglandin.

Cryptic t(12;21)

Definition

Cryptic t(12;21) is a chromosomal translocation that is not visible by routine chromosomal analysis, requiring molecular methods for accurate identification. It fuses the AML1 gene on chromosome 21 with the TEL gene on chromosome 12.

CSP-B

Synonyms

- CSPB
- GZMB
- → granzyme B
- (granzyme 2)
- (cytotoxic T-lymphocyte-associated serine esterase 1)
- CGL-1
- CTLA1
- CCPI

Definition

Cytotoxic T-lymphocyte-associated granule serine protease B. This enzyme is necessary for targeted cell lysis in cell-mediated immune responses. It cleaves behind asparagine and seems to be linked to an activation cascade of caspases (aspartate-specific cysteine proteases) that are responsible for apoptosis execution. CSP-B cleaves caspase-3, -7, -9 and 10 to give rise to active enzymes that mediate → apoptosis.

c-SRC

Definition

→ Src.

CT

Definition

→ Computed tomography.

CT Scan

Definition

A computerized tomography (CT) scan is a computerized-assisted evolution of the simple X-Ray exam that allows three-dimensional reconstruction of the studied organ; can be performed using contrast materials.

CTLA1

Synonyms

- → CSP-B
- GZMB
- → granzyme B
- granzyme 2
- CGL-1
- CSP-B
- CCPI

Definition

Cytotoxic T-lymphocyte-associated serine esterase 1.

CTLs

Definition

→ Cytotoxic T lymphocytes.

Cyclin B1

Definition

Cyclin B1, also known as Ccnb1 and Ccnb is a protein of 433 amino acids and 48 kD. The human CCNB1 or CCNB gene locus maps to 5q12 and the mouse ccnb1 gene locus to chromosome 13 (56.00 cM). Cyclin B1 is essential for the control of the cell cycle at the G2/M (mitosis) transition. It interacts with the Cdc2 protein kinase to form MPF (mitosis promoting activator). G2/M cyclins accumulate steadily during G2 and are abruptly destroyed at mitosis. Increased expression is often found in cancer cell lines and tumors.

Cyclin D

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Definition

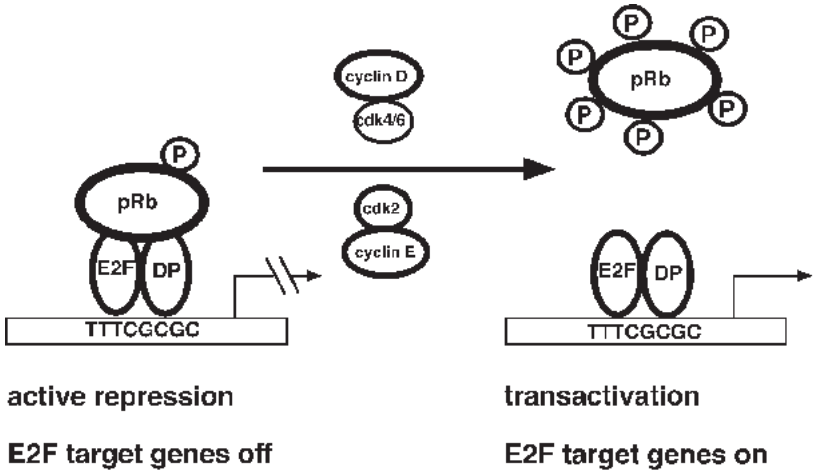
D type cyclins belong to a family of related proteins that bind to and activate several protein kinases named cyclin-dependent kinases (CDKs), which are involved in regulation of the cell division cycle.

Characteristics

D-type cyclins are encoded by three closely related genes (cyclins D1, D2 and D3) that are expressed in a tissue-specific fashion. Biochemically, D type cyclins act as regulatory subunits of a group of related protein kinases (CDKs), primarily the CDKs 4 and 6. Cyclin D/CDK4/6 complexes, together with cyclin E/CDK2, cause phosphorylation of the family of retinoblastoma proteins (→ pRb, p107 and p130) in the G1 phase of the cell cycle, resulting in abrogation of their growth inhibitory activity. Phosphorylation of the retinoblastoma proteins leads to release of → E2F transcription factors from the retinoblastoma proteins and to progression to the S phase of the cell cycle (Fig. 1).

Regulation of D cyclins

D type cyclins are major downstream targets of extracellular signaling pathways, which act to transduce mitogenic signals to the cell cycle machinery. Transcriptional induction of D type cyclins occurs in response to a wide variety of mitogenic stimuli, including the → Ras signaling cascade and the → APC-β-catenin-Tcf/



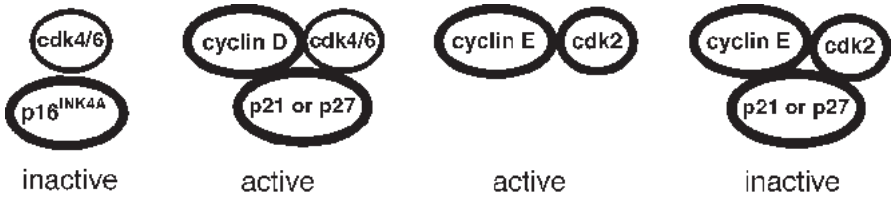
Cyclin D. Fig. 1 – Regulation of E2F activity through pRb phosphorylation. In the G1 phase of the cell cycle the retinoblastoma protein pRb is hypophosphorylated, allowing it to bind E2F transcription factors. E2F/pRb complexes are able to bind DNA but are inactive in transcription activation. Phosphorylation of pRb by cyclin D/CDK4 and cyclin E/CDK2 complexes causes the release of E2F from pRb. Free E2F is then able to activate transcription of E2F target genes (genes with TTTCGCGC-like E2F sites in their promoters), allowing cells to enter the DNA synthesis phase (S phase) of the cell cycle.

Lef pathway. In addition, cyclin D1 protein turnover and subcellular localization is highly regulated during the cell cycle. Phosphorylation of cyclin D1 by GSK-3β in resting cells renders the protein a target for rapid destruction by the proteasome. In contrast, mitogenic stimulation of cells leads to inhibition of GSK-3β and stabilization of cyclin D1 protein. In response to DNA damage, cells initiate an immediate G1 arrest, which is caused by rapid proteolysis of cyclin D1. Together with activation of the p53 tumor suppressor protein, cyclin D1 destruction causes a fast withdrawal from the cell cycle to allow repair of the damaged DNA before DNA synthesis resumes.

Binding of D type cyclins to their CDK partner is antagonized by the → INK4 family of CDK inhibitors (→ CKI). INK4 proteins bind to CDK4 and 6 and thereby prevent association of D type cyclins to these CDKs (Fig. 2). The most prominent member of this family is $p16^{INK4A}$. Mutations in $p16^{INK4A}$ (also known as → CDKN2A) are found in a variety of spontaneous tumors, and heterozygosity for $p16^{INK4A}$ in the germ line predisposes to melanoma. A second family of CKIs consists of three related proteins that bind to cyclin/CDK complexes. Members of this family include $p21^{cip1}$ and $p27^{kip1}$. This class of CKIs has quite divergent effects on the different cyclin/CDK complexes. Whereas cyclin E/CDK2 is inhibited by both $p21^{cip1}$ and $p27^{kip1}$, cyclin D/CDK4/6 complexes are active when complexed with this class of inhibitors (Fig. 2). In fact, formation of active cyclin D/CDK4/6 complexes requires the presence of $p21^{cip1}$ or $p27^{kip1}$ to act as 'assembly factors' of cyclin D/CDK complexes. These opposing effects of $p21^{cip1}$ and $p27^{kip1}$ on cyclin E/CDK2 and cyclin D/CDK4 complexes endows cyclin D/CDK4 complexes with an important second, non-catalytic function during the G1 phase of the cell cycle. Synthesis of cyclin D1 by mitogenic stimulation leads to absorption of $p21^{cip1}$ or $p27^{kip1}$ into active ternary complexes, thereby facilitating activation of cyclin E/CDK2 by removal of inhibitors.

CDK-independent activities of D type cyclins

Apart from their role in activation of CDKs, D type cyclins can have several profound effects on cellular physiology independent of their CDK partners. In → breast cancer, cyclin D1 can bind directly to the estrogen receptor, thereby causing hormone-independent activation of the estrogen receptor. This activity of



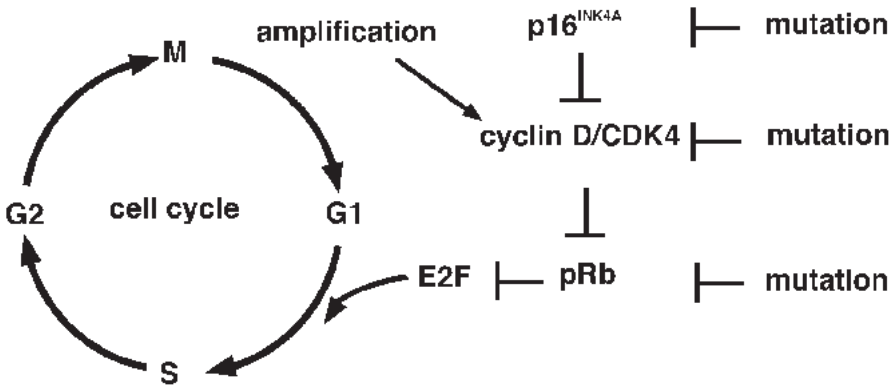
Cyclin D. Fig. 2 – Effect of CDK inhibitors on cyclin/CDK complexes. CDKs 4 and 6 are activated by binding of D type cyclins. Association of cyclin D to CDKs 4 and 6 is prevented by $p16^{INK4A}$ that binds with high affinity to these CDKs. Thereby, binding of cyclin D to these CDKs is prevented. The CDK inhibitors $p21^{cip1}$ and $p27^{kip1}$ bind both to cyclin E/CDK2 and to cyclin D/CDK4 complexes, although with different consequences. Even though these inhibitors antagonize cyclin E/CDK2 activity, they are required for proper assemblage and activity of cyclin D/CDK4/6 complexes.

cyclin D1 may contribute to resistance to antihormonal therapy that is often seen in the clinic. In addition, D type cyclins can modulate the activity of Myb transcription factors. Of particular interest in this respect is the Myb-like transcription factor DMP1, which has anti-proliferative activity. Expression of cyclin D inhibits this effect on cell proliferation of DMP1 through direct binding to DMP1, which prevents DNA binding by DMP1.

Clinical Relevance

Because of their critical role in linking cytoplasmic signals to nuclear responses it is perhaps not surprising that D type cyclins are frequently deregulated in several types of cancer. *Cyclin D1* → amplification or overexpression is found in a number of human malignancies, the most prominent being breast cancer, in which up to 50% of all cases have elevated levels of cyclin D1 protein. Chromosomal translocations involving *cyclin D1* are found in parathyroid adenoma and in mantle cell lymphoma.

Not only is *cyclin D1* itself often directly mutated in human cancer, its upstream regulators such as $p16^{INK4A}$ and its downstream target pRb are frequent targets in human carcinogenesis as well. It is generally believed that this $p16^{INK4A}$-cyclin D1-pRb pathway is deregulated in virtually all human cancers (Fig. 3).



Cyclin D. Fig. 3 – The p16-cyclin D-pRb pathway: a frequent target in human cancer. E2F transcription factors contribute to G1-S phase progression through the activation of specific target genes. E2F activity is negatively regulated by its binding to the retinoblastoma tumor suppressor gene product, pRb. The ability of pRb to bind E2F is regulated by cyclin D/CDK complexes. The activity of cyclin D/CDK complexes in turn is negatively regulated by $p16^{INK4A}$ that is encoded by the CDKN2A tumor suppressor gene.

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Cyclin D2

Definition

Cyclin D2 is a member of the growth factor inducible family of D-type cyclins, which in concert with cyclin dependent kinases (CDK) 4 and 6 promote G_0 exit and early G_1 progression in the cell cycle.

Cyclin-dependent Kinase

Definition

Cyclin-dependent kinases (CDK) are complexes that consist of two different proteins: one molecule of cyclin (which is the regulatory subunit) plus one molecule of the actual cyclin-dependent kinase (cdk, which is the catalytic subunit). CDKs are the 'engine of the cell cycle' and they direct the events required for cellular proliferation. Without CDK activity, cell growth does not take place.

- cyclinD/cdk4: CDK complex consisting of one molecule cyclinD plus one molecule cdk4
- cyclinD/cdk6: CDK complex consisting of one molecule cyclinD plus one molecule cdk6
- cyclinE/cdk2: CDK complex consisting of one molecule cyclinE plus one molecule cdk2
- cyclinA/cdk2: CDK complex consisting of one molecule cyclinA plus one molecule cdk2

Cyclin-dependent Kinase (CDK) Inhibitor

Definition

There are two groups of cyclin-dependent kinase (CDK) inhibitors (CKIs); the → Cip/Kip family and the cyclin → INK4 family.

Cyclin-dependent Kinase Inhibitor 1B

Definition

Cyclin-dependent kinase inhibitor 1B (CDKNB1B) is also known as p27 and Kip1; Cip/Kip family [→ CIP/KIP family].

Cyclobutane Pyrimidine Dimers

Definition

Cyclobutane pyrimidine dimers (CPD) are UV-induced DNA lesions between two adjacent pyrimidines (C or T).

Cyclooxygenase-2 in Colorectal Cancer

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Synonyms

- cyclooxygenase-2 (COX-2)
- cyclooxygenase-prostaglandin endoperoxide synthase

Characteristics

Colorectal cancer [→ colon cancer] remains a significant health concern for much of the industrialized world, even though mortality rates are beginning to decline in the USA. Diagnosis often occurs at a late stage in the progression of this disease, which reduces the likelihood of treatment being effective. Current treatment strategies often involve a combination of surgical resection and adjuvant chemotherapy [→ adjuvant therapy]. Because of the unsatisfactory outcome of existing treatment methods, much emphasis has been placed on developing new treatment, prevention and screening strategies. Numerous population based studies indicate that use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the risk for colorectal cancer and decrease the incidence of adenomatous polyps. NSAIDs have been shown to induce polyp regression in familial adenomatous polyposis (FAP [→ APC gene in Familial Adenomatous Polyposis]) patients and reduce tumor burden in animal models of colorectal cancer.

Cyclooxygenase-2 and colorectal cancer prevention

COX-2 mRNA and protein levels are increased in intestinal tumors that develop in rodents following carcinogen treatment and in adenomas taken from multiple intestinal neoplasia (→ Min) mice. When intestinal epithelial cells are forced to express COX-2 constitutively, they develop phenotypic changes that include increased adhesion to → extracellular matrix (ECM) and resistance to butyrate-induced → apoptosis. Both of these phenotypic changes are consistent with an increased tumorigenic potential. COX-2 expression has been detected in 80 – 90% of colorectal adenocarcinomas but in only 40 – 50% of premalignant adenomas. These data suggest that elevation of COX-2 expression is secondary to other initiating events such as dysregulation of the APC signaling pathway and/or dysfunction of other genes affected during the adenoma to carcinoma sequence (→ multistep development).

The observation of elevated COX-2 expression in three different models of colorectal carcinogenesis has led to consideration of the possibility that COX-2 expression may be related to colorectal tumorigenesis in a causal way. Recent studies have demonstrated a significant reduction in premalignant and malignant lesions in carcinogen-treated rats that were given a selective COX-2 inhibitor.

Tumor growth requires the maintenance and expansion of a vascular network. It has been demonstrated using *in vitro* assays that COX-2 can influence → angiogenesis, and treatment with selective COX-2 inhibitors blocks angiogenesis. COX-2 appears to contribute to tumor vascularization and there seems to be a link between COX-2 and regulation of → VEGF expression.

Summary

Both preclinical and clinical data indicate that selective COX-2 inhibitors have anti-neoplastic activity. The precise role of COX-2 and the → prostaglandins produced by this enzymatic pathway in carcinogenesis remains to be clearly delineated. Overexpression of COX-2 in epithelial cells leads to inhibition of apoptosis and increased adhesiveness to extracellular matrix. Inhibition of COX-2 activity leads to a marked reduction of tumor growth in a number of experimental models. Treatment with selective COX-2 inhibitors have been clearly shown to inhibit tumor-induced angiogenesis. The most effective role for selective COX-2 inhibitors for prevention and treatment of human cancers is currently under investigation.

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CYP

Definition

→ Cytochrome P450.

Cystectomy, radical

Definition

A radical cystectomy

- in males, is the wide excision of bladder, prostate and seminal vesicles, and
- in females, is the excision of bladder, uterus, ovaries and anterior vaginal wall.

Cysteine Switch

Definition

The cysteine switch model for proMMP (matrix metallloproteinase) activation is based on the observation that an unpaired cysteine residue in the propeptide region of pro→ MMPs coordinates the catalytically essential active site zinc atom, preventing access of a water molecule to the enzyme active site. Perturbation of the Cys-Zn interaction, using denaturing agents such as SDS, mercurial compounds like phenylmercuric acetate or limited proteolytic cleavage within the propeptide domain, leads to disruption of the Cys-Zn bond, propeptide processing and acquisition of catalytic activity.

Cytochemistry

Definition

Cytochemistry is the microscopical biochemistry of cells. The reagents most often used to distinguish the enzymes present in acute lymphoid leukemia (→ ALL) cells from those in acute myeloid leukemia cells are Sudan black, myeloperoxidase and nonspecific esterases (α-naphthyl butyrate and α-naphthyl acetate esterase); these reagents do not react with leukemic lymphoblasts.

Cytochrome P450

Definition

Cytochrome P450 is a highly versatile heme-containing superfamily of enzymes that catalyses the incorporation of molecular oxygen into an enormous spectrum of substrates. In addition to catalysing the oxidation of xenobiotics, these enzymes are involved in the biosynthesis of steroid hormones, bile acids, fatty acids and eicosanoids; → detoxification.

Cytogenetics

Definition

Cytogenetics is the laboratory analysis of chromosome structure and morphology. This analysis requires first growing cells, then 'freezing' them in metaphase just prior to the completion of cell division in order to get a good view of condensed, paired chromosomes.

Cytokine

Definition

A cytokine is any of a variety of secreted polypeptides that control the development, differentiation and proliferation of hematopoietic cells. The effects of cytokines on lymphocytes are usually mediated through membrane bound cytokine receptors and are especially critical during immune responses. They bind to specific cell surface receptors and induce activation of specialized functions usually in hematopoietic cell types, such as activation of immune responses. They are usually small, soluble and secreted by cells, and act in a → paracrine or → autocrine manner to control cellular responses.

Cytomegalovirus

Definition

Cytomegalovirus is a herpes virus that can reactivate and cause significant morbidity and mortality after bone marrow transplant.

Cytoplasm

Definition

The matter between the nucleus and the plasma membrane is called cytoplasm. It accounts for most of the cell mass and is a complex mixture of all kinds of molecules. Its highly organized structure consists in part of complex internal membranes, a basic feature of all eukaryotic cells. Membranes surrounding the nucleus form a 'labyrinth' referred to as the endoplasmic reticulum (ER), where lipids and proteins of the cell membrane are synthesized. Membranes also surround the mitochondria and form the Golgi apparatus, which is involved in synthesis and transport of various molecules. Membrane covered lysosomes contain enzyme systems, required for the intracellular digestion of exogenous agents. Also surrounded by membranes are peroxisomes, in which reactice peroxides are generated as well as degraded. The remaining area of the cytoplasm, which includes all the other non-membrane bound organelles, is referred to as the → cytosol.

Cytoskeleton

Definition

The cytoskeleton is a network of polymeric proteins organized in filaments (microtubules, microfilaments and intermediate filaments) that gives shape to the cell and contributes to transport of molecules inside the cell. It is an intracellular network of structural protein filaments (including actin filaments, microtubules and intermediate filaments like keratins, vimentin, desmin and neurofilaments). The cytoskeleton directs cell shape, mediates the anchoring and movement of cell organelles and is necessary for cell division. It is linked to cell-cell and cell-substrate → cell adhesion molecules and increases the structural integrity of tissues and organs.

Cytosol

Definition

The cytosol describes the general volume of → cytoplasm and includes everything other than the membrane-bound organelles. Here, the majority of chemical reactions of the intermediary metabolism take place; the cell synthesizes (e.g. macromolecule-precursors, needed for the structure, function, and growth of the cell) and also degrades molecules. The cytosol is the site of glycolysis, gluconeogenesis, biosynthesis of sugars, fatty acids, nucleotides and amino acids; contains also a variety of cytoskeletal proteins that confer shape to the cell and cause coherent cytoplasmic movements.

Cytotoxic Chemotherapy

Definition

Cytotoxic chemotherapy involves treatment with chemicals having selective toxicity for cancer cells; → chemotherapy.

Cytotoxic T Lymphocytes

Definition

Cytotoxic T lymphocytes (CTLs) are effector T cells (usually CD8+) that can lyse appropriate target cells (virus infected or tumor cells) in an antigen-specific → MHC (major histocompatibility complex)-restricted manner.

D

Damage Response

Definition

→ Stress response.

DAP

Definition

Death associated proteins (DAPs) are proteins involved in apoptosis. The genes have been isolated by the functional approach of → technical knock out.

DAP-3

Definition

→ Death-associated protein 3.

DBL Family Proteins

Definition

Dbl family proteins share sequence identity with Dbl, the first identified member of this family. Dbl was identified as a transforming protein that was detected in gene transfer transformation assays using genomic DNA isolated from a human diffuse B-cell lymphoma. These proteins also share a tandem Dbl homology and pleckstrin homology domain structure, → RHO family proteins.

DBL Homology Domain

Definition

The Dbl homology domain has approximately 180 amino acids that catalyses the GDP/GTP exchange activity of → Rho family proteins.

DCC

Definition

DCC (deleted in colorectal carcinoma) is implicated as a tumor suppressor gene. The gene maps to 18q21.3 and encodes a membrane protein preferentially found in axons of the neurons of the central and peripheral nervous system and in differentiated cell types of the intestine. Colorectal tumors that have lost their capacity to differentiate into mucus producing cells, uniformly lack DCC protein expression. Inactivation of *DCC* due to allelic deletion and/or point mutations may also be involved in both lymphatic and hematogenous metastasis of oesophageal squamous cell carcinomas.

Deamination

Definition

Deamination of DNA is a process which converts a 5-methyl-cytosine into a thymine. This is a spontaneously occurring process that is believed to be the cause for the loss of → CpG dinucleotides from the genome.

Death-associated Protein 3

Definition

Death-associated protein 3 is a ubiquitously expressed protein of 398 aa and 45 kD involved in mediating interferon-γ induced cell death. The gene maps to 1q21; → DAP.

Deleted in Malignant Brain Tumors 1

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Synonyms

Deleted in malignant brain tumors 1 (DMBT1; human) is also known as glycoprotein-340 (gp-340; human), H3 (rhesus monkey), CRP-ductin (mouse), muclin (mouse), vomeroglandin (mouse), ebnerin (rat), hensin (rabbit).

Definition

Deleted in Malignant Brain Tumors 1 (*DMBT1*) was initially identified as a gene located at human chromsome 10q25.3-q26.1 that shows frequent deletions on both of its chromosomal copies in malignant brain tumors. *DMBT1* codes for an extracellular glycoprotein (a protein to which carbohydrates are attached) belonging to the superfamily of scavenger receptor cysteine-rich (SRCR) proteins. Various isoforms of the protein exist with relative molecular weights ranging from about 200 kD to 340 kD. gp-340 (DMBT1/gp-340) represents one of these isoforms and is defined by its ability to interact with the surfactant protein D (SP-D) and surfactant protein A (SP-A). The homologues of *DMBT1* in various species have been identified (see: synonyms). The mouse homologue has been designated in the literature as CRP-ductin, muclin and vomeroglandin.

Characteristics

General characteristics

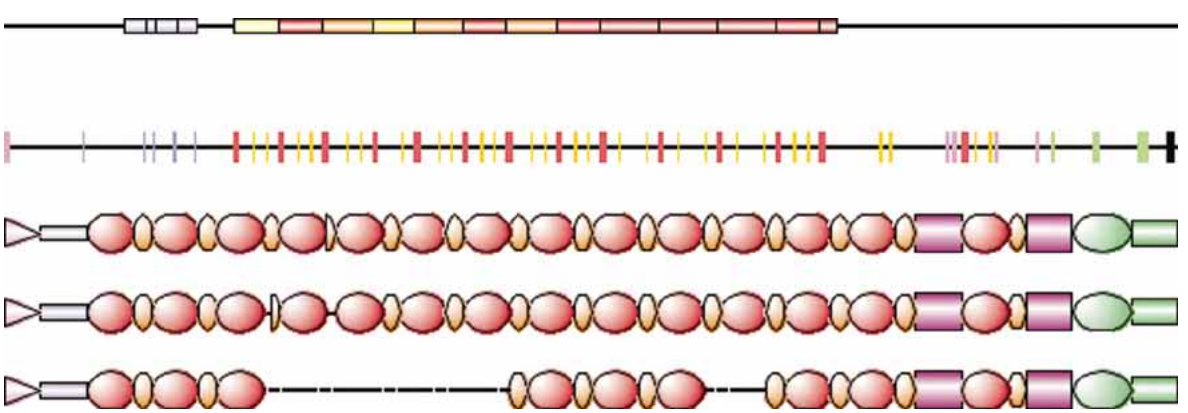
DMBT1 consists of a putative signal peptide, followed by a region with no homology to known proteins and a variable number (up to 13) of SRCR domains (Fig.). The SRCR domains are separated by short amino acid stretches (20-24 amino acids) that have been designated as SRCR interspersed domains (SIDs). SRCR13 is followed by a threonine-rich stretch. Towards the carboxyterminal end, a fourteenth SRCR domain precedes a serine-threonine-proline rich motif and is flanked by two C1r/C1s Uegf Bmp1 (CUB) domains. At the carboxyterminus there is a short stretch of amino acids with homology to rat ebnerin (ebnerin homologous domain; EHD) that is preceded by a zona pellucida (ZP) domain. So far, alternative splicing has only been reported for the exons encoding the repetitive SRCR domains and SIDs (Fig.). The *DMBT1* locus is built up by tandem-arrayed repeats with extensive homologies in the coding and non-coding regions (Fig.).

Alterations in cancer

A reduction or an absence of *DMBT1* expression has been reported for 4/5 brain tumor cell lines, 55/76 (72%) lung tumors (cell lines and primary tumors), 35/58 (60%) oesophageal tumors, 5/40 (12.5%) gastric and 4/24 (17%) colorectal cancer cases.

Homozygous deletions (deletions concerning both copies of the gene) of or within *DMBT1* have been observed in 2/20 (10%) medulloblastomas, 17/60 (28%) glioblastoma multiformes, 6/77 (8%) lung cancers and 7/58 (12%) oesophageal tumors. Genomic rearrangements of *DMBT1* have been found for 16/18 (89%) tumor cell lines (brain and lung tumors). These findings have led to the proposal that *DMBT1* might represent a tumor suppressor gene.

In the majority of the cases, tumors with homozygous deletions also display a reduction or an absence of mRNA expression. However, only the minority of tumors with a diminished



Deleted in Malignant Brain Tumors. Fig. – Genomic organization and domain structure of *DMBT1*. The *DMBT1* gene contains multiple repeating units. The top line depicts the relative lengths and positions of these units (*DMBT1* repeats) in the genomic DNA. Each unit consists of one to three exons and the highly homologous intronic sequences. Subtypes of *DMBT1*-repeats are indicated by different colours. The repeating units are drawn to scale with the exon-intron structure in the second line. The *DMBT1* gene consists of at least 54 exons. The exons are coloured according to the domain that they code for (see below). The presence of a further exon (black box) with coding potential for a transmembrane domain and a short cytoplasmic tail is predicted by homology searches with the cDNA sequences of the rodent homologues. The bottom three lines depict (from top to bottom) the domain organisation of a prototype protein assembled and conceptually translated from the genomic exons, from one of the 8kb transcripts isolated from human adult trachea (*DMBT1*/8kb.1) and one of the 6kb transcripts isolated from human fetal lung (*DMBT1*/6kb.1). Broken lines indicate regions of alternative splicing. Pink triangle, signal peptide; blue box, unknown motif; red circles, SRCR domains; orange circles, SIDs, threonine- and threonine-serine-proline-rich domain, respectively; violet boxes, CUB domains; green circle, ZP domain; green box, Ebnerin homologous domain.

expression also display deletions, indicating that the two mechanisms do not depend on each other.

The repetitive structure is thought to represent the driving force for the multiple deletions and rearrangements in tumors. It is likely that an enhanced inter- and/or intrachromosomal recombination takes place at the *DMBT1* locus and eliminates different parts of the gene during tumorigenesis. However, about one fourth of normal individuals is hemizygous for different alleles with pre-existing alterations. The complex genotypes that are observed in the tumors are therefore anticipated to be a result of both pre-existing deletions, which have been uncovered by a loss of the full-length copy of the gene and secondary rearrangements acquired during tumorigenesis.

The exact role of *DMBT1* in tumorigenesis has not yet been defined. Oesophageal tumors with diminished *DMBT1* expression show an increased tendency for vascular vessel invasion. The data values, however, have been below statistical significance. The transfer of chromosomal segments containing the *DMBT1* locus has not led to a change of the tumorigenic phenotype of a glioblastoma cell line. However, in this case it was not defined whether the cell line has defects in the gene or is deficient for expression. Moreover, it was not tested whether the chromosomal segments contained a full-length copy of the gene. Chromsomal losses at the *DMBT1* locus (i.e. the loss of one copy of the gene) appear not to be specific for the progression to the most malignant form of gliomas, glioblastoma multiforme. In this study it has been found that these losses occur at equal and high frequency (72 – 90%) in all stages of gliomas.

Functional characteristics

General features.

The three known motifs in DMBT1, the SRCR, the CUB, and the ZP domain have in common that they are involved in the media-

tion of protein-protein interactions. ZP domains have been found in the sperm receptors ZP2 and ZP3 of the zona pellucida, the outer envelope of the oocyte, but also in the TGF-ß type III receptor. CUB domains have been recognized as a motif common to developmentally regulated proteins that are involved in processes of embryogenesis and organogenesis. The SRCR domain is an amino acid motif common to a group of proteins that have mainly been implicated in diverse functions within the immune system. The SRCR family is further subdivided in group A and group B SRCR proteins according to the number and spacing of invariant cysteine residues that are most probably involved in intra- or intermolecular bonding. DMBT1, together with, for example, CD5, CD6, WC1/T19, and CD163/M130 belong to the group B SRCR proteins.

Involvement in the immune defense/tumor surveillance.

DMBT1-related proteins play a role in diverse aspects of the immune defense. For example, CD6 is involved in the regulation of lymphocyte proliferation by interacting with the ALCAM receptor. The interaction is mediated by the membrane proximal SRCR domain. To date this is the only function that has been demonstrated for any of the known SRCR domains. WC1/T19 was originally proposed as a homing receptor for T-cells. It appears to be the case that isoforms differing in the utilization of highly homologous SRCR domains are specifically expressed by different T-cell subpopulations. CD163/M130 is a macrophage specific differentiation antigen. The splicing of exons equivalent to the SIDs of DMBT1 seems to be regulated in a glucocorticoid dependent manner. Spalpha (also known as apoptosis inhibitor expressed by macrophages, AIM) is a group B SRCR protein that inhibits apoptosis of thymocytes during thymic selection. An example of the group A of SRCR proteins is the Mac-2 binding-protein (Mac-2bp, also known as the 90k tumor antigen). The Mac-2bp is a multifunctional protein. It is involved in cell-cell adhesion, cell-extracellular matrix adhesion, the mucosal immune defense, probably by its interaction with Mac-2 (also known as galectin-3) that is a carbohydrate binding protein (a lectin) that binds to various pathogens. Mac-2bp is found at elevated levels in cancer and AIDS patients and has been demonstrated to have a tumor suppressive effect when injected into tumor-bearing mice. It is anticipated that Mac-2bp exerts its tumor suppressive effect by activating natural killer cells to eliminate the tumor cells. DMBT1 shares a set of features with the Mac-2bp, so that it has been proposed that DMBT1 might represent a kind of group B counterpart of Mac-2bp. Both proteins are expressed in organs with large mucosal surfaces and are secreted as oligomers either towards the lumen or to the extracellular matrix. Moreover, it recently has been demonstrated that isoforms of DMBT1 are identical to a protein formerly known as gp-340. DMBT1/gp-340 likewise has been shown to have a stimulatory effect on immune cells (lung macrophages), and DMBT1/gp-340 likewise interacts with lectins, namely the lung lectins SP-D and SP-A. These lectins are known to play an important role in innate immunity that is a kind of first frontline of the organism in the defense against invading pathogens such as bacteria, viruses, funghi and allergens. However, lectins have also been linked to tumor surveillance by the recent finding that the mannose-binding protein (MBP) can bind to tumor cells and activate a cellular anti-tumor response *in vivo*. Two sources of DMBT1 expression have been identified in tumor tissues: i) the tumor cells itself, and ii) tumor associated macrophages. These observations and the properties of DMBT1 have led to the proposal that it might be involved in mechanisms of tumor surveillance by acting as an alarm signal when expressed by tumor cells and/or by facilitating the elimination of tumor cells via indirect or direct binding when expressed by tumor associated macrophages.

Involvement in epithelial differentiation.

DMBT1 has a specific temporal and spatial distribution when comparing fetal and adult epithelial structures. Hensin, the DMBT1 homologue of the rabbit, has been demonstrated to induce epithelial terminal differentia-

tion when present in multimeric form in the extracellular matrix. It therefore is likely that DMBT1 plays a role in processes of epithelial differentiation and/or regeneration in the human organism. A loss of DMBT1 expression or genomic rearrangements of the gene could thus also interfere with these processes and facilitate tumorigenesis at epithelial surfaces.

Involvement in cytoprotection/wound healing.

Recently, the porcine variant of DMBT1 has been co-purified with trefoil factor 2 (TFF2), and it has been postulated that it fulfills the criteria for a TFF2 receptor. TFFs are known to be cytoprotective and to promote healing in the gastrointestinal tract. They stimulate cell migration, inhibit apoptosis of certain cells and increase the barrier function of mucus. TFFs are ectopically expressed in many epithelial cancers and it has been proposed that they may contribute to tumorigenesis by the inhibition of the programmed cell death and by increasing the metastatic capacity of tumor cells. The disruption of the TFF1 gene leads to adenoma formation in the stomach, indicating that TFFs can potentially exert tumor suppressive functions. Taken together, these observations raise the possibility that alterations of DMBT1 or its loss of expression favours tumorigenesis or promotes tumor progression by interfering with or modulating processes that are mediated by TFFs.

Cellular and molecular regulation

Studies with a reporter gene suggest the presence of a silencer and an enhancer of transcriptional activity within the *DMBT1* promotor region. The exact sequences and factors that regulate the expression of the gene remain to be identified. However, it has been found that *H3*, the rhesus monkey homologue of *DMBT1*, is upregulated by progesterone in the endometrium, so that it is likely that also *DMBT1* is subjected to such a regulation. There is initial evidence that a multitude of *DMBT1* variants can be generated by alternative splicing of the exons that code for the SRCR domains and SIDs. The different variants, their expression pattern and regulation as well as the differences in their functional properties have not yet been characterised.

Synopsis

Evidence is accumulating that *DMBT1* is a polymorphic gene that might be involved in multiple fundamental processes within the living organism. *DMBT1* is highly unstable in cancer and its expression is frequently suppressed in tumors. It could act at different stages of tumorigenesis possibly depending on the time point and the quality of the alterations as well as the source (tumor cells or associated immune cells) that expresses aberrant *DMBT1* variants.

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Deleted in Pancreatic Carcinoma Locus 4

Definition

Deleted in pancreatic carcinoma locus 4 (→ DPC4).

Deletion

Definition

Deletion is the loss of a chromosomal segment or gene. A chromosomal deletion can be terminal, i.e. involve the end of a chromosome, or it can be interstitial, in which case a segment from within the chromosome is lost.

Denaturation

Definition

Denaturation of DNA or RNA describes its conversion from double-stranded to single-stranded. The separation of the strands is most often accomplished by heating.

Density-dependent Inhibition

Definition

Density-dependent inhibition occurs when cells grow to a limited density then growth becomes inhibited, possibly by cell-cell contacts. Tumor cells have often lost density-dependent inhibition.

Depurination of DNA

Definition

Depurination of DNA is the loss of purine residues from the DNA structure.

Dermatofibrosarcoma Protuberans

Definition

Dermatofibrosarcoma protuberans is a soft tissue tumor of the dermis that is locally, highly invasive and aggressive. It is characterized by a reciprocal t(17;22) translocation or by ring chromosomes containing chromosome 17 and 22 DNA sequences.

Desmoid

Definition

Desmoid tumors are a form of fibromatosis found either in the retroperitoneal tissue or in the abdominal wall of FAP patients (familial adenomatous polyposis). Their appearance is usually associated with abdominal surgical trauma. They are composed of sheets of elongated myofibroblasts with variable amounts of collagens between cells, and are mostly vascularized, which often precludes surgical resection. Desmoid tumors are benign lesions, however, their infiltrative nature make them a frequent cause of morbidity and mortality among FAP patients.

Desmoplastic Medulloblastoma

Definition

Desmoplastic medulloblastoma is a histological subtype of medulloblastoma characterized by a network of reticulin fibres leaving pale islands of typical medulloblastoma cells. It is the predominant histological medulloblastoma type in → BCNS or → Gorlin syndrome.

Desmosome

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Synonyms

- macula adherens (plural: maculae adherentes)

Definition

Desmosomes are spot-like intercellular adhesive junctions of epithelia with dense cytoplasmic plaques linked to the intermediate filament cytoskeleton. Intercellular → adhesion in desmosomes is mediated by desmocollins and desmogleins (→ cell adhesion molecules, members of the cadherin family). Desmosomes are crucial to withstand the tensile and shearing forces to which tissues, like epithelia, are subjected.

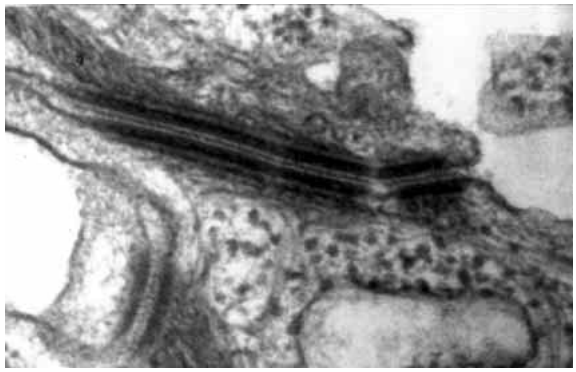
Characteristics

Desmosomes are believed to be responsible for strong intercellular adhesion. They co-ordinate a cytoskeletal scaffold throughout a tissue by linking the intermediate filament cytoskeletons of adjacent cells. They are present in almost all epithelia, cardiac muscle, the arachnoid and pia of the meninges and on the processes of follicular denditric cells of the lymphoid system, being especially abundant in tissues that are subject to high stress, such as the epidermis (Fig. 1).

Desmosomal adhesion is mediated by two transmembrane glycoproteins, desmocollin (Dsc) and desmoglein (Dsg), which are members of the cadherin family of calcium dependent cell-cell adhesion molecules. Both molecules appear to be required for adhesion and they are believed to interact heterophilically, with desmocollin binding to desmoglein. Electron microscopy has shown that the desmosomal adhesion molecules are arranged in a highly ordered fashion in the intercellular space, though precise details of this arrangement are not understood.

The cytoplasmic plaque consists of two regions, an outer dense plaque (ODP) that is adjacent to the inner leaflet of the plasma membrane, and an inner dense plaque (IDP) that is separated from the outer plaque ODP by an electron-lucent region. The intermediate filaments of the cytoskeleton appear to interact with the IDP. The ODP, which is extremely electron-dense, contains the cytoplasmic domains of the desmosomal glycoproteins and two proteins, plakoglobin and plakophilin, which are members of the armidillo protein family. The ODP also contains the amino-terminus of desmoplakin, a member of the plakin family. This molecule extends across the electron-lucent region to the IDP where its carboxy-terminus resides, interacting with the intermediate filaments.

Each of the desmosomal glycoproteins and the armadillo protein plakophilin exists as



Desmosome. Fig. 1 – Electron micrograph of human epidermis showing desmosomes in cross section. The diameter of the largest is approximately 0.5 μm.

three distinct isoforms, being the products of different genes. These show tissue specific expression. For example, Dsc 1 and Dsg 1 are expressed in cornified stratified epithelia, Dsc 2 and Dsg 2 are ubiquitous, and Dsc 3 and Dsg 3 are expressed in stratified epithelia as well as myoepithelial cells of the mammary gland. Plakophilins 1, 2 and 3 are distributed similarly to their glycoprotein counterparts.

The importance of desmosomal adhesion in tissue structure is apparent in human autoimmune and genetic disease. In pemphigus vulgaris and pemphigus foliaceus, autoantibodies directed against Dsg 3 and Dsg 1, respectively, cause severe epidermal blistering. Heterozygous null mutation of plakophilin 1 gives rise to an epidermal fragility syndrome, hair loss and abnormal nail morphology. Null mutations of plakoglobin and desmoplakin in mice cause embryonic fatality, while null mutation of Dsg 3 generates lesions resembling those seen in pemphigus vulgaris.

Assembly of desmosomes is a calcium dependent process, as would be expected from a cadherin-mediated system. Surprisingly, however, desmosomal adhesion in confluent cell sheets and animal tissues is largely calcium independent, being resistant to the depletion of extracellular calcium. Under experimental conditions, conversion of desmosomes from the calcium independent to the calcium dependent state can be initiated by wounding the cell sheet or activation of the signalling enzyme protein kinase C.

Immunohistochemical studies have shown that the expression of desmosomal components is reduced or lost in some human cancers, including transitional carcinoma of bladder, oral squamous cell carcinoma, basal and squamous cell carcinoma of epidermis and oesophageal carcinoma. However, in other cancers, such as colorectal carcinoma, no loss of desmosome expression can be detected. These observations suggest that desmosomes may play a role in suppression of invasion and metastasis in some carcinomas. Experimental evidence for this is currently sparse but it has been shown that desmosomal adhesion can inhibit the invasion of cells into collagen gels in an experimental system. As ubiquitous epithelial proteins, desmoplakin and desmoglein can be used as diagnostic markers of carcinomas. In combination with other markers they can also be used as markers for tumours of the nervous system (Fig. 2).

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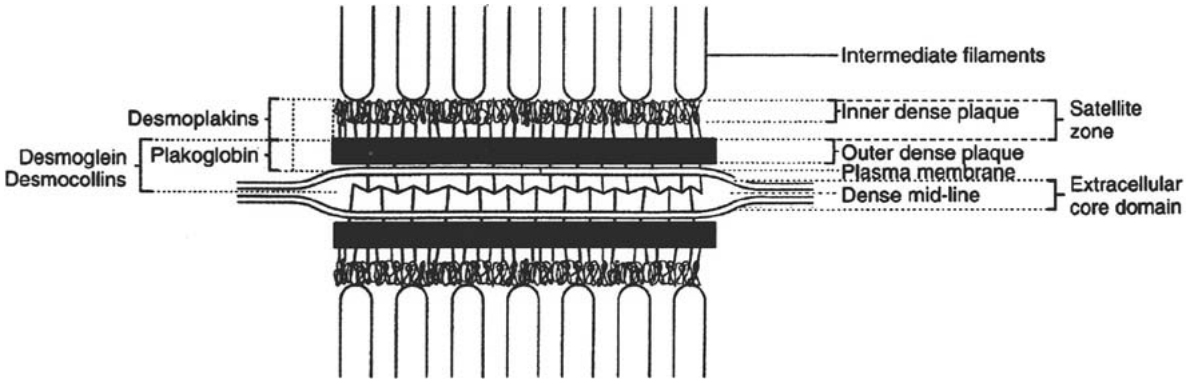

Desmosome. Fig. 2 – Diagram showing interpretation of desmosome structure and location of some of the major components.

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Detoxication

Definition

→ Detoxification.

Detoxification

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Synonyms

- detoxication
- drug metabolism
- xenobiotic metabolism
- carcinogen metabolism
- xenobiotic biotransformation

Definition

Metabolic and transport processes used to chemically inactivate noxious compounds and eliminate them from cells for subsequent excretion from the body.

Characteristics

Humans are continuously exposed to foreign chemicals (xenobiotics [→ xenobiotic]) through administration of medicines, the consumption of food and drink, and air breathed. Protection against the detrimental effects of xenobiotics is achieved by the concerted actions of a battery of proteins that metabolise, transport and ultimately pump out of cells modified forms of the compounds originally encountered. This process is called detoxification, or detoxication (in instances where no toxicity occurs). Although detoxication occurs primarily in the liver, all cells possess some capacity to metabolise and eliminate unwanted chemicals. The xenobiotics subject to this process are numerous and include mycotoxins, phytoallexins, pesticides, herbicides, environmental pollutants, cytotoxic anti-cancer agents and many pharmacologically-active drugs. Detoxication processes also confer protection against harmful compounds of endogeneous origin, many of which arise as a consequence of interaction with reactive oxygen species, such as the superoxide anion, produced normally in the body.

Detoxication is achieved in two distinct stages, the first involving metabolism of the xenobiotic, and the second involving energy-dependent efflux of the xenobiotic from the cell. Historically, description of xenobiotic biotransformation has been divided into phase 1 and phase 2 metabolism, and consequently efflux of xenobiotics is referred to as phase 3 of detoxication.

- Phase 1 drug metabolism involves an initial chemical modification of the xenobiotic that results in the introduction, or exposure, of a functional chemical group (e.g. -OH, -NH_2, -SH, -COOH) into the compound. This usually entails enzyme-catalyzed oxidation reactions by → cytochrome P450 (CYP) or flavin monooxygenase (1).
- Phase 2 drug metabolism often involves a second chemical alteration of the xenobiotic, usually at the same region of the molecule where the functional group was introduced. This is performed by enzymes catalyzing conjugation reactions (such as → glutathione S-transferase (GST), → N-acetyltransferase (NAT), → sulphotransferase (SULT) and → UDP-glucuronosyl transferase (UGT) (2, 3). It should be noted that use of the terms phase 1 and phase 2 to define the detoxication enzymes is somewhat arbitrary and does not necessarily reflect the pathway of biotransformation of all chemicals. Thus, a number of xenobiotics are subject to several

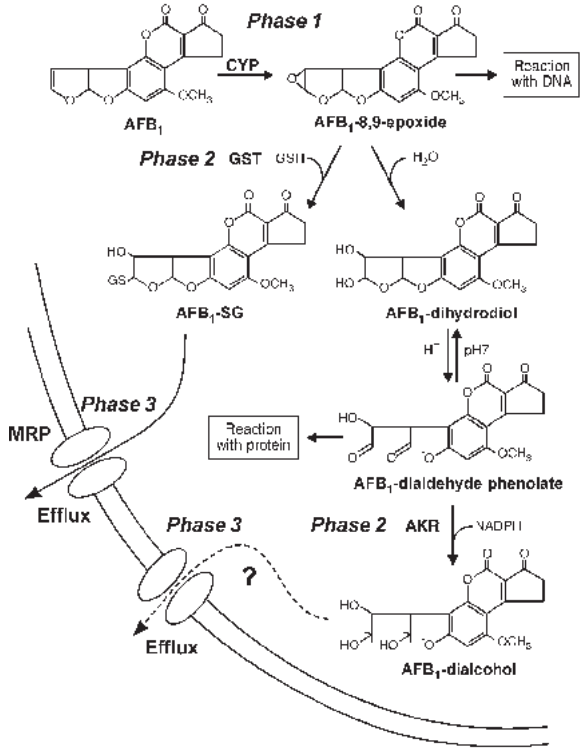
modifications by the phase 1 CYP isoenzymes before serving as substrates for the phase 2 enzymes (3). Alternatively, some xenobiotics do not require modification by phase 1 enzymes before metabolism by phase 2 enzymes, and others are subject to modification by more than one phase 2 drug-metabolizing enzyme. As a result of differences in drug metabolism, the group of enzymes catalyzing reduction of hydrolysis reactions (e.g. → aldehyde dehydrogenase (ADH), → aldo-keto reductase (AKR), → epoxide hydrolase (EPHX) and → NAD(P)H-quinone oxidoreductase (NQO)) are variously referred to as phase 1 or phase 2 detoxication, depending on the individual xenobiotic being considered and the preferences of research workers (4). Clearly, these enzymes provide a highly flexible metabolic defense that has evolved to protect against a diverse spectrum of chemicals.

- Finally, phase 3 of detoxication involves ATP-dependent elimination of the parent compound or modified xenobiotic by proteins that are drug efflux pumps (e.g. → multidrug resistance protein (MDR) and → multidrug resistance-associated protein (→ MRP)) (5, 6). As a consequence of the combined actions of phase 1 and phase 2 enzymes, a diverse spectrum of xenobiotics acquires a limited number of molecular 'tags' (i.e. acetate, glutathione, glucuronide or sulphate moieties) that are recognized by the MRP trans-membrane pumps. Furthermore, the xenobiotic metabolites produced by phase 1 and phase 2 are usually more soluble, and easily excreted, than the parent compound.

Whilst the ability of CYP to oxidize xenobiotics is generally desirable, as it facilitates further metabolism and elimination of harmful chemicals, it can sometimes result in the generation of highly reactive products that may not be readily detoxified (3). In such instances, modification of intracellular macromolecules will occur resulting in necrosis, → apoptosis or malignant → transformation. As an example of the interplay between toxification and detoxification reactions, a scheme depicting metabolism of → aflatoxin B_1 (AFB_1), modification of macromolecules by AFB_1 metabolites, and efflux of the AFB_1-glutathione conjugate from a cell is shown in the illustration (Fig.).

Genetic variation

Numerous proteins have envolved that detoxify drugs, and certain of the families listed above comprise over twenty genes. In total, the



Detoxification. Fig. – Detoxification pathways for aflatoxin B1. The mycotoxin is converted to the ultimate carcinogen AFB1-8,9-epoxide, by the actions of the hepatic phase 1 CYP enzyme system. The epoxidated AFB1 is highly reactive, and if it is not detoxified it will form DNA adducts that may cause hepatocarcinogenesis. The phase 2 GST enzymes can achieve detoxification of this unstable intermediate, and the resulting AFB1-glutathione conjugate is eliminated from the liver cell by MRP. In addition, AFB1-8,9-epoxide can rearrange to form a dialdehyde-containing metabolite which will covalently modify proteins by forming Schiff's bases. The dialdelhyde can be reduced by phase 2 AKR to yield a dialcohol that may be a substrate for SULT or UGT before being transported out of the cell, presumably by MRP.

human probably possesses between 100 and 150 genes encoding detoxication proteins. Substantial variation can occur in the levels of these proteins in tissues from different individuals, and this can result in increased sensitivity of cells to chemical insult. In part, this inter-individual variation is due to → genetic polymorphisms. By definition, such differences must be present in at least 1% of the population in order to be considered a genetic polymorphism. In some instances the variation involves deletion of detoxication genes with complete loss of specific functions, whereas in other instances point mutations result in alteration of protein structure causing only a modest attenuation of activity. In other cases mutations alter the regulatory regions of genes causing altered expression of normal protein. Detoxication genes that are polymorphic in the human include those for the enzymes CYP3A4, CYP2C9, CYP2C19, CYP2D6, CYP2E1, AKR1C4, GSTM1, GSTP1, GSTT1, NAT2, SULT1A1, SULT1E1, SULT2A1, UGT1A1, UGT1A4, UGT1A6 and UGT2B7, EPHX and NQO1, as well as the MRP2 efflux pump. It is clear additional polymorphisms remain to be identified.

Cellular & Molecular Regulation

In addition to genetic polymorphisms, induction of detoxication proteins by xenobiotics and environmental agents is a further mechanism that can cause inter-individiual differences in detoxification capacity. Induction of detoxication proteins represents an → adaptive response to chemical and → oxidative stress, which can be brought about by synthetic drugs or by naturally occurring compounds such as coumarins, indoles and isothiocyanates that are found in edible plants (4, 5). Increased expression provides short-term resistance to toxic xenobiotics. Enzyme induction also results in increased metabolism of therapeutic drugs. Many of the enzymes and pumps such as CYP, GST, ADH, AKR, NQO and MRP are inducible, often by transcriptional activation of genes encoding the proteins. The promoters of these genes contain enhancers that enable a transcriptional response to a diverse spectrum of chemical agents. The enhancers that are involved in induction of detoxication proteins include → AP-1 binding sites, the antioxidant responsive element, the xenobiotic responsive element, the phenobarbital responsive enhancer module, progesterone X receptor and peroxisome proliferator-activated receptor enhancer (3, 4, 5).

Clinical Relevance

It is apparent from studies into the mechanisms of selective toxicity between species that variation in the activity of detoxication proteins influences sensitivity to chemical insult. Increasing evidence suggests that genetic polymorphisms in detoxication enzymes can confer an inherited predisposition to a number of malignant diseases that are influenced by environmental factors (e.g. lung and colorectal cancer). They may also confer a predisposition to adverse drug reactions.

Induction of some phase 2 detoxication systems is believed to represent a major mechanism of → cancer chemoprevention, and is thought to explain in part the epidemiological data suggesting that consumption of diets rich in fruit and vegetables protect against certain malignant diseases.

Acquired → drug resistance to chemotherapy is a major problem in the treatment of many cancers. There is overwhelming evidence that the overexpression of several detoxication proteins, particularly GST, MDR and MRP, contributes to the drug-resistant phenotype (2, 5, 6).

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De-ubiquitinating Enzyme

Definition

De-ubiquitinating enzymes are a large family of enzymes that cleave chemical bonds formed at the C-terminus of → ubiquitin. By virtue of their action, the → ubiquitination of a given protein is reversible. Ubiquitin-protein conjugates are not always degraded by the proteasome, an alternative fate is the protein is spared from degradation through the activity of any of a large family of de-ubiquitinating enzymes. De-ubiquitinating enzymes can remove ubiquitin from ubiquitin-protein conjugates. These enzymes break down abundant multiubiquitin chains that are nor attached to any substrate and produce mature ubiquitin from the precursor forms in which it is synthesized. A second alternative fate for ubiquitin-protein conjugates is that ubiquitinated cell surface proteins may be targeted for endocytosis and eventual degradation via the lysosome rather than the proteasome.

Development of Tumors

Definition

→ Multistep development.

DFFB

Definition

DNA fragmentation factor (DFFB) is a DNAse (40 kD) that acts downstream of, and is activated by, caspase 3 to trigger DNA fragmentation during → apoptosis.

Dicentric Chromosome

Definition

A dicentric chromosome is the product of fusing two chromosome fragments, each of which has a centromere. It is unstable and may be broken when the two centromeres are pulled to opposite poles in mitosis.

Diet and Cancer

Definition

Dietary factors may contribute to enhancing risks for the various cancers. These factors are heterogeneous. In many cases individual compounds have been suggested to be involved but little definitive evidence is available. The individual risk factors are specific for different tissues, and the state of current knowledge has recently been published. In general diets high in total fat or animal fat are considered causative for several common tumours including those arising from tissues of the gastrointestinal tract. In contrast, vegetables and fruits are considered to be protective for many tissues and for all cancers discussed in the review listed below. Phytoprotectants are implicated as contributing to risk reduction by different mechanisms, but it has not been possible to pinpoint individual compounds as the responsible factors. Tissues that seem to be most protected are oesophagus, stomach, colon, lung, pancreas and bladder. The least clear cut protection is achievable in the hormone dependent tissues,

prostate and breast, although a dietary component can not be excluded for these tumours. Altogether the estimates indicate that at least 35% of all human tumours are dietary related, which means a large proportion of tumours could be prevented by adequate dietary regimens; → biomarkers.

See also: World Cancer Research Fund and American Institute for Cancer Research. Food, Nutrition and the Prevention of Cancer: a global perspective.Washington DC: American Institute for Cancer Research, 1997.

Differential Display

Definition

Differential display is a polymerase chain reaction (→ PCR)-based method used to compare differential mRNA expression in two cell types or two cells exposed to different stimuli.

Differentiation

Definition

Differentiation is the process of becoming specialized, as during embryonic development when cells take on a certain form and function and become ordered into tissues and organs that perform specific activities.

Diffuse Large B-cell lymphoma

Definition

Diffuse large B-cell lymphoma (DLBCL) is the most common subcategory of → NHL, but apparently a heterogeneous subgroup in terms of cell morphology, genetic abnormality and clinical pictures. → *MYC*, *BCL2* and → *BCL6* are rearranged in proportions of the cases. The disease sometimes involves a single nodal site or extranodal organ. DLBCL shows an aggressive clinical behavior, but is potentially curable by appropriate chemo-radiotherapy.

Diffusional Flux

Definition

Diffusional flux is the amount of a substance or gas diffusing from one location to another.

Digital Rectal Examination

Definition

Digital rectal examination is the finger palpation of the prostate gland.

Diploid

Definition

A diploid set of chromosomes contains two copies of each autosome and two sex chromosomes.

Diplopia

Definition

Diplopia is objective double vision.

Direct Repeats

Definition

Direct repeats are identical (or related) sequences present in two or more copies in the same orientation in the same molecule of DNA, which are not necessarily adjacent.

DISC

Definition

DISC stands for death inducing signalling complex. The formation of a DISC is an initiating event of apoptosis and occurs within seconds after binding of a ligand to the death receptor, CD95/FAS/APO-1. DISC formation involves the association of a complex of proteins with activated CD95. First, the adaptor protein FADD (Fas-associated death domain protein; alias MORT-1) binds to the death domain of CD95. FADD, via its so-called death effector domain (DED), recruits procaspase-8 (caspases) into the DISC. Procaspase-8 is then activated proteolytically, active caspase-8 is released from the DISC into the cytoplasm and activates downstream procaspases, particularly procaspase-3.

Disjunction

Definition

Disjunction describes the movement of members of a chromosome pair to opposite poles during cell division. At mitosis and the second meiotic division disjunction applies to sister chromatids; at first meiotic division it applies to sister chromatid pairs.

DLBCL

Definition

→ Diffuse large B-cell lymphoma.

DM

Definition

→ Double minute; dmin.

DMBT1

Definition

→ *Deleted in malignant brain tumors 1* (*DMBT1*) is a tumor suppressor gene candidate located on chromosome 10.25.3-26.1 that has homology to the scavenger receptor cysteine-rich (SRCR) superfamily. Homozygous deletions have been described in medulloblastoma and glioblastoma multiforme.

DMIN

Definition

→ Double minute.

DNA Adducts

Definition

→ Adducts to DNA are covalent binding products formed between a chemical and DNA.

DNA Double-strand Break Repair

Definition

DNA double-strand break repair in mammalian cells are the pathways for the repair of DNA double-strand breaks that include simple rejoining by the non-homologous end-joining reaction, or homology-directed processes including single-strand annealing or homologous recombination.

DNA Interstrand Cross-links

Definition

DNA interstrand cross-links are the covalent links formed between bases on opposite strands of a DNA molecule, which impair processes that require the separation of the two strands of DNA, such as replication and transcription.

DNA Methylation

Definition

DNA methylation is an epigenetic modification of DNA involving the addition of a methyl (CH3) group to the 5′ position of a cytosine residue. The majority of methylation events in humans occur in cytosines that are located next to a guanine (5′-CpG-3′); → methylation.

DNA Polymerase

Definition

DNA polymerase is an enzyme that can catalyze the polymerization of nucleotide triphosphates and is responsible for the synthesis of DNA.

DNS

Definition

→ Dysplastic naevus syndrome.

Domain

Definition

A domain is a functional and folding unit of protein tertiary structure.

Domain of Protein

Definition

The domain of a protein is a region with a specific amino acid sequence that serves as an interface for binding to other proteins or DNA.

Dominant

Definition

A dominant allele is an allele that determines the phenotype displayed in a heterozygote with another (recessive) allele.

Dominant-negative

Definition

Dominant-negative refers to a molecular mechanism by which a protein A binds to a protein B, thereby antagonizing the activity of protein B but without expressing its own phenotype. This is an important mechanism in cancer development. Proteins that due to gene alterations, are damaged or expressed in truncated versions, may loose their own normal function but still induce a phenotype (in a 'dominant' form) by binding to and inactivating other proteins.

Dormancy

Definition

Dormancy is the property of a few cancer or precancerous cells to exist for long periods of time without consequently evolving to relapse.

Double Minute

Definition

Double minutes (DM), also known as dmin, are extrachromosomal small paired chromatin bodies. In tumor cells they indicate → oncogene → amplification and are also seen in cells resistant against certain cytostatic drugs, where genes involved in drug metabolism are amplified (e.g. dehydrofolate reductase gene in methotrexate resistance).

Double Strand Break

Definition

Double strand breaks (DSB) of DNA are opposite breaks in both of the sugar phosphate backbones of the DNA molecule. DSBs are caused directly by ionising radiation, some chemicals and free radicals. DSBs are also natural intermediates during meiotic recombination and immune gene rearrangements.

Downstream

Definition

Downstream identifies sequences proceeding farther in the direction of expression, for example, the coding region is downstream of the initiation codon.

Doxorubicin

Definition

→ Anthracycline.

DPC4

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Definition

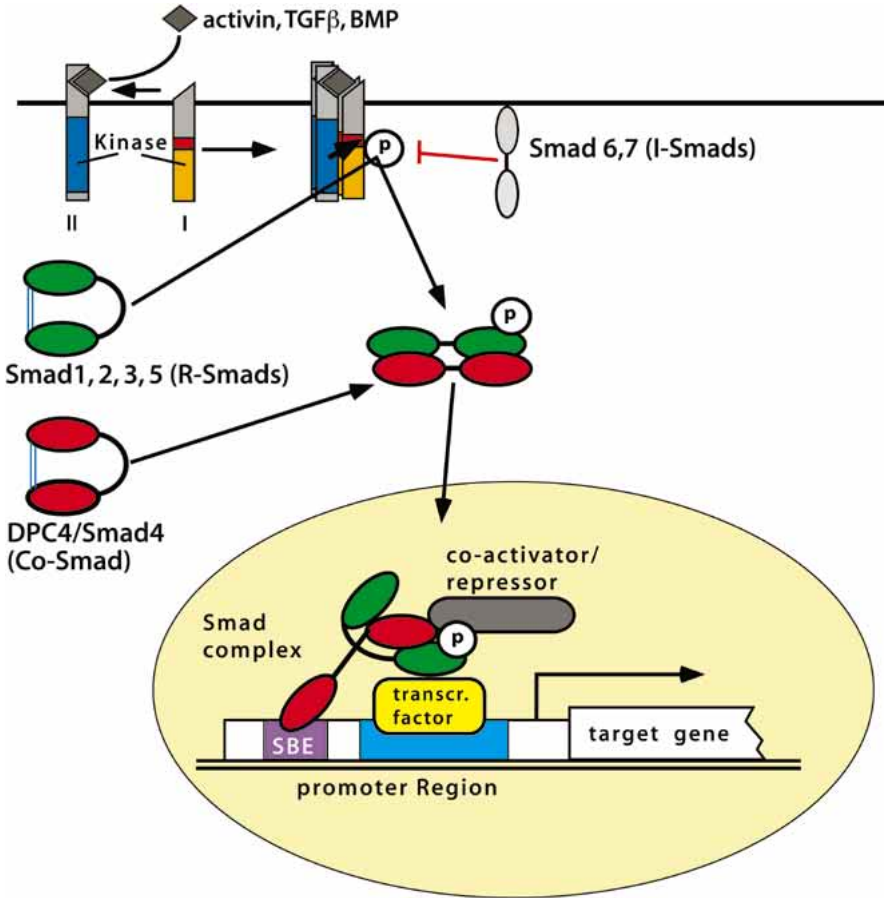
Deleted in Pancreatic Carcinoma Locus 4 (DPC4), belongs to the class of → tumor suppressor genes. It was identified within chromosome band 18q21.1 which is frequently deleted in pancreatic carcinoma. DPC4 is also known as Smad4 or MADH4. DPC4 is a component of the → transcription complex that mediates cell surface signals to the nucleus which are initiated by transforming growth factor β (TGF [→ TGF-β]β)-related growth and differentiation factors.

Characteristics

The open reading frame of DPC4 spans 1656 nucleotides and comprises 11 exons that code for a 60 kD protein (552 amino acids). DPC4 belongs to the highly conserved family of Smad genes that has been identified by protein sequence homology studies. The founding member Mad (Mothers against decapentaplegic), was identified in *Drosophila melanogaster*.

Mad and its homologs mediate signals from cell surface receptors to the cell nucleus. Involved in this signaling cascade are serine/threonine kinase receptors of the TGFβ family that become activated upon binding of polypeptides of the TGFβ cytokine family (Fig. 1). At least 25 diffferent cytokines from various species are currently known. They include the TGF-β's, activins, inhibins, bone morphogenic proteins (BMP's) and Müllerian-inhibiting substance and control, among others, important biological functions such as embryonic development, cell growth and cell differentiation, modulation of immune responses and bone formation.

The number of Smad [→ Smad proteins in TGFβ signalling] genes identified in humans has grown to a total number of eight (Fig. 2).



DPC4. Fig. 1 – A model for DPC4 signaling. In response to ligand binding to the TGFβ receptor complex, receptor regulated Smads (also called R-Smads) become C-terminally phosphorylated through the receptor kinase. The phosporylated Smads change their folding pattern and form a hetero oligomeric complex with DPC4. The newly formed Smad complex is then translocated into the nucleus. In the nucleus the Smad complex will make contact to transcription factors as well as bind directly to DNA through the Smad binding element (SBE), thus stabalizing the higher order DNA binding complex. In addition, transcriptional co-activators or co-repressors may be recruited into the complex ultimately leading to either activation or repression of target gene expression.

Common to all of them is a characteristic three-domain structure (Fig. 3): a highly conserved region, the Mad homology domain 1 (MH1), is located at the amino (N) -terminal end. Next to it is a poorly conserved, proline-rich linker region which is adjacent to a second highly conserved domain (MH2), that is located at the carboxy (C) -terminal end of the protein.

A link between the DPC4 protein and TGFβ signaling cascade was initially established by sequence homology studies of the proteins DPC4 and Mad. At the time of DPC4 discovery its potential tumor suppressor function was hypothesized; it was believed that the loss of DPC4 function in tumors is the reason for the observed resistance towards TGFβ mediated growth inhibition of many tumor types. Although this hypothesis has only been partly proved, a wealth of information regarding the signaling pathway has been collected in recent years. This gave rise to a model of DPC4 protein function within the TGFβ signaling pathway.

TGFβ-Smad Signaling Cascade (Fig. 1)

The Smad signaling cascade involves three different classes of Smad proteins: the receptor

regulated Smads (R-Smads), the common mediator Smad (Co-Smad) and the inhibitory Smads (I-Smads).

Upon ligand induced TGFβ receptor stimulation, R-Smads can transiently interact with the type I receptor. They become C-terminally phosphorylated by the receptor kinase and, once phosphorylated, are able to form a hetero oligomeric complex with the 'common-mediator' DPC4/Smad4 which then translocates into the nucleus. Here it can either up- or down-regulate the transcription levels of target genes by interacting with other nuclear factors and by recruiting transcriptional co-activators or co-repressors.

This signaling cascade can be negatively interfered with at the level of R-Smad activation. I-Smads can compete with R-Smads for type I receptor binding and can therefore prevent the phosphorylation-dependent activation of R-Smads. Direct binding of I-Smads to R-Smads has also been shown, yielding R-Smads inactive. The expression of I-Smads appears to be regulated by TGFβ and BMP via an autoregulatory feed back loop. Furthermore, it has been shown that epidermal growth factor (→ EGF) and interferon-γ (IFN-γ) can induce I-Smads expression to antagonize TGFβ signaling.

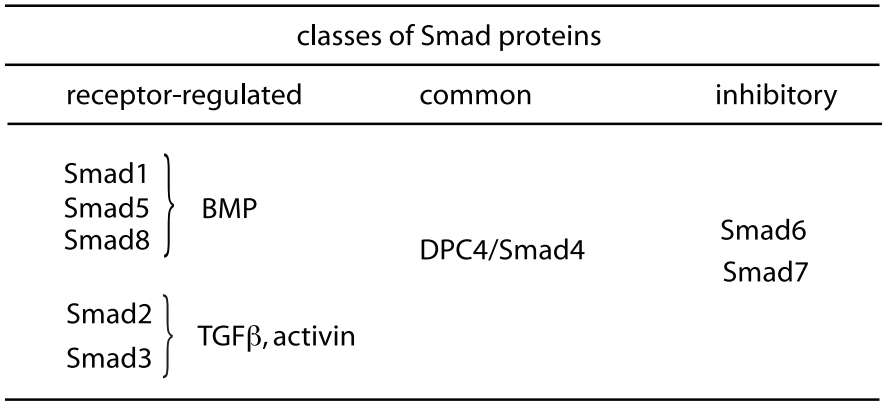
Transcriptional Regulation through Smads (Fig. 1)

Since Smad proteins have no intrinsic enzymatic activity, they exert their effector function as transcriptional regulators either directly by binding to specific promoter consensus sequences termed Smad binding elements (SBE) and/or indirectly by associating with transcription factors already bound to the promoter. Therefore, many but not all Smad responsive promoters have two adjacent DNA sequences. One provides the binding site for transcription factors that are cooperating with the Smad complex, the other allows direct binding of the Smad complex to the DNA. While R-Smads provide the interface for the binding to transcription factors, DPC4 makes the contact to SBE elements. DPC4 thereby stabilizes the formation of a higher order DNA binding complex which is able to recruit transcriptional co-activators or co-repressors.

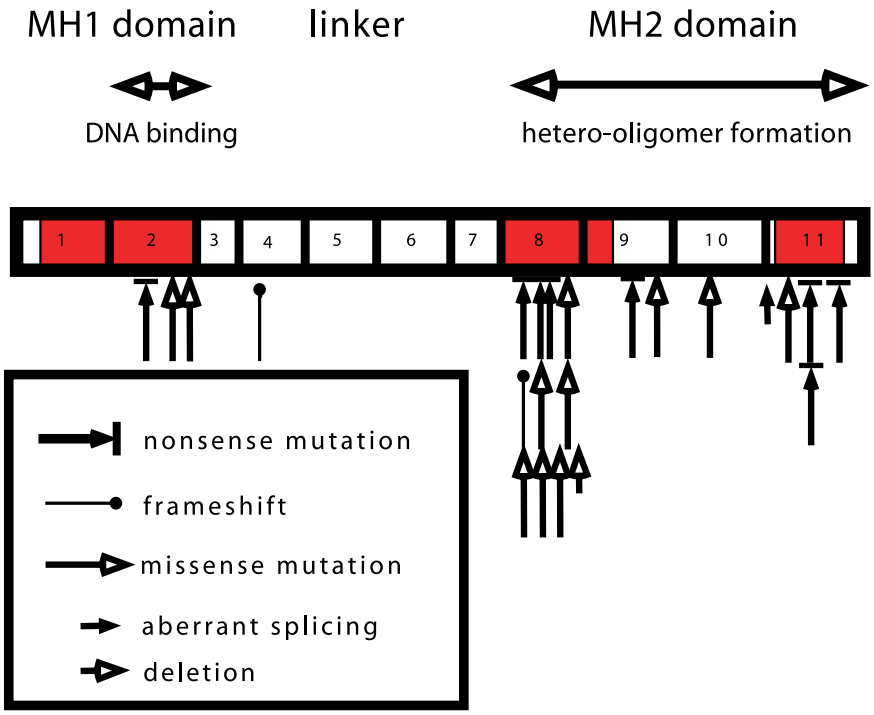
Many of the factors that cooperate with the Smad complex are regulated independently by other signaling cascades. The function of an active Smad complex can therefore be described as a co-modulator of transcription. It can modulate gene expression positively as well as negatively by integrating various incoming signals - including those mediated by the TGFβ ligand family.

What makes DPC4/Smad4 unique among the other Smad family members?

- DPC4 is the only human Co-Smad that is currently known.
- It seems particular to DPC4 that it is, almost without exception, essential for the establishment of a functional active Smad com-



DPC4. Fig. 2 – Summary of functional classes of human Smads.



DPC4. Fig. 3 – Functional domains and DPC4 mutations. The majority of DPC4 mutations identified to date are located within the C-terminal MH2 domain. There they appear to impair the formation of a functional active Smad complex by preventing hetero-oligomerization of DPC4 with R-Smads. Few mutations are confined to the N-terminal MH1 domain and are thought to interfere with the ability of the Smad-complex to directly interact with DNA, therefore rendering the DPC4 containing transcriptional complex unstable.

plex, a fact that emphasizes its role as a 'master switch' in the regulation of TGF-β-like signals.

- Most somatic and all germ line mutations in human Smad genes identified to date, did target DPC4. Only very few somatic mutations were found in the human Smad2 gene, none were found in the other members of the human Smad gene family.

Bioactivity

Which human tumors show alterations of the DPC4 gene?

Changes, resulting in the inactivation of the DPC4 gene were found in approximately 50% of pancreatic carcinomas. Research, carried out in a variety of other cancer types suggested that DPC4 may contribute primarily to the formation of pancreatic neoplasia, and to a lesser extend to colon and biliary cancer as well as the induction of non-producing neuroendocrine tumors. However, such changes appear to play only a minor role in the development of other tumor types such as head and neck, lung, ovarian, breast and bladder cancer. It was recently shown that the frequency of DPC4 mutations is markedly increased in metastatic colorectal carcinoma (35%) compared to non-metastatic colorectal carcinomas (7%), suggesting the loss of DPC4 function as a critical parameter of metastasis formation in colorectal carcinomas. In addition, germline mutations of the DPC4 gene have been identified in patients with familial juvenile polyposis, an autosomal dominant disorder that is characterized by a predisposition to hamartomatous polyps as well as an increased risk for gastrointestinal carcinomas.

How are naturally occuring DPC4 mutations interfering with the Smad signaling cascade?

Most DPC4 mutations identified to date are located within the C-terminal MH2 domain. Functional studies identified the MH2 domain as providing the binding properties to R-Smads, the latter being important for a functionally active Smad complex. It is therefore likely that compromising mutations of the MH2 domain structure restrict the formation of a functional Smad complex, thus preventing signal transduction to downstream components. In addition, a few mutations have been identified within the N-terminal MH1 domain which was shown to mediate the direct binding of DPC4 to DNA promoter sequences. Such mutations might interfere with Smad signaling by rendering the formation of the higher order Smad-DNA complex unstable.

Does DPC4 contribute to the familial risk for pancreatic cancer?

Although the DPC4 gene is frequently altered in sporadic pancreatic carcinoma, to date no germline mutations were found in families with an increased risk of this type of carcinoma. DPC4 is therefore unlikely to play an important role as a heritable genetic risk factor in pancreatic carcinoma.

How does DPC4 contribute to tumor formation?

At present there is no conclusive answer to this question, not at least because only limited information about DPC4 regulated target genes is available. Nevertheless, based on a few studies and our knowledge that Smad family members mediate TGFβ signals to the nucleus, some preliminary models can be shaped: Loss of DPC4 function may either have a more or less direct effect on tumor cell growth rate, as suggested by some experiments, or, as other experiments support, a more indirect role by altering the extracellular matrix components, thereby supporting tumor invasion and metastasis. Furthermore, recent data provide evidence that loss of DPC4 expression might promote tumor angiongenesis by causing an increase in the concentration of angiogenic factors and/or a decrease its corresponding inhibitors. In addition, it was speculated that DPC4 might be involved in negatively modulating immune responses towards tumor cells.

Additional insight of DPC4 function was provided by targeted mutagenisis in mice. Mice with two mutated alleles for DPC4 die at embryonic day 7.5, a result that underlines the importance of DPC4 in early embryonic development. DPC4 heterozygous mice develop gastric polyps which eventually become tumors. Furthermore, knockout mice experiments have demonstrated a functional cooperation between the DPC4 and the APC (adenomatous polyposis coli) gene. In mice that were carrying defect copies of both genes, compared to mice carrying only the mutated APC gene, the induced colonic tumors displayed a much more agressive phenotype. These data clearly support the importance of DPC4 in the suppression of tumorigenesis.

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Drosophila melanogaster

Definition

Drosophila melanogaster is the fruit fly commonly used in genetic studies and introduced by Thomas Hunt Morgan in the early 1900s.

Drug Metabolism

Definition

→ Detoxification.

Drug Resistance

Definition

Drug resistance is a major problem in the treatment of many cancers. There is overwhelming evidence that the overexpression of several detoxication proteins, particularly → GST, → MDR and → MRP, contribute to the drug resistant phenotype. It is the biochemical mechanism whereby cancer cells fail to respond to chemotherapy, such that there is growth of cancer despite therapy. There are many mechanisms, but the final endpoint is that cancer cells are unresponsive to therapy.

DSB

Definition

→ Double strand break.

Dynamin

Definition

Dynamin is a guanosine triphosphatase (GTPase) capable of self-assembling into oligomeric 'collars' around the necks of coated pits. It is responsible for the pinching off of a vesicle from the plasma membrane.

Dysphagia

Definition

Dysphagia is a difficulty in swallowing.

Dysphonia

Definition

Dysphonia is altered vocal sounds.

Dysplasia

Definition

Dysplasia is abnormal tissue development.

Dysplastic Naevus Syndrome

Definition

Dysplastic naevus (us spelling: hevus) syndrome is a more general term for the → B-K mole syndrome, defining a syndrome of large numbers of atypical moles/ dysplastic naevi.

Dysplastic Naevi

Definition

Dysplastic naevi (DN) is multiple naevi of atypical appearance; → mole; → melanoma.

Dysuria

Definition

Dysuria is pain during or after urination.

E1A

Definition

E1A is an → adenovirus oncogene product that regulates viral gene expression in a lytic infection; it has the potential to interfere with cellular gene expression.

E2F

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Definition

Family of transcription factors involved in cell-cycle regulated transcription; they control the expression of genes coding for growth regulatory proteins.

Characteristics

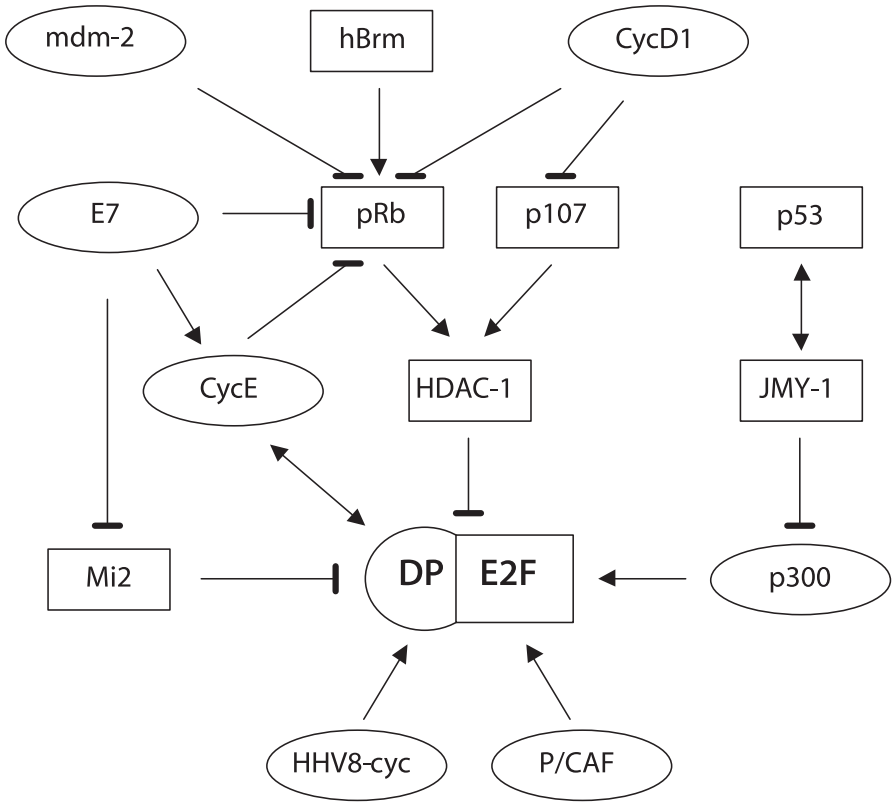
The E2F family of transcription factors has the five members, E2F-1 to 5, which are capable to either activate or repress transcription, depending on the promoter context and the composition of E2F complexes (see below). A sixth member of the E2F family, referred to as EMA or E2F-6, also binds E2F recognition sites but only acts as a repressor of transcription. Members of the E2F family form heterodimers with members of the DP family. The latter consists of two genes, DP1 and DP2, that encode several isoforms. Heterodimerization is required for DNA binding. E2F/DP heterodimers function as transcriptional regulators of a variety of cellular genes, some of which are involved in cell-cycle checkpoint control.

E2F target genes can be grouped as follows:

1. Genes that code for cell-cycle regulators (Fig. 1), such as cyclin E, cyclin A, the retinoblastoma protein, p107 and Ran BP1. Expression of these genes is driven by E2F. E2F thereby directly controls the progression from one cell cycle phase into the other, particularly at the G1/S and the G2/M transition. E2F also activates expression of the p14 (ARF) gene, an upstream regulator of p53.
2. Genes that code for enzymes involved in DNA replication, such as thymidilate synthetase, thymidine kinase, dihydrofolat reductase, etc. It is believed that up-regulation of these genes prepares cells for DNA synthesis which are in the G1 phase of the cell-cycle.

Cellular and molecular regulation

- E2F proteins are regulated by cellular tumor suppressor proteins (Fig. 2). Members of the retinoblastoma protein [→ retinoblastoma protein, biological and clinical functions] family (pRB, p107 and p130) associate with the E2F/DP heterodimers, block their transactivation capacity and, in some cases, convert these activators of transcription into transcriptional repressors. The latter convertion appears to involve the tethering of histone deacetylases to E2F/pRB complexes.
- E2F regulates the activation of the tumor suppressor p53 via p14 (ARF). In turn, E2F function is regulated by p53 via a competition for the p300 coactivator protein.

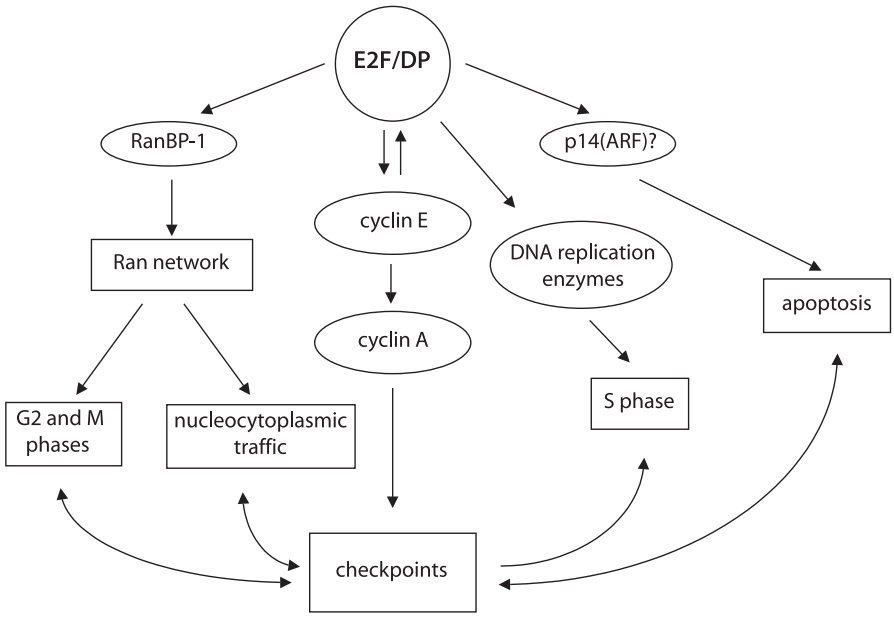


E2F. Fig. 1 – Control of E2F activity in cancer cells. Signals and regulatory pathways that induce or repress the activity of the E2F/DP transcription factors are indicated. The picture contains only proteins with a known function in human cancer; although other proteins that can modulate E2F/DP activity have been identified their role in cancer is not yet clear. The activity of E2F/DP transcription factors is controlled by chromatin-modifying enzymes such as HDAC-1 and Mi2, which block the transcription of E2F-dependent genes by deacetylating histones. In contrast, histone acetyl transferases such as p300 and P/CAF, act as potent co-activators of E2F. These proteins acetylate histones and thereby release E2F-driven genes from chromatin repression. p300 and P/CAF also upregulate the activity of E2F-driven genes by directly acetylation of the E2F partner in the E2F/DP heterodimer. p53 and E2F compete for the adapter protein p300. This interaction is mediated by the currently identified protein JMY1. Finally, human tumor viruses such as HPV-16 or HHV-8, encode genes that directly modulate E2F activity, leading to aberrant activation of E2F target genes.

- The function of E2F is positively regulated by cyclin-dependent kinases. This, at least in part, relies on the phosphorylation and the resulting inactivation of members of the retinoblastoma protein family. However, cdk-dependent phosphorylation of the E2F subunit also contributes to E2F activation.
- E2F activity is up-regulated by cellular and viral oncogenes such as MDM2, adenovirus E1A, papillomavirus E7 and SV40 T antigen. Activation of E2F by these oncogenes is thought to be mediated by a displacement of the retinoblastoma protein or its relatives from E2F/DP complexes.

Clinical Relevance

E2F-1 acts as a tumor suppressor and at the same time as an oncogene in the mouse, as was examplified by the complex phenotype of E2F-1 knock out mice. It appears that tumor suppression by E2F depends on its interaction with the retinoblastoma protein by enhanced transcriptional silencing. The oncogenic activ-



E2F. Fig. 2 – E2F regulation of cell-cycle checkpoints. The role of E2F/DP genes in co-ordination of cell-cycle checkpoint control is schematically depicted. Apart from its role in S-phase entry, E2F acts at the G2/M checkpoint in mammalian cells, which is mediated by Ran-dependent modulation of nucleocytoplasmic traffic. Induction of apoptosis by certain E2F family members is believed to depend on transcriptional activation of the gene encoding p14(ARF). The major groups of E2F target genes are shown and the interplay of their gene products is indicated by arrows.

ity of E2F-1 is mediated by free E2F-1/DP complexes which enforce the transcription of E2F-dependent target genes, some of which are required for cell proliferation.

→ Amplification of the *E2F-1* gene was described in human erythroleukemia cells; the *E2F-5* gene is amplified in some human breast cancers.

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E12/47 Family

Definition

E12/47 family is a → bHLH protein family that comprises E12 and E47 and is involved in myogenesis, neurogenesis and immunoglobulin gene expression. E2A immunoglobulin enhancer binding factor E12/E47, also known as E2A, Itf1 and transcription factor 3 (Tcf3) is a → bHLH protein member of the E12/E47 family (654 amino acids, 67 kD). The human *TCF3*, *E2A* or *ITF1* gene locus maps to 9p13.3 and the mouse *tcfe2a* gene locus to chromosome 10 (43.00 cM). E12 and E47 are two proteins that bind to the κ-e2 site in the κ immunoglobulin gene enhancer. E12 and E47 are splice isoforms and form heterodimers with bHLH proteins of the Achaete-scute family.

Early Detection

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Definition

Early detection is the defining signatures of cells for earlier cancer detection and risk assessment.

Characteristics

All cancers evolve over time by the accumulation of molecular, genetic and biochemical changes. During the course of cancer progression, specific genes are over- or under-expressed or modified. Only those cells that are capable of preferential proliferation and accumulation are biologically selected. It is generally believed that the clinically apparent tumor represents the descendent of one cell. This forms the basis that the cancer is a monoclonal disease.

Genes associated with monoclonal evolution and progression have been used as biomarkers. In practical terms, biomarkers are defined as morphological, biochemical or genetic alterations by which a physiological or pathological process can be identified or monitored. To be clinically useful, biomarkers should be measurable in tissues, cells or fluids. Such biomarkers have been used in conducting early phases of clinical/epidemiological studies, e.g., cross-sectional and retrospective, and for evaluating their predictive value in risk-assessment. However, the clinical applications of biomarkers at the population level remain to be seen. Prior to such application, biomarkers must be proven to have high predictive accuracy, and be easily measurable and reproducible. Tests for biomarkers in most settings must be minimally invasive and acceptable to patients and physicians.

The potential for biomarker use has never been greater. The lack of non-invasive screening tools for some cancer sites such as pancreas, ovary and bladder, has forced us to look for appropriate alternate sites that are easily accessible. The concept of identifying surrogate sites is to assess cancer risk without directly accessing the organ or tissue. A surrogate endpoint, defined as an endpoint measured in lieu of the so called 'true' endpoint, can be particularly useful if it is easily measured and highly correlated with the 'true' endpoint. Usually, the 'true' endpoint has clinical importance for the patient whereas a surrogate endpoint reflects a biological or molecular alteration. Surrogate markers are used when a clinical outcome is substantially delayed, in comparison with predictive biochemical or molecular changes, which can be promptly measured. Little progress has been made towards evaluating molecular profiles for determining cancer detection and risk assessment in accessible anatomical sites rather than in less accessible organs. In this direction, highly specific and sensitive assays should be developed to detect molecular products, including epigenetic changes, such as → methylation, of tumor cells in body fluids with an emphasis on identifying molecular determinants (risk factors).

To help accelerate the discovery, identification and validation of clinically useful biomarkers, the National Cancer Institute has established a multi-institutional and multi-disciplinary consortium, called the Early Detection Research Network (EDRN). The EDRN provides a platform for developing, cataloging and supporting the systematic validation of biomarkers for earlier detection and risk assessment for human cancers.

Biomarkers in early detection

Investigators participating in EDRN have already begun identifying molecular signatures for cancer detection and risk assessment. Microsatellite instability (MIN) and mutations in mitochondrial DNA (mt DNA) are being tested for early detection of colorectal, bladder, head and neck, and lung primary cancers (1-4). → Microsatellites are tandem repeats of simple sequences that are highly polymorphic and randomly interspersed throughout the human genome. They consist of 10-15 copies of 1-6 base-

pair motifs, and it is estimated that there are on the order of 50,000-100,000 (CA)n elements scattered throughout the human genome. Microsatellites are replicated faithfully at each cell division with a new mutation rate of approximately 10^{-3} to 10^{-6}. While no specific function has been assigned to these repeat sequences, it has been postulated that they act as promoters, sites of recombination or as binding sites for DNA topoisomerase. Continuous efforts are being undertaken to catalog and characterize MIN and microsatellite alterations in normal and preneoplastic tissues. Mutated genes that have been detected in tumors associated with MIN include → TGF-β type II receptor, IGF-IIR [→ insulin-like growth factor-I receptor], Bax, MSH3/MSH6, PTEN, → APC and β-microglobulin.

Genes encoded by mtDNA are required to maintain proper function of the organelle, and slight alterations in their DNA sequence could have a profound effect. mt DNA mutations are generated during oxidative phosphorylation through pathways involving reactive oxygen species. Since mitochondria lack protective histone proteins, effective DNA repair mechanisms do not operate in mitochondria. Over a period of time, uncorrected mutations accumulate. For instance, several mtDNA mutations have been detected in colorectal, bladder and lung cancers (1). The hot spot of somatic mutations is located in the D loop region of the mitochondrial genome. The majority of these somatic mutations are homoplasmic in nature; thus, the mutant mtDNA is dominant in tumor cells. Due to their high copy number (compared to nuclear genome) and clonality, mtDNA mutations may provide excellent biomarkers for noninvasive detection of early cancer.

Other promising biomarkers are being validated and their assays refined to achieve high-throughput testing suitable for the clinical setting (4). These biomarkers are evaluated for their effectiveness in identifying individuals at risk for cancer, for earlier detection and for predicting metastatic phenotypes. Some of the biomarkers used for the early detection of cancer are listed in a Table. In the clinical community, the consensus is to utilize multiple biomarkers for risk assessment, earlier cancer detection and prevention trials.

Newer technologies, such as Surface Enhanced Laser Desorption Ionization (SELDI), Matrix Assisted Laser Desorption Ionization (MALDI), two-dimensional gel electrophoresis, Laser Capture Microdissection (LCM) and tissue microarrays are accelerating the discovery process of finding sensitive biomarkers. For example, the sensitivity of the proteomics technologies is in the femtomole range. Proteomic approaches attempt to systematically catalog protein levels quantitatively in order to characterize biological processes and pathways. Informatics and computer analysis play a significant role in the application of these techniques in early detection of cancer. Chip technology can be harnessed for high-through-

Early Detection. Table – Biomarkers for the early detection of cancer.

proliferation	cyclin D, PCNA, creatine kinase, Ki67, bcl1
oncogenes	p185erbB2, cKi-ras, c-myc, erbB1, erbB2
tumor suppressor genes	p53, Rb
growth factors	TGF-α, TGF-βR, IGF 1R, IGF2R, ERP28
differentiation	CD44v6, CDw75ag
apoptosis	Bcl2, Bax
senescence	→ telomerase
DNA repair	MSH3, MSH6, PMS1
unclassified	PTEN

put analyses with sufficient sensitivity to detect molecular alterations in early cancer detection. The information gained is invaluable in devising intervention strategies to alter disease course, reveal insights into the fundamental mechanisms of tumor progression and serve as new drug targets, cancer vaccine antigens and markers for early detection and risk assessment. The application of these emerging technologies in the field of early detection and risk assessment might help reduce mortality linked to cancer.

Clinical relevance

Data show that detection and prompt treatment of pre-malignant or small lesions can prolong survival and reduce mortality (5, 6). Early detection of cancer helps in designing prevention and treatment strategies. Most therapeutic intervention strategies or early detection technologies target expressed proteins. Therefore, proteomic-based studies can provide fundamental information to characterize cancer progression at the molecular level (7), and improved analytical tools are allowing a more detailed examination of the molecular pathogenesis.

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Early Genes of Human Papillomaviruses

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Definition

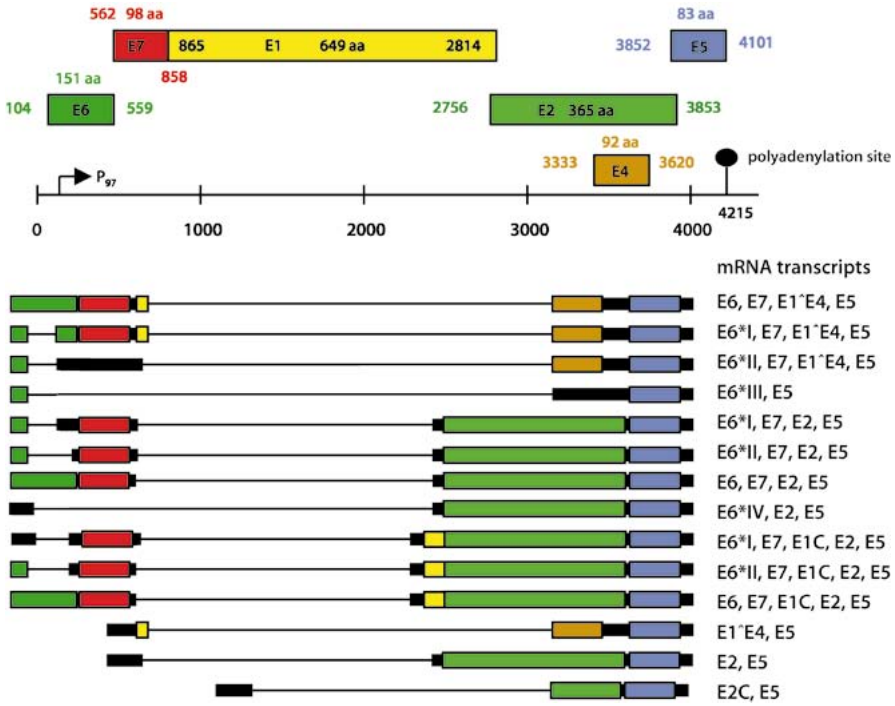
The →human papillomavirus 16 (HPV 16) is the most frequently found HPV genotype in cervical cancers worldwide, and thus its early gene products are the best studied and characterized. HPV 16 has six early genes that are transcribed from the same DNA strand (Fig. 1). As in other viruses, the function of the early proteins is to alter several cellular events to guarantee the completion of the virus life cycle. In addition, three early proteins, E6, E7 and E5 are also involved in the induction of malignant transformation of the infected cells.

Characteristics

HPV16 E6 and E7 are the major transforming proteins

Three different lines of evidence have demonstrated the involvement of E6 and E7 in cervical carcinogenesis.

- The first indication came from the analysis of HPV-infected cells, which showed that viral DNA is randomly integrated in the genome of the majority of cervical carcinomas. Integration leads to disruption of several viral genes with preservation of only the E6 and E7 genes, which are actively transcribed.
- The discovery that E6 and E7 proteins are able to induce cellular transformation *in vitro* confirmed their oncogenic role. Im-



Early Genes of Human Papillomaviruses. Fig. 1 – Organization of the early region of HPV16. The six early genes are represented in the figure in different colors, nucleotide positions of each early gene and their predicted size in amino acids are shown. Several polycistronic transcripts have been identified that comprise 2-3 early genes in different combinations and that are most likely transcribed from the P^{97} promoter. The function of each transcript is not known but some of them are only transcribed at different stages of differentiation. There is evidence that alternative splicing in E6 and E7 transcripts plays an important role in the translational regulation of these viral proteins.

mortalized rodent fibroblasts can be fully transformed by expression of HPV 16 E6 or E7 protein. These rodent cells acquire the ability to grow in an anchorage-independent manner and to be tumorigenic when injected in nude mice. In addition, HPV 16 E6 and E7 together are able to immortalize primary human keratinocytes, the natural host cell of the virus. In agreement with the *in vitro* assays, transgenic mice coexpressing both viral genes exhibit epidermal hyperplasia and various tumors. Independent studies have demonstrated that E6 and E7 proteins do not induce cellular transformation via a 'hit and run' mechanism, but that continuous expression of both proteins is required for the maintenance of the malignant phenotype.

- Finally, biochemical studies have clarified the mechanism of action of E6 and E7. The viral oncoproteins are able to form stable complexes with cellular proteins and alter, or completely neutralize, their normal functions. These events lead to the loss of control of cell-cycle checkpoints of → apoptosis and differentiation.

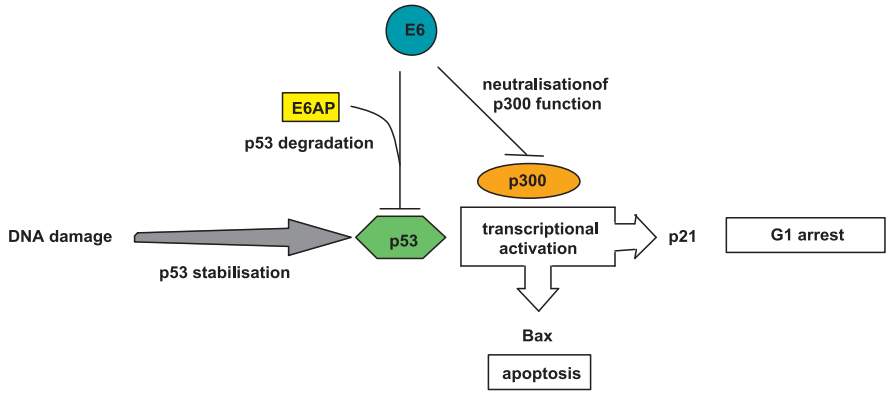
E6 protein

HPV 16 E6 is a small basic protein of 151 amino acids. The major structural characteristic of E6 is the presence of two atypical zinc fingers. At the base of each of the zinc fingers are two motifs containing two cysteines (Cys-X-X-Cys), which are conserved in all E6 HPV types. The best characterized HPV16 E6 activity is

its ability to induce degradation of the tumor suppressor protein p53 via the ubiquitin pathway. This cellular protein is a transcription factor that can trigger cell-cycle arrest or apoptosis in response to stress or DNA damage. E6 binds to a 100 kD cellular protein, E6AP (E6 associated protein), a member of the ubiquitin protein ligases (als called E3 proteins). The E6/E6AP complex then binds p53, which becomes very rapidly ubiquitinated and targeted to proteosomes for degradation (Fig. 2). Since the major role of p53 is to safeguard the integrity of the genome by inducing cell-cycle arrest or apoptosis, cells expressing HPV16 E6 show chromosomal instability that greatly increases the probability that HPV-infected cells will evolve towards malignancy. Recent findings have demonstrated that HPV16 E6 also associates with the transcriptional regulators, CBP and p300, with resulting inhibition of p53-driven transcription. Thus, E6 neutralizes p53 by two distinct mechanisms, the first is mediated by the p300/CBP association, while the second occurs via binding to E6AP to promote p53 degradation (Fig. 2).

HPV16 E6 can also interfere with the apoptosis via its association with Bak, a member of the → Bcl-2 family. Analogously to its effect on p53, E6 induces Bak degradation via the ubiquitin-mediated pathway. Recently, several p53-independent cellular pathways, which are altered by the E6 molecule, have been identified. HPV16 E6 induces, by an unkown mechanism, up-regulation of telomerase activity, a key event in the immortalization of primary cells. In addition, HPV16 E6 is able to interfere with cell mobility through interaction with the human homologue of the *Drosophila* discs large protein (DLG). Also in this case, E6 binding leads to degradation of the cellular protein. However, different E6 domains are required to induce degradation of p53 and DLG. Deletion of the carboxy terminus of HPV16 E6 abolishes its binding to DLG without influencing its ability to promote p53 destabilization.

Another cellular target of HPV16 E6 is paxilin, a protein involved in transducing signals from the plasma membrane to focal adhesions and the actin cytoskeleton. The fact that E6 from the oncogenic HPV16 but not E6 from the low risk HPV types 6 and 11 is able to bind paxilin suggests that this interaction has a role in the carcinogenesis of HPV infection. It has also been shown that HPV16 E6 interacts with interferon regulatory factor-3 (IRF-3), a positive transcriptional regulator of the INFβ promoter, which is activated in response to virus infection. E6 binding inhibits IRF-3 transactivation function. Thus, this E6-induced event may enable the virus to circumvent the antiviral response of the infected cell. Finally, E6 associates with ERC 55, a putative calcium



Early Genes of Human Papillomaviruses. Fig. 2 – p53 pathways targeted by HPV16 E6. When cells are exposed to DNA damaging agents, e. g. X-rays, the half-life of p53 can be greatly increased by post-translational modification (phosphorylation). p53 can then activate the transcription of the cyclin dependent kinase (CDK) inhibitor, $p21^{WAF1/CIP1}$, leading to a G1 arrest and DNA repair before replication, or can activate the transcription of the pro-apoptotic *Bax* gene, consequently inducing apoptosis. Cells expressing HPV16 E6 protein are resistant to the cell-cycle arrest and to apoptosis induced by DNA damaging agents.

binding protein located in the endoplasmic reticulum. However, the biological significance of this interaction is unclear.

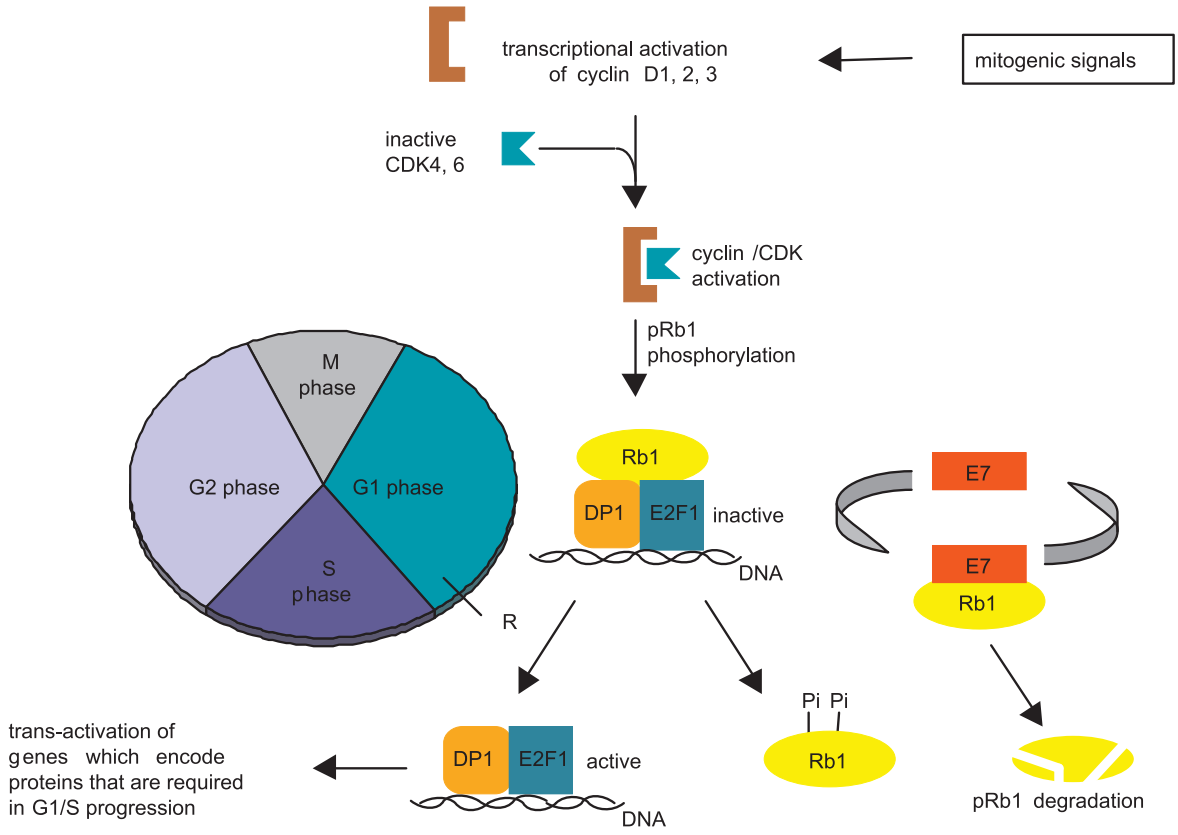
E7 protein

HPV16 E7 is an acidic phosphoprotein of 98 amino acids, which is structurally and functionally related to a gene product of another DNA tumor virus, the Adenovirus E1A protein. On the basis of the similarity in primary structure between the two viral proteins they can be divided into three domains: conserved region 1-3 (CR1-3). Mutational analysis of HPV16 E7 has demonstrated that all three regions are important for the *in vitro* transforming activity of the molecule. CR3 contains two CXXC motifs involved in zinc binding and are essential for the stability of the protein. Independent studies have demonstrated that this viral protein is located in the nucleolus, nucleus and cytoplasm. Indeed, it has been shown that E7 molecule associates with cytoplasmic and nuclear proteins. The best understood interaction of E7 with a cellular protein is that involving the 'pocket' proteins, pRb, p107 and p130. The pocket proteins are central regulators of cell cycle. They negatively regulate, via direct association, the activity of several transcription factors including members of the E2F family (E2F1-5), which are associated with their partners, DPs. Under normal cell-cycle regulation, phosphorylation of pRb, which is mediated by cyclin-dependent kinase (CDK) activity, leads to the disruption of pRb/E2F complexes with consequent activation of E2Fs. HPV16 E7 binds the pocket proteins and, analogously to the CDK-mediated phosphorylation, results in the release of active E2Fs that in turn activate transcription of a group of genes encoding proteins essential for cell-cycle progression, such as cyclin E and cyclin A (Fig. 3). As described for the interaction between E6 and p53, HPV16 E7 protein is able to promote the destabilization of pRb through the ubiquitin-proteasome pathway. This property is not shared by E7 from all the different HPV genotypes. Indeed, E7 from the benign HPV1 can efficiently associate with pRb without inducing its degradation. It is likely that the E7-induced pRb degradation represent a more effective way to neutralize the function of the cellular protein. The other two members of the pocket protein family, p107 and p130, are involved in controlling additional cell-cycle checkpoints. p130 exerts its transcriptional regulatory function during the G0/G1 transition, while p107 is active in the G1/S transition and in the G2 phase. Analogously to pRb, HPV16 E7 protein associates with p107 and p130, inactivating the cell-cycle checkpoints in which they are involved. It is not clear yet whether the E7 binding to p107 and p130 leads to their destabilization, as shown for pRb.

Besides the targeting of the pocket proteins, E7 can alter cell-cycle control by additional mechanisms. The HPV16 E7 protein is able to associate with the CDK inhibitors $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ causing neutralization of their inhibitory effects on the cell cycle. Cells expressing HPV16 E7 and high levels of $p21^{WAF1/CIP1}$ or $p27^{KIP1}$ are still able to enter S phase, while in the absence of E7 cells are arrested in G1 phase. HPV16 E7 can also directly and/or indirectly interact with cyclin A/CDK2 complex. The biological function of this interaction remains to be elucidated, but it is possible that E7 may act by redirecting the kinase complexes to a different set of substrates.

Other cellular proteins involved in transcriptional regulation have been identified as HPV16 E7 targets. HPV16 E7 binds the TATA-box binding protein (TBP) and the TBP-associated factor TAF110, indicating that the viral protein is able to interfere with the basic transcriptional machinery of the host cell. Furthermore, E7 associates with the AP1 complex activating its transcriptional activity.

Similarly to other stimulators of proliferation, the HPV16 E7 protein promotes apoptosis in addition to its ability to deregulate the cell cycle. Expression of HPV16 E7 in normal human fibroblasts or in human keratinocytes results in a cytocidal response, which displays the typical features of apoptosis and is much more evident in the absence of mitogenic signals. This E7-induced apoptosis requires pRb inactivation and is mediated by p53-dependent and p53-independent pathways. It is likely that the E7-induced apoptosis represents a cellular response elicited by the loss of cell cycle control.



Early Genes of Human Papillomaviruses. Fig. 3 – Deregulation of the restriction point (R) by HPV16 E7. E2F transcription factors form heterodimers with members of the DP family and regulate the transcription of several genes during the cell cycle. In quiescent cells, pRb is present in a hypophosphorylated form and is associated with E2F molecules, thereby inhibiting their transcriptional activity. When quiescent cells are exposed to mitogenic signals, genes encoding the G1 specific D-type cyclins (D1, D2 and D3) are activated. Subsequently, cyclins associate with catalytic subunit of CDK4 or 6. After their transport into the nucleus, the kinase complexes phosphorylate pRb in mid-G1 phase, causing release of active E2F/DP1 complexes and progression through the restriction point (R). E7 binding to pRb mimics its phosphorylaion. E7 expressing cells can thus enter the S phase in the absence of a mitogenic signal.

Interestingly, E6 protein is able to completely abrogate the E7 activity in promoting apoptosis. Thus, both viral proteins are required to induce full transformation of the host cells.

E5 protein plays an early role in the HPV-induced transformation

Studies on bovine papillomavirus (BPV) have provided evidence that E5 is a potent oncoprotein. Recently, it has also been shown that HPV16 E5 is able to induce cellular transformation, although with less efficiency than BPV E5. HPV16 E5 is a small hydrophobic protein, which is located in the endoplasmatic reticulum (ER), nuclear membrane and cytoplasmic vesicles. E5 is able to enhance growth factor-mediated signal transduction to the nucleus, with resulting stimulation of cellular proliferation. BVP1 and HPV16 E5 associate with the 16 kD subunit c of the vacuolar H^+-ATPase, which is responsible for acidification of membrane bound organelles such as Golgi, endosomes and lysosomes. It has been recently shown that BPV1 E5 induces alkalinization of Golgi and that this activity is linked to its *in vitro* transforming activity. Mutations in E5 that abolish the interaction with 16 kD subunit c ab-

rogate Golgi alkalinization and cellular transformation.

Since the integration of viral DNA, which occurs in tumour cells, results in a loss of E5 gene expression, it is clear that E5 is involved in early events during the multi-step process of cervical cancinogenesis, and that its function is no longer required after the establishment of the transformed phenotype.

E1 and E2 are involved in the regulation of viral DNA replication

The function of E1 is to control the replication of viral DNA. E1 contains the cyclin-binding RXL motif and is able to associate with cyclin E and cyclin A. Consistent with these findings, E1 is phosphorylated by cyclin E or cyclin A-associated kinase. Mutation of E1 phosphorylation sites results in a reduction of HPV DNA replication, supporting the idea that the E1/cyclin association play an important role in viral DNA replication. Moreover, it has been shown that E1 has an ATPase and helicase activity. E1 forms a stable complex with E2 and binds to the replication origin of HPV in order to recruit cellular factors essential for DNA replication. Furthermore, E1 has been found associated with components of the cellular DNA replication machinery, e.g. DNA polymerase α.

E2 regulates the transcription of the early genes

In addition to controlling viral DNA replication together with E1, E2 is able to negatively or positively regulate the transcription of the early genes. Like all transcription regulatory factors, E2 has an amino-terminal trans-acting domain and a carboxy-terminal DNA binding domain, which recognizes four cis elements (ACCN6GGT) in the long control region (LCR) of the HPV genome. These two domains are separated by a central region (hinge), which is important, together with the amino-terminal domain, for the nuclear localization of the molecule. Whether E2 binding results in repression or activation of the promoter of the early genes is dependent on the position of the E2-binding site in the LCR. E2 binding to the promoter-distal or promoter-proximal elements leads to a positive or negative regulation of transcription, respectively.

E4 is probably involved in virus maturation and/or replication

E4 is a late protein expressed from the early region of the genome. Most of the studies have been performed on E4 from the cutaneous HPV type 1. In these lesions E4 is present at very high levels and several E4-derived proteins have been detected. The primary product is a 17 kD protein, which is expressed from E1^E4 transcript (Fig. 1). E4 associates with and disrupts the cytoplasmic keratin network. The biological significance of this E4-induced event is not fully understood. It has been proposed that E4 plays a role in the productive phase of the infection establishing a favorable condition for viral maturation.

Clinical Relevance

HPV16 infection results in the induction of a benign proliferation, which after a long latent period can progress to invasive cancer. Persistent HPV infection is necessary for the development of the malignant lesion. This requirement is explained by the fact that viral proteins, in order to induce full malignant transformation of the host cells, have to cooperate with an activated cellular oncogene. Accumulation of mutations in cellular genes, which possibly lead to activation of an oncogene, requires continuous proliferation. This is achieved by the abilities of E6 and E7 to respectively neutralize apoptotic pathways and to induce unscheduled proliferation. Therefore, a possible approach to induce regression of a HPV-positive lesion is to target the biological functions of E6 and E7. This possibility is supported by findings that clearly demonstrate that continuous expression of the two viral genes is necessary for the maintenance of the host cell transformed phenotype. Thus, we can predict that blocking the activity of E7 can lead to a rapid exit from the cell cycle. Neutralization of E6 function should result in an even more efficient way to induce regression of the HPV-lesion. As described above, E7 has a

dual activity being able to induce proliferation and apoptosis. E6, acting upon p53-dependent and p53-independent pathways, completely abolishes E7-induced apoptosis. Thus, we could imagine that in cells expressing E6 and E7 genes, the block of only E6 functions may push the balance between proliferation and apoptosis in favor of the latter causing regression of the HPV lesion.

Alternative targets are E1 and E2, which are involved in viral transcription and replication. Several approaches to neutralize the early viral proteins are under investigation. These include strategies to block the transcription or translation of viral genes or to identify small molecules able to specifically associate with and inactivate the viral proteins.

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Early Virus Genes

Definition

Early virus genes are genes whose expression occurs before the onset of replication of the viral genome. Early proteins are usually required for replication of the virus nucleic acid. In contrast to the structural ('late') proteins, early proteins are usually not incorporated into the virus particle.

Ebnerin

Definition

Ebnerin is the rat homologue of deleted in malignant brain tumors 1 (→ DMBT1) which is specifically expressed by the von Ebner glands of the tongue and is suggested to play a role in taste perception.

E-box

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Synonyms

- E box
- E motif

Definition

E-box is the collective term for DNA motifs with the consensus sequence CANNTG. E-boxes appear in a broad variety of promoters and enhancers and serve as protein binding sites. Proteins with affinity to this motif all belong the basic helix-loop-helix (bHLH) class of transcription factors, wich can act as activators of transcription as well as repressors. More than 240 bHLH proteins are known to date in eukaryotes ranging from yeast to human, and the number continues to grow. Their binding specificity depends on both the nature of the 'NN' nucleotides and sequences in the vicinity of the E-box. Genes containing E-boxes are activated during important developmental processes and some are described to be involved in cancer development and/or progression.

Characteristics

The DNA sequence 'CAGGTGGC' was originally identified in 1985 within the sequence of the immunoglobulin enhancers, the first enhancers found to be activated in a tissue specific

manner. Later the name E-box, (with 'E' for enhancer), was used as a common term for all motifs with the consensus sequence 'CANNTG' and in 1989 the first two E-box binding proteins, E12 and E47, were identified. Both bind their recognition site as dimers and dimerisation is promoted by the helix-loop-helix (HLH) domain. HLH domains were subsequently found in other transcription factors such as MyoD and Myc proteins.

Genes containing E-boxes and thebHLH proteins that control them

To date an overwhelming number of E-box containing genes, regulated by bHLH proteins have been identified (Table 1), they include:

- Muscle (smooth and skeletal) specific genes that control myogenis and are regulated by the → bHLHs of Muscle Regulatory Factors (Mrf).
- Genes involved in heart development that are regulated by the gene products of the → *Hand* gene family, Hand1 and Hand2.
- Genes involved in neuronal development and differentiation are regulated by different bHLH proteins such as the *Achaete-scute* family; *Atonal* family; *E12/47* family; *Hen* family as well as by *Nex*; *Hairy/E(Spl)* and *Id* [→ Id proteins]).
- Insulin inducible genes controlling pancreatic development, are regulated by a member of the *Atonal* family, *NeuroD*. The latter is also a key player in neurogenesis.
- Genes involved in B- and T-cell development, regulated by bHLHs of the *E12/E47* family.

Genes and gene products that are related to growth control and cancer

- The proto-oncogenes → *BCL6* and → *FOS* that encode transcription factors.
- CDC2 and CYCLIN B1 gene products, involved in cell-cyle regulation.
- Cathepsin B and Cathepsin D, Fatty acid synthase and HMG-CoA reductase, These enzymes appear to be involved either in the process of tumor cells invading healthy tissue (Cathepsins) or seem to support the growth of cancer cells (Fatty acid synthase and HMG-CoA reductase).
- Overexpression of the *HB-EGF* gene, encoding a growth factor, promotes cancer cell growth.
- COX-2 and SP-A gene products may be involved in tumor promotion. Underlying mechanisms are still unknown.

Many E-box containing genes that are thought to be involved in cancer development provide binding for members of the Myc protein family, which play an important role in many cancer types. Being transcription factors they can activate transcription of the genes *CAD*, *CDC25A*, *CYCLIN B1*, *ORNITHINE DECARBOXYLASE*, *PROTHYMOSIN ALPHA*, *RCC1*, *ID2* and *TERT*.

Members of the Usf (upstream transcription factor) protein family also contain the bHLH motif and bind to a variety of genes correlated with cancer. However, to date no oncogenic behavior has been described for these proteins and since Usfs are ubiquitously expressed, they may only play a less specific role in mechanisms of gene regulation (Table 2).

How do bHLH proteins bind to E-boxes?

bHLH proteins bind to the E-box only as dimers, either as homodimers or heterodimers with other members of the bHLH protein family. The helix-loop-helix motif within the protein allows dimerization: Two amphipathic alpha-helices are separated by a short stretch of amino acids, forming one or more β-turns, the 'loop'. Similar to the leucine-zipper motif, hydrophobic amino acid residues on one face of the helix interact with similar, also hydrophobic, residues of the helix from a second protein thus stabilizing the dimer. Some proteins possess a leucine-zipper as a second dimerization motif; these include members of the Mad, Srebp, Tfe and Myc family (Fig.).

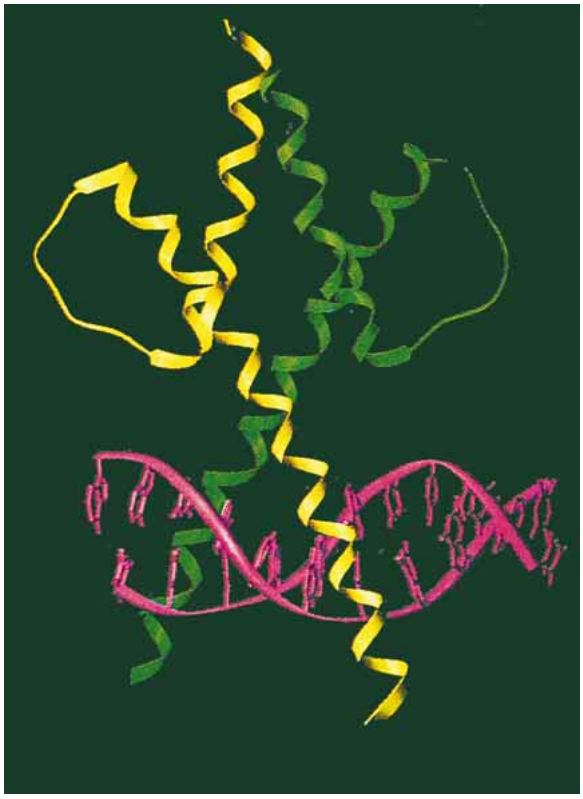
Dimerization is necessary but not sufficient for DNA binding. A sequence specific E-box recognition is mediated by a pattern of basic amino acids (Table 3). Since this basic region is localized N-terminal to the HLH region, it was

E-box. Table 1 – Genes that contain E-boxes and that are involved in cell growth control or cancer development.

gene	function/role in cancer development	E-box regulating protein
Bcl-6	protooncogene coding for Krüppel-like zinc finger transcription factor, genetic rearrangements frequently found in lymphoma	?
carbamoyl-phospate synthase/ aspartate carbamoyltransferase/ dihydroorotase (CAD)	enzyme involved in de novo pyrimidine biosynthesis, essential for cell growth	Myc
cathepsin B	protease, overexpression causes ability of cancer cells to metastasize	?
cathepsin D	estrogen induced protease, same mechanism as cathepsin B but mainly in breast cancer	USF1, USF2
Cdc2	kinase, associates with Cyclin A and Cyclin B1, regulator of cell cycle progression through G2 and M phase	USFs, Myogenin
Cdc25A	oncogene coding for cdk activating phosphatase, cell-cycle progression	Myc
c-Fos	protooncogene coding for a transcription factor	E12, E47, Myogenin
cyclin B1	activates cdc2 kinase which regulates cell-cycle progression through G2 and M phase, increased expression often found in cancer cell lines	Myc
cyclooxygenase-2 (COX-2)	enzyme involved in prostaglandine synthesis in inflammatory processes, overexpression is correlated with tumor promotion	USFs
fatty acid synthase (FAS)	main enzyme in lipogenesis, overexpressed in wide variety of cancer types, mainly breast and prostate cancer, identical with prognostic molecule OA-519	USF
heparin-binding epidermal growth factor like growth factor (HB-EGF)	growth factor, member of the EGF family, postulated role in developement of hepatocellular carcinoma, prostate cancer, breast cancer, esophageal and gastric cancer	MyoD
3-hydroxy-3-methylglutyl coenzme A reductase (HMG-CoA reductase)	rate limiting enzyme in isoprenoid biosynthesis, inhibitions blocks Ras activation and inhibits growth of ras-transformed cells	SREBP
ornithine decarboxylase (ODC)	rate limiting enzyme in polymamine synthesis, essential for cell growth	Myc, MycN
prothymosin alpha	protein of unclear function, related to cell growth	Myc, MycN
pulmonary surfactant protein A (Sp-a)	lung specific phospholipid-associated glycoprotein, mediates pathogen defence, frequently overexpressed in lung adenocarcinomas	USF1
regulator of chromosome condensation-1 (Rcc1)	guanin exchange factor, necessary for cell proliferation	Myc
telomerase reverse trascriptase (Tert)	catalytic subunit of telomerase which maintains chromosome ends and is an immortalizing enzyme	Myc

E-box. Table 2 – List of bHLH protein families in animals and their E-box binding specificity (extended, updated and modified after W.R. Atchley & W.M. Fitch, 1997).

E-box sequence	bHLH family	family members	function
CAG (CTG)	Achaete/Scute	Achaete, Scute, Mash-2, Hash-2, Fash-2	neurogenisis
	Atonal	Atonal, Lin-32, Hath1, Math1, Math2, NeuroD, NeuroD2, NeuroD3, NeuroM	neurogenesis; pancreatic development
	Delilah	Delilah	differentiation of epidermal cells into muscle in Drosophila
	Hand	Hand1, Hand2	cardiac morphogenesis; trophoblast cell development; neural crest development
	E12/E47	E12, E47, Pan1, Pan2	ubiquitously expressed; myogenesis, neurogenesis; immunoglobulin gene expression
	Hen	Hen1, Hen2, Nhlh1, Nhlh2	neurogenesis
	Lyl	Lyl-1, Nscl1, Nscl2, Scl/Tal-1, Tal-2	hematopoietic proliferation and differentiation
	Mrf	MyoD, Myogenin, Myf5, Myf6, Mist1, Mrf4	myogenesis
	Nex	nex-1	neurogenesis
	Twist	Twist, Paraxis, Scleraxis, Dermo-1, Tcf21	specification of mesoderm lineages, myogenesis
CAC (GTG)	bHLH/Pas	Arnt, Arnt2, trh, Hif-1alpha, Sim, ahr, BMAL1, clock, tim, per	reaction to aromatic hydrocarbons; regulation of circadian rhythm; hypoxia response
	Hairy/E(Spl)	Hairy, E(Spl), Deadpan, Hes1, Hes5, Her1, Her4, Hesr-1, Sharp-1, Sharp-2	neurogenesis; segmentation
	Mad	Mad, Mad3, Mad4, Mnt, Mxi1	regulation of cell proliferation
	Myc	Myc, Mycn, Mycl, Max	cell proliferation; differentiation
	Srebp/Add	Srepb1, Srebp2, Add1, HLH106	cholesterol homeostasis; sterol synthesis; adipocyte determination
	Tfe	Tfe3, Tfeb, Tfec, Mitf, Mi	placenta vascularization; development of melanocytes, osteoclasts, masts cells
	USF	Usf1, Usf2, Spf1	ubiquitous transcription factors
no binding	Id	Id1, Id2, Id3, Id4, TId1, TId2, XIdl, XIdx, XId2, emc	negative regulators of myogenesis, neurgenesis



E-box. Fig. – Shown is an E47 bHLH dimer bound to an E-box element (sequence: CACCTG). Each monomer consists of two α-helices that are separated by a loop; hydrophobic interactions between the α-helices stabilize dimerization. The basic region of each bHLH monomer makes contact with the the major groove of the DNA molecule, each covering one half of the provided DNA binding site (from T. Ellenberger et al., (1994) with permission).

this order which gave the name to a whole class of proteins, the 'basic helix-loop-helix proteins'. bHLH proteins that rather bind to the 'CAGCTG' DNA motif have different set of basic residues than those recognizing the DNA sequence 'CACGTG'. The change of only a single amino acid residue can alter binding specificity. The MyoD basic region usually recognizes the DNA sequence 'CAGCTG'. Replacement of the leucine at position 13 by an arginine within the basic MyoD region, changed its binding preference to the 'CACGTG' sequence.

Regulation of E-box containing genes is also determined by the composition of the bHLH dimer bound to the DNA. Max, a member of the Myc family, can heterodimerize with Myc and activate target genes. If Myc is replaced by one of the Mad family members, the resulting heterodimer now represses the same target genes. Whereas Max is an ubiquitously expressed protein, the expression patten of its potential partners are tissue-, developmental- or cell cyle-dependent. Consequentely, transcriptional activation or repression of the target gene is determined by the ratio of Myc to Mad proteins, competing for Max dimerisation.

E-box. Table 3 – Amino acid sequence comparison of bHLH proteins that bind the DNA sequences CAC GTG and CAG CTG. [B = basic residue].

	bHLH	BB---N--ER-R---
CAC GTG	Myc	KRRTHNVLERQRRNE
	Max	KRAHHNALERKRRDH
	Usf	RRAQHNEVERRRRDK
	Tfe3	KKDNHNLIERRRRFN
CAG CTG	MyoD	RRKAATMRERRRLSK
	E12	RRVANNARERLRVRD
	E47	RRMANNARERVRVRD

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EBV

Definition

→ Epstein-Barr virus.

E-cadherin

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Synonyms

- Epithelial cadherin

Definition

Epithelial/E-cadherin is a prototype member of the classical cadherin family of single-pass transmembrane proteins that mediate calcium-dependent cell-cell adhesion. Normally, → epithelial cells are tightly interconnected through several junctional structures, including → tight junctions, → adherens-type junctions and → desmosomes, which are intimately associated with the actin and the intermediate filament cytoskeleton. Crucial for the establishment and maintenance of these junctional complexes are the Ca^{2+}-dependent homophilic interactions mediated by the cell-adhesion molecule E-cadherin in the zonula adherens.

The majority of human cancers (80 – 90%) is of epithelial origin, and extensive clinical and experimental studies have revealed that loss of E-cadherin expression correlates with late stage tumorigenesis, characterized by tumor cell dedifferentiation, invasive tumor growth and the formation of metastasis. Moreover, studies in a transgenic mouse model of tumorigenesis have demonstrated that the loss of E-cadherin is a rate-limiting step in the transition from → benign tumors to → maligant tumors and the subsequent formation of metastases. Notably, germline mutations of E-cadherin have been shown to predispose to diffuse gastric cancer.

Characteristics

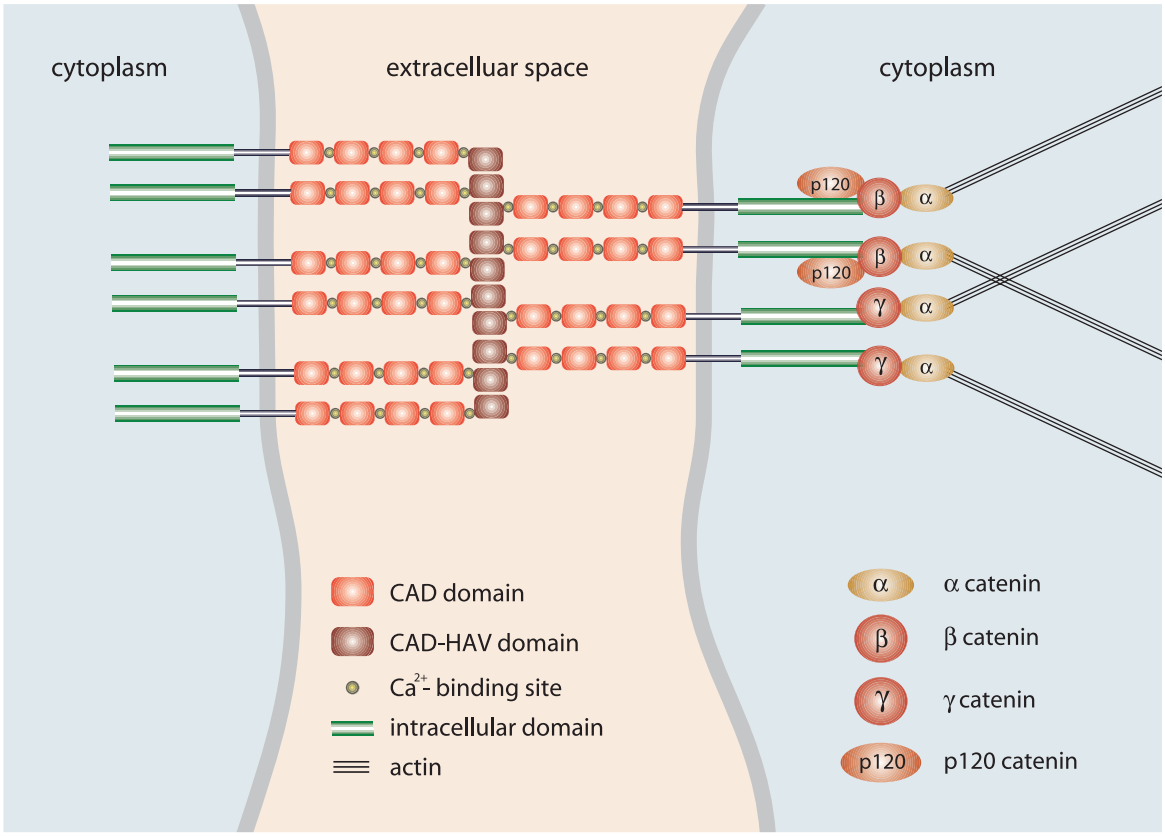
The extracellular region of E-cadherin consists of five highly conserved cadherin (CAD) domains that coordinate with calcium ions to form rod-like structures (Fig. 1). The outermost CAD domain contains a conserved HAV motif (→ amino acid single letter code) that is thought to mediate homotypic binding in *trans* to an E-cadherin molecule on the surface of an adjacent cell. Dimerization of E-cadherin molecules in *cis* appears to be mediated by their transmembrane domains. The combination of dimerization in *cis* and homotypic binding in *trans* results in the formation of zipper-like E-cadherin adhesion structures (Fig. 1). Depletion of calcium ions results in the disassembly of these structures and, hence, in the loss of cell-cell adhesion.

Also critical for E-cadherin-mediated cell-cell adhesion is the interaction of its cytoplasmic domain with catenins [α-, β-, and γ-catenin (plakoglobin)]. β-catenin and γ-catenin/plakoglobin associate with the distal cytoplasmic domain of E-cadherin. β-catenin and γ-catenin/plakoglobin also bind to α-catenin, which in turn connects the cadherin cytoplasmic complex to the actin cytoskeleton (Fig. 1). An additional catenin, p120 catenin, was originally identified as a substrate of the non-receptor tyrosine kinase → $pp60^{c\text{-}Src}$ and binds to the juxtamembrane region in the cytoplasmic tail of classical cadherins. By linking cadherins to the cytoskeleton the β-catenin/α-catenin bridge increases adhesive strength. In contrast, depending on the cell type, p120 catenin has been found to positively or negatively modulate the strength of cell-cell adhesion.

Deletion of the cytoplasmic catenin-binding domain or any disruption of the intracellular E-cadherin-catenin complex results in loss of cell-cell adhesion. Hence, mutations in genes other than E-cadherin itself might also affect E-cadherin function. Finally, E-cadherin-mediated cell adhesion might also participate in the transduction of transmembrane signals that regulate gene expression and cell fate.

Function

Historically, E-cadherin has been perceived to physically link neighboring cells and stabilize these contacts by connecting the cadherin cell adhesion complex to the actin cytoskeleton. However, the function of E-cadherin is not limited to the establishment of physical interactions between adjacent cells; it also affects dif-



E-cadherin. Fig. 1 – E-cadherin-mediated cell-cell adhesion. E-cadherin homodimers on the plasma membranes of adjacent cells interact in a zipper-like fashion. The most N-terminal CAD domain on each E-cadherin molecule contains a HAV motif thought to interact with an E-cadherin molecule on an adjacent cell. The cytoplasmic cadherin complex, which consists of a catenin, b catenin, g catenin and p120 catenin, links E-cadherin to the actin cytoskeleton.

ferentiation, migration, and proliferation of cells. For example, loss of E-cadherin function does not only result in a loss of cell-cell adhesion but also induces a migratory and invasive cellular phenotype. Conversely, establishing E-cadherin-mediated cell-cell adhesion in migratory, invasive cells promotes epithelial polarity.

Precise and rapid regulation of cell-cell adhesion is crucial for various cellular processes including cell migration, cell sorting, invasion and differentiation during early embryonic development and organogenesis. Considering the complexity of the cytoplasmic cadherin complex, regulation of cell adhesion usually does not occur at the level of E-cadherin gene expression. Rather recruitment of pre-existing E-cadherin at the plasma membrane and assembly of the cadherin-catenin complex are most important in determining the adhesive capabilities of a particular cell. For example, compaction of the mouse embryo at the morula stage, where a collection of loosly adherent blastomeres packs into a tight epithelium, is independent of major changes in E-cadherin gene expression.

Regulation of the E-cadherin adhesion complex. Recruitment of pre-existing E-cadherin molecules to the sites of cell-cell adhesion occurs upon formation of membrane contacts with adjacent cells. An increase of calcium to physiological concentrations also induces the assembly of cadherin at contact sites, leading to the formation of E-cadherin-mediated adherens junctions. Initiation of cell-cell adhesion

leads to recruitment of additional cadherin molecules to the adhesion site and to the formation of the cytoplasmic adhesion complex.

Once formed, the E-cadherin-mediated cell-cell adhesion can be rapidly dismantled by growth factor-mediated signals. Members of the cytoplasmic adhesion complex, in particular p120 catenin and to a lesser extent β-catenin and γ-catenin, are phosphorylated upon treatment of cells with → growth factors such as → hepatocyte growth factor (HGF), epidermal growth factor (EGF), or → platelet-derived growth factor (PDGF). Phosphorylation occurs either directly by the respective receptor tyrosine kinases or indirectly by downstream effector kinases such as $pp60^{c\text{-}src}$. Phosphorylation of the catenins causes their release from the cell adhesion complex resulting in the disruption of cell adhesion and eventually in scattering of cells. Similarly, treatment of cells with potent inhibitors of tyrosine phosphatases induces dissociation of the cytoplasmic cell adhesion complex and the loss of cell adhesion.

Signals elicited by the loss of cell adhesion. The fact that the loss of E-cadherin-mediated cell-cell adhesion causes a defect in cell adhesion as well as inducing a migratory and invasive cellular phenotype raises the possibility that abrogation of E-cadherin function may actively transmit signals and elicit various cellular responses.

Besides being a major component of the cytoplasmic cadherin complex β-catenin is also part of the → WNT-signalling pathway. In the absence of a WNT-signal non-sequestered β-catenin is rapidly phosphorylated by glycogen synthetase kinase 3β (GSK-3β) in the adenomatous polyposis coli (→ APC)-GSK-3β complex and subsequently degraded by the → ubiquitin-proteasome pathway. If the tumor suppressor APC is non-functional, as in many colon-cancer cells, or GSK-3β activity is blocked by the activated WNT signalling pathway, β-catenin accumulates at high levels in the cytoplasm. Subsequently, it translocates to the nucleus, where it binds to a member of the TCF/LEF-1 family of transcription factors and modulates the expression of TCF/LEF-1-target genes. Loss of function mutations of APC are a hallmark of colon cancers, and stabilizing mutations of β-catenin are frequently found in a number of cancer types, making these genes an important tumor suppressor and oncogenes, respectively. However, it remains to be determined whether β-catenin, which upon loss of E-cadherin function is released from the E-cadherin cell adhesion complex, directly feeds into the WNT-signalling pathway.

Since α-catenin not only connects adherens junctions with the actin cytoskeleton but interacts with several actin binding proteins including vinculin, ZO-1 and α actinin, it may also be involved in communication between cell adhesion and changes in cellular phenotype. Part of the regulation of the actin cytoskeleton is mediated by members of the Rho family of small → GTPases, and recent work suggests that Rho family GTPases including Rho, Rac and Cdc42 are required for cadherin mediated cell-cell adhesion.

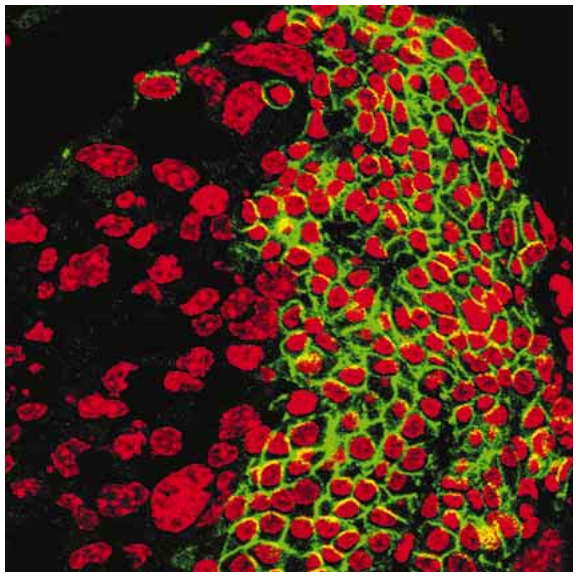
Inhibition of Rho reduces the accumulation of E-cadherin at cell contact sites in keratinocytes. Moreover, forced expression of constitutively-active forms of Rac or Cdc42 inhibits HGF-induced scattering of epithelial cells, probably by increasing E-cadherin-mediated cell adhesion. Similarly, overexpression of Tiam-1, a nucleotide exchange factor for Rac, enhances E-cadherin-mediated adhesion. These effects are explained by the known function of these small GTPases on the actin cytoskeleton. However, recent experiments suggest a direct influence of Rac and Cdc42 on the cadherin-catenin complex via the protein IQGAP1. This downstream effector of small GTPase has been shown to be a novel regulator of E-cadherin activity. Not only does it accumulate at contact sites of cells expressing E-cadherin, it also directly interacts with the amino-terminus of β-catenin and the cytoplasmic tail of E-cadherin. Active Rac and Cdc42 prevent the interaction of IQGAP1 with β-catenin by sequestration of IQGAP1. However, upon release of IQGAP1 from GDP-bound inactive Rac and Cdc42, IQGAP1 displaces α-catenin from β-catenin resulting in the dissociation of α-catenin from the cytoplasmic cadherin complex and concomitant loss of E-cadherin-mediated cell adhesion. Thus, Rac and Cdc42 seem to positively regulate cell adhesion by suppression of IQGAP1 activity.

Bioactivity

A multitude of clinical and experimental studies have revealed that the adhesive function of E-cadherin is frequently lost during the development of most human epithelial cancers including carcinomas of the breast, colon, prostate, stomach, liver, esophagus, skin, kidney and lung (Fig. 2). In general, decreased E-cadherin function correlates with the de-differentiation of tumor cells, infiltrative tumor growth and metastasis. In some instances, reduced expression of E-cadherin at the cell-cell boundaries correlates significantly with tumor grading and, thus, is a marker for poor prognosis. Several different mechanisms appear to cause the loss of E-cadherin function, including deletion or mutational inactivation of the E-cadherin gene. Notably, germline mutations in the E-cadherin gene are evident in cases of familial gastric cancers, indicating that mutation of the E-cadherin gene is sufficient to predispose to the development of cancer. Moreover, changes in the expression of proteins that are part of the E-cadherin adhesion complex impair E-cadherin-mediated cell-cell adhesion; for example, expression of truncated α-catenin or truncated β-catenin abrogate E-cadherin function. Whereas mutations in the α-catenin gene or reduced α-catenin protein levels have thus far only been found in cultured tumor cell lines, mutations in the β-catenin gene are evident in many primary tumors including melanoma, colon cancer, gastric cancer and prostate cancer.

Besides the genetic impairment of E-cadherin-mediated cell adhesion caused by deletion or mutation, several other mechanisms directly affect E-cadherin expression and function. For example, chromatin rearrangement and loss of transcription factor binding coincide with suppression of E-cadherin promoter activity in invasive carcinoma cells. Notably in many tumor types, → hypermethylation of the regulatory region of the E-cadherin gene and thus transcriptional silencing of the gene appears to be a major mechanism underlying E-cadherin loss of function. Finally, proteases that are up-regulated during tumor progression are able to degrade the extracellular portion of the E-cadherin molecule resulting in the disruption of cell-cell adhesion and the cytoplasmic cadherin complex.

The observation that E-cadherin function is frequently lost in malignant cancers prompted an examination of the functional role of E-cadherin in tumor progression. Using tumor cell lines in culture, several groups demonstrated that re-establishing the functional cadherin complex, for example by forced expression of E-cadherin, results in a reversion from an invasive to a benign epithelial tumor cell phenotype. Recently, the causal role for E-cadherin in the transition from adenoma to carcinoma *in vivo* has been demonstrated in a transgenic mouse model of multistage tumorigenesis. Maintenance of E-cadherin expression during tumorigenesis results in arrest of tumor development at the adenoma stage. By contrast, expression of dominant-negative E-cadherin induces early invasion and metastasis, indicating that the loss of E-cadherin-mediated cell adhesion is a rate limiting step in the progression from benign to malignant tumors. It remains to be elu-



E-cadherin. Fig. 2 – Loss of E-cadherin expression during tumor progression. E-cadherin (green) is expressed at the plasma membranes of tumor cells with benign, epithelial phenotype and normal nuclei (red). In contrast, E-cadherin expression is focally downregulated within the portion of the tumor that exhibits invasive growth and atypic nuclei (red).

cidated how the loss of E-cadherin can actively support tumor invasion and metastasis, i.e. the signals that are elicited by the loss of cell adhesion and that induce a change in cellular phenotype (see above).

Other family members of classical cadherins are expressed in epithelia, however, only few have been implicated in human carcinogenesis. One exception is the inactivation of H-cadherin in human lung cancer. Notably, deletion of the H-cadherin locus is accompanied by hypermethylation of the promoter region on the remaining allele. Recently, it was observed that in several cancer types expression of N-cadherin was upregulated during tumor progression concomitant with the loss of E-cadherin function, refered to as the 'cadherin switch'. In these cases, N-cadherin appeared to induce tumor cell invasion and metastasis.

Clinical Relevance

Based on the fact that E-cadherin expression is lost during the development of most, if not all, epithelial tumors, it is used as a diagnostic and prognostic marker for several human cancers. Moreover, mutations in the E-cadherin gene are found in a subset of gastric cancer. Therapeutic approaches that are based on the loss of E-cadherin function will have to await the identification of downstream effector genes that as appropriate targets are more amenable to therapeutic intervention.

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ECM

Definition

→ Extracellular matrix.

ECSA

Definition

→ Erythroid colony stimulating factor.

EEN

Definition

The EEN gene encodes a partner of the MLL gene in a case of t(11;19)(q23;p13) translocation. The 19p13 band is where two other partners of MLL also map, ENL and ELL/MEN. For this reason, the new fusion gene was defined EEN (extra eleven-nineteen gene).

Effector Cell Protease Receptor-1

Definition

Effector cell protease receptor-1 (ERP-1) is a cellular receptor involved in mitogenic signalling by the coagulation protease factor Xa.

EFT

Synonyms

- Ewing tumor (ET)

Definition

→ Ewing sarcoma family tumor (EFT), the generic term for Ewing sarcoma, is a peripheral primitive neuroectodermal tumor and → Askin tumor.

EGF

Definition

Pro-epidermal growth factor precursor (EGF) is a membrane protein of 1207 amino acids and 133 kD. The human gene *EGF* locus maps to 4q25. The EGF growth factor stimulates the growth of various epidermal and epithelial tissues *in vivo* and *in vitro* and of some fibroblasts in cell culture.

EGF Receptor

Definition

→ Epidermal growth factor receptor (EGF receptor; EGFR) is a transmembrane receptor that binds epidermal growth factor *via* its extracellular domain and transmits growth signals intracellularly by means of a cytoplasmic tyrosine kinase domain. This domain phosphorylates the C-terminal tail of the molecule at multiple tyrosine residues, which become docking sites for various signaling molecules that bind to and subsequently become phosphorylated by the receptor. There are four members of the receptor family (Her1-4 or ErbB1-4), all of which are implicated in the etiology or progression of cancer.

EGFR

Definition

→ Epidermal growth factor receptor, → EGF receptor.

EGL

Definition

→ External granular layer.

Eker Rat

Definition

The Eker rat carries a *Tsc2* germ-line mutation that confers a strong predisposition for renal carcinoma development; → modifier loci.

Electromagnetic Fields and Cancer

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Definition

Magnetic fields are generated by the movement of any electrical charge. A continuous electric current passing through a conductor creates a static magnetic field, while an electric current changing in time creates a variable magnetic field, which radiates electromagnetic waves spreading around the surrounding space at light speed. These electromagnetic fields enter living tissue but are known as non- ionizing radiation since they are weak and unable to break molecular bonds. Metals such as iron, zinc, manganese and cobalt are sensitive to electromagnetic fields that may exert their effects on

proteins and cellular components containing these metallic elements.

Characteristics

Few environmental issues are as contentious as the question of whether exposure to electromagnetic fields affects biological systems. Considering the widespread use of electromagnetic radiation generating devices such as radio, television, wireless communications etc., the health hazard implications of any connection between electromagnetic fields and cancer risk have raised a growing interest in the potential biological effects of electromagnetic fields on the mammalian cell growth, viability and response to genotoxic injury. This topic is still a subject of repeated argument, and caution in the interpretation of the effects of static and variable magnetic fields on cellular behaviour needs to be claimed. A measurable magnetic field is created even by the residential electric current. It is noteworthy that we are pervaded by the Earth's static magnetic field that is hundreds of times greater than the low frequency electromagnetic fields created by current within homes.

Epidemiological and clinical evidence

The first connection between human disease and electromagnetic fields was suggested by the observation of a higher incidence of cancer in children living near power distribution lines. Afterwards major power lines have been held responsible for the occurrence of different cancer varieties. Results of different studies of a possible link between exposure to electromagnetic fields and childhood cancer, namely leukemia, have been rather inconsistent. One large study found no association between electromagnetic field exposure and an increased risk of childhood leukemia, in contrast to previous reports showing that the exposure to electromagnetic fields resulted in nearly a 20 percent increase in the risk of leukemia. This case-control investigation did not find a significant link between the risk of childhood leukemia and the actual measurement of magnetic fields in children's current and former homes, including homes their mothers lived in during pregnancy of the affected subjects. Electromagnetic field exposure has also been associated with the risk of breast cancer, mainly in men. Epidemiological studies have shown that in industrialized countries, where the electromagnetic field generating devices are in use on a large scale, breast cancer risk is higher. It has been suggested that electromagnetic field exposure might promote breast neoplasm through inhibition of melatonin release. Different occupational epidemiological studies have shown an increased incidence of breast neoplasm in women employed in occupations with high electromagnetic field exposure as well as in male electrical workers. However, other investigations, not producing any significant correlation, failed to confirm these suggestive data from occupational studies. In a large Swedish cohort study, an approximate 10% increase in the risk of cancer was documented in people in the medium and high exposure levels. Several types of cancer including skin, digestive, respiratory, reproductive and urinary organs, were linked with occupational magnetic field exposure, suggesting an involvement of the endocrine and immune systems. Discrepancies in epidemiological studies dealing with this matter have involved different estimates of electromagnetic field exposure, measurement and characteristics; the statistical analysis performed with data obtained in such epidemiological reports is another Achilles heel and considerable biases can create misleading conclusions. Higher exposure has been associated with an increase in the cancer risk even though care needs to be taken in drawing any conclusion, because no dose-response relation has been documented so far.

Experimental evidence

In case of high-frequency magnetic fields, biological effects and health risks are related to the thermal effect associated with sources emitting fields high enough to cause a significant temperature rise in living tissue. Carcinogenesis is a multistep process of accumulating mutations and promoting events. It has been proposed that electromagnetic field exposure

might potentiate the effects of other carcinogens, provided that both exposures are chronic. The potential for genotoxicity of electromagnetic fields has been investigated and several negative studies in several exposure categories have presented sound and independent, reproducible data. Using *in vivo* animal models of carcinogenesis the assessment of the potential carcinogenic activity of electromagnetic fields have yielded negative results in different studies, while using the rat mammary carcinoma model results seem to be conflicting. According to available data it is unlikely that long-term exposure to electromagentic fields is carcinogenic per se in animal models. However, a promoting effect in the development of cancer under certain exposure conditions cannot be ruled out. Since exposure conditions vary widely in the different models thus far proposed, independent replication of experimental results is absolutely crucial. Exposure to electromagnetic field, alone or in combination with ionizing radiation, appears to induce an insult at the cellular level; to inhibit DNA synthesis and the growth of human tumor cell lines *in vitro*. However, controversies still exist about the possibility that electromagnetic fields may influence tumor promotion. Different *in vitro* studies have failed to demonstrate any detectable effect of electromagnetic fields on the rate of DNA synthesis and cultured cell growth. Moreover, exposure of cultured mammalian cells to electromagnetic fields has not resulted in the production of detectable DNA lesions and has not affected intracellular ATP levels, suggesting that electromagnetic fields are not genotoxic and cytotoxic. On the other hand, investigating the genotoxic potential of electromagnetic fields using *in vitro* experiments, statistically significant and suggestive positive results have been reported. Following electromagnetic field exposure, enzymatic activity induction, DNA mutation in human and non-human cells, and DNA strand breaks in rat brain cells have been demonstrated. The static magnetic field has been shown to induce a remodelling and differentiation of human neuronal cells in the absence of any alteration of DNA, thus ruling out a direct effect of the magnetic field on DNA stability. Investigating the effects of a static magnetic field on the ability to proliferate of human breast cancer cells *in vitro*, it has been observed that magnetic field exposure only temporarily slows down cellular growth, which then eventually fully recovers. The reduced cell growth caused by the magnetic field could be explained by a temporary effect on some cellular metabolic events leading to the reduced DNA synthesis. Alternatively, it could be ascribed to a transient cellular differentiation, since induction of differentiated phenotype often correlates with decreased cell proliferation. These results are consistent with the observation that magnetic field induces time-dependent developmental effects on the process of differentiation of the chick cerebellar cortex.

Clinical relevance

In conclusion, different studies have yielded information about the apparent lack of a strong association between electromagnetic fields and cancer. Available data from other reports suggest that the exposure to electromagnetic fields brings about a weak, not statistically powerful, increase in the risk estimates of neoplasm. Considering that a weak association is not synonymous with a negligible or negative effect, additional more methodologically rigorous studies are warranted.

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Electroporation

Definition

Electroporation is a method to introduce biological molecules, such as exogenous genetic material (DNA) into cultured cells. During this physical process the membranes of prokaryotic or eukaryotic cells are permeabilized by a short electric pulse, thus permitting the uptake of the molecule(s) of choice into the cell. Electroporation is very effective and provides a good alternative to other chemical or physical methods of that may be ineffective or toxic to particular cell types.

ELISA

Definition

Enzyme-linked immunosorbent assay (ELISA) is a method for detecting proteins in body fluids or cell lysates.

Elongin BC Complex

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Definition

The mammalian elongin BC complex is a heterodimer, composed of the 118 amino acid, ubiquitin-like elongin B protein and the 112 amino acid elongin C protein. The elongin BC complex interacts with multiple proteins, including the transcription factor elongin A, the von Hippel-Lindau (VHL) tumor suppressor protein and members of the the SOCS-box protein family.

Characteristics

The elongin B and C proteins were initially identified as positive regulatory subunits of the 3-subunit elongin complex, which is one of several transcription factors capable of controlling the activity of the RNA polymerase II elongation complex *in vitro*. The elongin B and C proteins were subsequently found to be present in cells as components of a variety of multiprotein complexes, including the VHL tumor suppressor [→ von Hippel-Lindau tumor suppressor gene] and SOCS-box protein complexes. The elongin B and C proteins form a stable elongin BC subcomplex that functions through interaction with a short, degenerate sequence motif, referred to as the 'BC-box' present in elongin A, VHL and SOCS-box proteins. The BC-box motif has the consensus sequence LxxxCxxx(A,I,L,V). Interaction of elongin BC with the BC-box is governed by interaction of the highly conserved leucine, found at the N-terminus of the BC-box with a hydrophobic pocket created by residues in the C-terminal half of elongin C. A short N-terminal elongin C region binds to the N-terminal ubiquitin-like domain of elongin B.

The elongin complex

The 3-subunit elongin complex was originally purified from rat liver nuclei by its ability to activate the overall rate of elongation by RNA polymerase II, by suppressing transient polymerase pausing at many sites within transcribed sequences. The elongin complex is composed of a transcriptionally active A subunit of approximately 770 amino acids and the elongin BC subcomplex. The latter positively regulates the activity of the elongin complex by binding to a BC-box in the elongin A elongation activation domain and potently inducing elongin A transcriptional activity. Elongins B and C perform different functions in regulation of elongin A transcriptional activity. Elongin C functions as the inducing ligand and is capable of binding directly to the BC-

box and maximally activating elongin A transcriptional activity in the absence of elongin B. Elongin B binds to elongin C and promotes stable binding of elongin C to elongin A.

The VHL tumor suppressor complex

The VHL gene on chromosome 3p25.5 is mutated in the majority of sporadic clear cell → renal carcinomas and in the → VHL disease, an autosomal dominant familial cancer syndrome that predisposes affected individuals to a variety of tumors including clear cell renal carcinomas, cerebellar hemangioblastomas and hemangiomas, retinal angiomas and pheochromocytomas. A substantial fraction of VHL mutations found in sporadic clear cell renal carcinomas and in VHL kindreds results in mutation or deletion of the BC-box and disruption of the VHL-elongin BC interaction. Renal carcinoma cells expressing VHL mutants lacking a functional BC-box exhibit remarkably pleiotropic phenotypes, including:

- transcriptional defects or defects in regulation of mRNA stability resulting in misregulation and constitutive expression of hypoxia-inducible genes like vascular endothelial growth factor (VEGF);
- cell-cycle defects resulting at least in part from misregulation of the degradation of the Cdk inhibitor p27;
- defects in ubiquitin-dependent degradation of improperly processed or folded proteins;
- extracellular matrix defects resulting from failure of cells to secrete fibronectin.

Although the exact function of the VHL protein is presently unknown, the VHL complex has associated E3 ubiquitin ligase activity and may therefore function at least in part to control the levels of proteins that participate in these diverse cellular processes. The VHL complex contains, in addition to VHL and elongins B and C, the Cullin family member Cul2 and the RING-H2 finger protein Rbx1 (also referred to as Hrt1 or ROC1). Cul2 and Rbx1 form a stable subcomplex that activates ubiquitination of VHL-bound target proteins by recruiting and activating the E2 ubiquitin conjugating enzymes Ubc5 and Cdc34. Within the VHL complex, the elongin BC subcomplex functions as an adaptor that links VHL to the Cul2/Rbx1 subcomplex; as a consequence, VHL mutations that disrupt the VHL-elongin BC interaction also prevent association of VHL with the Cul2/Rbx1 subcomplex.

Although the repertoire of cellular proteins targetted for ubiquitination and destruction by the VHL complex is presently unknown, recent evidence suggests that the DNA-binding transcriptional activators hypoxia-inducible factors 1 and 2 (HIF1 and HIF2) are among those targets. HIF1 and HIF2 activate transcription of a large number of hypoxia-inducible genes including those, such as VEGF, which are known to be negatively regulated by the VHL protein. The cellular levels of HIF1 and HIF2 are tightly controlled by ubiquitin-dependent proteolysis. Under normoxic cell growth conditions, HIF1 and HIF2 are rapidly ubiquitinated and destroyed by the proteasome. Under hypoxic conditions, ubiquitination of HIF1 and HIF2 is inhibited and their concentrations rise to sufficient levels to activate hypoxia-inducible genes. VHL has been shown to interact with HIF1 and HIF2 *in vitro* and in cells. Furthermore, in cells expressing a variety of VHL mutants that do not bind HIF1 and HIF2 or that lack a functional BC-box and do not assemble into the VHL complex, HIF1 and HIF2 fail to be properly ubiquitinated and destroyed and hypoxia-inducible genes are constitutively expressed. Thus, the VHL complex may target HIF1 and HIF2 for ubiquitination and destruction by the proteasome and, in so doing, may maintain VHL-regulated hypoxia-inducible genes like VEGF in a repressed state under normal cell growth conditions.

SOCS-box proteins and the elongin BC complex

The class of SOCS-box proteins includes the SH2 domain-containing suppressors of cytokine signalling (SOCS) proteins and nearly 30 previously uncharacterized members of the ras, WD-40 repeat, ankyrin repeat, and SPRY domain families. SOCS-box proteins are modular and are composed of an N-terminal

SH2, ras, WD-40 repeat, ankyrin repeat, or SPRY domain and a C-terminal SOCS-box. The SOCS-box is an ~50 amino acid motif composed of an N-terminal consensus BC-box and a short C-terminal L/P-rich region of unknown function. The elongin BC complex interacts *in vitro* and in cells with representative members of each of the SOCS, Ras, WD-40 repeat, ankyrin repeat and SPRY domain families of SOCS-box proteins.

The founding members of the SOCS-box protein family were the SH2 domain-containing SOCS proteins, which are negative regulators of cytokine-induced Jak/STAT signalling. SOCS proteins appear to inhibit phosphorylation and activation of STATs by binding to and inhibiting Jak or receptor tyrosine kinases. The elongin BC complex functions as a component of a multiprotein SOCS-1 complex that attenuates cytokine-induced Jak/STAT signalling by binding to and inhibiting Jak2 kinase. SOCS-1 mutants that do not bind stably to the elongin BC complex are still able to inhibit Jak kinase, indicating that SOCS-1 does not require a functional BC-box for inhibition of kinase. SOCS-1 mutants lacking a functional BC-box do, however, appear to have substantially shorter half-lives than wild type SOCS-1. Thus, the elongin BC complex may control SOCS-1 activity by regulating the level of SOCS-1 protein in cells. The potential importance of properly regulating the cellular levels of SOCS proteins is underscored by evidence that the leptin resistance and consequent obesity of Ay/a mice is due at least in part to inappropriately elevated SOCS-3 levels.

Clinical Relevance

Although mutation of the elongin A or SOCS genes have not yet been identified in human disease, mutation the VHL gene on chromosome 3p25.5 is responsible for the majority of sporadic clear cell renal carcinomas and for VHL disease, an autosomal dominant familial cancer syndrome that predisposes affected individuals to a variety of tumors including clear cell renal carcinomas, cerebellar hemangioblastomas and hemangiomas, retinal angiomas and pheochromocytomas.

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Embryonal Cancer

Definition

Embryonal cancer is a malignant tumour usually occurring in childhood, where the tumour cells resemble their undifferentiated normal counterparts in embryogenesis, with varying degrees of mimicry of structures and cell types seen during normal differentiation. Such tumours usually carry the suffix '-blastoma', e.g. nephroblastoma (→ Wilms tumour), hepatoblastoma, retinoblastoma [→ retinoblastoma, cancer genetics], → neuroblastoma.

End Labeling

Definition

End labeling describes the addition of a radioactively labeled group to one end (5′ or 3′) of a DNA strand.

End Replication Problem

Definition

End replication problem is where all organisms with linear chromosomes show loss of telomeric DNA with each cell division; → telomerase.

Endocrine

Definition

Endocrine means involving hormones. Specialized endocrine cells secrete hormones that travel through the bloodstream to influence target cells which are widely distributed within the body. Each type of endocrine cell secretes a different type of hormone into the blood. The specificity of the response depends entirely on the hormone specific receptors of the target cells.

Estradiol for instance, causes breast epithelial cells to divide at puberty; the pituitary hormone somatotropin (also called growth hormone) indirectly stimulates cell division by inducing liver cells (and perhaps other cells) to divide. This in turn, stimulates the growth and metabolism of muscle and cartilage cells. Hydrophobic molecules, such as steroid hormones, diffuse through the plasma membrane of the cell and activate receptor proteins in the cell cytoplasm. The resulting receptor-hormone complexes migrate into the nucleus where they bind to chromatin and regulate the transcription of specific genes; → estrogenic hormones and cancer.

Endocrine Ablation

Definition

Endocrine ablation is the surgical removal of organs that produce hormones.

Endocytosis

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Synonyms

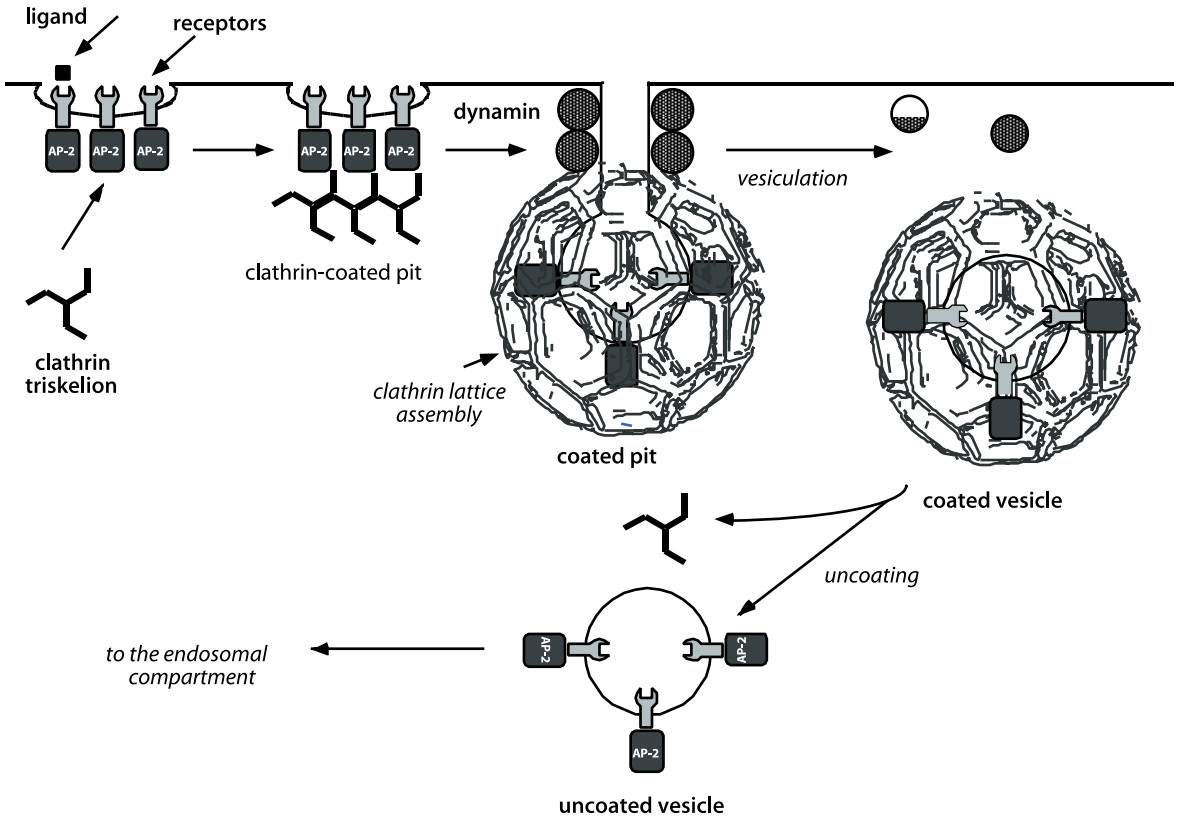
- receptor-mediated endocytosis
- clathrin-mediated endocytosis
- pinocytosis
- fluid phase endocytosis

Definition

Endocytosis is a process in eukaryotic cells consisting of a progressive invagination of a small region of the plasma membrane that is subsequently pinched off to form a cytoplasmic vesicle.

Characteristics

Eukaryotic cells use endocytosis to internalize plasma membrane, surface receptors and their bound ligands, nutrients, bacterial toxins, immunoglobulins, viruses and various extracellular soluble molecules. The molecular machinery of endocytosis is also largely overlapping that of → synaptic vesicle recycling. Once an intracellular vesicle is formed following the endocytic process, its content is trafficked through the → endosomal compartment and normally destined to either degradation in the lysosomal compartment, or to recycling to the cell surface. The definition of endocytosis should be limited to the process leading to the formation of vesicles of 100-200 nm, thus excluding the process known as phagocytosis.



Endocytosis. Fig. – Schematic representation of clathrin-mediated endocytosis. The temporal phases of endocytosis are depicted; from left to right: recrutiment of receptors into pits, clathrin assembly, vesiculation, uncoating and trafficking to the endosomal compartment.

Three different types of endocytic processes are described:

1. *Clathrin-mediated endocytosis* occurs through the formation of → clathrin-coated pits at the plasma membrane, followed by the generation of clathrin-coated vesicles of 100-150 nm in diameter. The process requires, in addition to clathrin, the presence of the adaptor protein complex Ap-2 [→ AP-2]. The formation of clathrin-coated pits at the plasma membrane shares structural and molecular similarities with the budding of clathrin-coated vesicles from the Golgi apparatus, which also requires clathrin and a different adaptor complex, AP-1 [→ AP]. In its simpler form, this process takes the name of pinocytosis or fluid phase endocytosis. This is a non-specific form of endocytosis in which any fluid extracellular material is taken up at a rate that is simply proportional to its concentration in the extracellular fluid. Fluid phase endocytosis does not require a specific interaction of extracellular material with surface-bound receptor structures. Pinocytotic vesicles are normally routed to the lysosomal compartment.
   The specialized form of clathrin-mediated endocytosis, on the other hand, requires interaction of an extracellular ligand with a surface receptor, and takes the name of receptor-mediated endocytosis (or internalization). Two types of receptor-mediated endocytosis are known.
   - In constitutive endocytosis, membrane receptors are continuously internalized and after sorting in the endosomal compartment, they are recycled back to the cell surface. When a ligand binds to the

receptor, the ligand is also internalized and can undergo different metabolic destinies. Two paradigmatic examples are provided by the constitutive endocytosis of the low-density lipoprotein (LDL) receptor and of the transferrin receptor. In the former case, LDL complexed to cholesterol is internalized with its receptor. In the endosomes, the LDL receptor dissociates from the LDL-cholesterol complex and it is re-directed to the cell surface for more cycles of internalization. The LDL-cholesterol complex is routed to the lysosomes, where LDL is degraded and free cholesterol made available to the cell. The cycle of the transferrin receptor is more complex. Transferrin, bound to iron, is also internalized together with its receptor. In the endosomal compartment, the acidic pH causes the dissociation of iron. Iron-free transferrin (apotransferrin) remains, however, bound to the receptor, and it is recycled to the plasma membrane. The process of constitutive internalization is thus used by the cell mostly for the uptake of nutrients.

- In the ligand-regulated process, internalization is triggered by the interaction of a ligand with its surface receptor. Both ligand and receptors are normally routed to the lysosomal compartment with ensuing degradation. The process of induced internalization therefore serves, in the majority of cases, as a down-regulation mechanism to extinguish signals originating on the plasma membrane from signaling receptors, for instance tyrosine kinase receptors.

2. *Caveolae-mediate endocytosis* occurs through flask-shaped, non-clathrin-coated surface structures called caveolae. The vesicles formed in this process are of 50-85 nm in diameter. By this process, cells are able to internalize certain glycosylphosphatidylinositol-linked proteins, albumin, bacterial toxins and MHC I. The molecular details and biological significance of this type of endocytosis are still largely obscure.
3. *Phagocytosis* is the intake of large particles, such as bacteria or parts of broken cells, and should not be considered an authentic endocytic process. It is used by many protozoans to ingest food particles and by blood cells (macrophages) to take in and destroy bacteria. After the binding of the target particle to the cell surface, the plasma membrane expands along the surface of the particle and eventually engulfs it. Vesicles formed by this process are much larger than those formed by endocytosis (1 to 2 μm).

Mechanisms

The structural and regulatory mechanisms of endocytosis (descriptions here will be limited to clathrin-mediated endocytosis) are being elucidated. Four major structural components are required in the formation of an endocytic vesicle (Figure): clathrin, AP-2, receptor tails and →dynamin. Polymerization of clathrin into a hexagonal/pentagonal array forms a cage-like lattice around the internalizing pit and provides the organizing framework of the pit. The AP-2 complex drives the polymerization of clathrin and serves as a recruiter of receptors to the forming pit, due to its ability to simultaneously interact both with receptor intracytoplamic tails and clathrin. Receptor tails contain endocytic codes, which are amino acid sequences capable by themselves of sustaining internalization. Endocytic codes are thought to be cryptic in receptor tyrosine kinases and to be unmasked by conformational changes that follow receptor activation and autophosphorylation. In other types of receptors, such as the transferrin receptor, endocytic codes are probably continuously exposed, thus determining constitutive internalization. In many cases endocytic codes contain a critical tyrosine residue, thus being known as 'tyrosine-based' signals. There is ample evidence that tyrosine-based signals bind directly to the clathrin adaptor protein complex AP-2, thus allowing receptor recruitment into the pit. Once a clathrin-coated pit is formed, dynamin is responsible for its release into the cytosol as a vesicle (vesiculation). Vesiculation is caused by conformational changes of dynamim, which require its intrinsic GTPase activity, and which lead to pinch off of the vesicle from the plasma

membrane. Once a vesicle is formed it appears as a coated vesicle that is still surrounded by a clathrin lattice. Subsequent shedding of clathrin and AP-2 leads to the formation of an uncoated vesicle. Uncoated vesicles fuse with other vesicles in the endosomal compartment, where decisions are made whether to recycle the content of the vesicles (or part of it) to the plasma membrane or to further process it in the lysosome. Several other proteins (AP180/CALM, Epsins, Eps15 and Eps15R, amphiphysins, synaptojanins etc.), globally referred to as accessory or regulatory proteins, participate in the various phases of the endocytic process, frequently entering in contact with a forming pit through AP-2 in a precise hierarchical fashion. Finally post-translational modifications such as phosphorylation, dephosphorylation and monoubiquitination are known to play a role in the various cycles of assembly and disassembly of structural and regulatory endocytic proteins.

Clinical aspects

Pathogenesis of a number of membrane receptor-linked diseases can be directly traced to primary defects in endocytosis. In familial hypercholesterolemia, genetic defects in the low-density lipoprotein receptor result in the absence of cholesterol internalization into the cell. Both receptor-absent and receptor-defective mutants occur. Among the latter, internalization-defective mutants can be caused by nonsense or frameshift mutations or by single amino acid substitutions. In insulin-resistant diabetes mellitus, mutations of the insulin receptor gene occur that fall into various categories, among which those involving accelerated receptor degradation and impaired transport of receptors to the cell surface. Another genetic disease that might be linked to the processes of endocytosis and of vesicle transport is Huntington disease. The disease is associated with increased length of a glutamine stretch located in the NH2-terminal region of the protein huntingtin. Abnormal protein interactions have been proposed as a pathogenetic mechanism. Huntingtin localizes to vesicles of the secretory and endocytic pathway and interacts with proteins involved in vesicle trafficking. Recent data suggest that huntingtin is a regulator of these pathways through association with clathrin-coated vesicles.

A remarkable overlap of molecular events between endocytosis and vesicle recycling in synapses is emerging, with increasing relevance for those conditions of neurological interest in which alterations affecting components of the synaptic vesicles were shown. Among those are the Lambert-Eaton myastenic syndrome, in which synaptotagmin might be a target of autoimmunity; Stiff-Man syndrome, in which amphiphysin and glutamic acid decarboxylase (GAD) (both of which are associated with the cytoplasmic surface of synaptic vesicles) are targets of autoimmunity; and possibly even Alzheimers disease, in which the progressive cognitive loss is associated with synaptic loss in the cortex and decrease in the levels of synaptobrevin and synaptophysin.

Alterations of the processes of endocytosis and intracellular vesicular sorting might play a role in cancer. Translocations are described in leukemia, which involve accessory endocytic proteins. The *Eps15* and *EEN* (extra eleven-nineteen) genes are translocated with the *MLL* (myeloid/lymphoid, or mixed lineage, leukemia) gene, resulting in the production of fusion proteins. The *CALM* gene is rearranged with the *AF-10* (*ALL1* fused gene from chromosome 10) gene, which in turn is a partner in *MLL*-involving translocations. Tumor suppressor and/or cancer predisposition genes are also linked to endocytic/sorting pathways, including *ATM* [→ ATM protein], the cancer predisposition gene mutated in ataxia-teleangectasia, and the two genes *TSC1* and *TSC2*, encoding hamartin and tuberin respectively, identified in tuberous sclerosis.

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Endometriosis

Definition

Endometriosis is a painful chronic disease that occurs when tissue, like that lining the inside of the uterus (the endometrium), is found outside of the uterus.

Endometrium

Definition

The endometrium is the epthelial cells that line the uterus.

Endonucleases

Definition

Endonucleases cleave bonds within a nucleic acid chain; they may be specific for RNA or for single-stranded or double-stranded DNA.

Endoplasmic Reticulum

Definition

The endoplasmic reticulum is a highly convoluted sheet of membranes, extending from the outer layer of the nuclear envelope into the cytoplasm. Together with the Golgi apparatus, are membrane organelles that function to sort and modify those proteins that are either secreted from the cell or become integrated into the plasma membrane.

Endorphin-β

Definition

→ POMC.

Endosomal Compartment

Definition

The endosomal compartment is a complex set of vesicles and tubules, of various sizes, extending from the cell periphery (early endosomes) to the peri-nuclear region (late endosomes). In the endocytic process, the endosomal compartment is the place where the ligand is dissociated from the receptor, and decisions are made about whether to destine the receptor for degradation or to recycle it to the cell surface; → endocytosis.

Endothelium

Definition

Endothelium is a single sheet of highly flattened cells (endothelial cells) that forms the lining of all blood vessels.

Enhancer Element

Definition

An enhancer element is a *cis*-acting sequence that increases the utilization of (some) eukaryotic promoters and can function in either orientation and in any lcoation (upstream or downstream) relative to the promoter.

Environmental Tobacco Smoke

Definition

Environmental tobacco smoke is a composite of sidestream smoke and the smoke exhaled by a smoker. Sidestream smoke is the material released into the air from the burning tip of the cigarette plus the material that diffuses through the paper.

EP300

Definition

E1A binding protein p300 (EP300) is a nuclear protein, a probable transcriptional adaptor required for the activity of certain complex transcriptional regulatory elements. It connects the basal transcriptional machinery to various DNA binding factors and connects this to its tissue specific expression. It may have a function in cell-cycle regulation, by preventing the G0/G1 transition. It binds to and may be involved in the transforming capacity of the → adenovirus E1A protein. The gene maps to 22q13 and is involved in acute myeloid leukemia with translocation t(11;22)(q23;q13).

Ependymoma

Definition

Ependymoma is tumor of ependymal cells; → brain tumors.

Epidemiology of Cancer

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Definition

Cancer epidemiology is the study of the incidence, distribution, and ultimately, the prevention and control of cancer within the general population.

Characteristics

The discipline of cancer epidemiology is a relatively young one, with much of the methodology developed over the past fifty years. Prior to the advent of formal methods of collection and analysis of cancer incidence and risk factor data, associations were generally the result of reports or observations of astute clinicians or scientists. The literature is full of fascinating stories of early attempts at epidemiologic cancer studies such as that of the nineteenth-century physician, Alfred Haviland, who created elaborate maps of cancer deaths in England and Wales using national mortality statistics. One of the first and probably most well-known reports of a relationship between a risk factor and the occurrence of cancer occurred in 1950, with the publication of several case-control studies detailing the association between cigarette smoking and the development of lung cancer.

Study design

There are a number of basic study designs in cancer epidemiology. Descriptive cancer epidemiology examines how cancer incidence and mortality rates vary according to demographic characteristics of the study population such as geographic location, race and sex. One may further analyze such information by categories of age as well as by birth cohort and time period. Ecological studies generally examine aggregate measures of risk and cancer outcome such as median income and cancer incidence across counties of a given state in an effort to identify an association between the two. Alternatively, many epidemiologic studies are based on the use of individuals as the study unit rather than larger groups, or populations of study subjects. Within this category of analysis there are essentially three study designs (with several variations) including the cross-sectional, case/control and cohort study design. Cross-sectional study designs allow for the consideration of a reference population at a given point in time. In a case-control study, the frequency of a particular risk factor among individuals with a cancer of interest (cases) is compared with that among individuals without cancer (controls). Case/control studies have been instrumental in the identification of numerous important cancer risk factors including the association between family history and breast cancer as well as between tobacco and lung can-

cer. In a prospective or cohort study, researchers assemble a cohort of healthy individuals who provide information on risk factors of interest at a baseline point in time. The study subjects are then followed prospectively until they develop cancer or the study is completed. An advantage of this type of study design is that risk factor data is collected before any cancer is diagnosed, thus reducing the amount of recall bias associated with disease status in the reporting of risk factor data such as family history information. There are many well-known examples of cancer cohort studies including the Atomic Bomb Casualty Commission established in 1947 to study the effects of exposure to radiation with outcomes such as leukemia, the British physicians cohort from the 1950s that examined the association between smoking and lung cancer and the Nurse's Health Studies I and II, started in 1976 and 1991, respectively, which have examined a wide range or hormonal and dietary risk factors (among others) in the development of breast and other cancers.

Risk factors

Categories of cancer risk factors are many and include infectious agents, diet and lifestyle factors, endogenous and exogenous hormonal components and genetic factors, to name a few. It is currently popular to divide cancer risk factors into two broad categories defined as genetic and environmental. This has come about because of the many laboratory-based advances in the identification of genetically transmitted or regulated diseases such as cancer, leading to the emergence of a new field of investigation, that is, the genetic epidemiology of cancer. For cancers, a small subset of cases exist that are attributable to rare inherited cancer susceptibility genes. The majority of cases appear to be due to sporadic mutations that may be a result of genetic or environmental events or the result of an interaction between genetic and environmental factors. Much of traditional epidemiologic methodology has been adopted for use in genetic epidemiology. In addition, new methods specific to genetic epidemiology have been developed including the twin, adoption, and pedigree study designs as well as segregation and linkage analyses.

The many advances in genetic testing, as well as in some instances, prevention or treatment options associated with particular cancer diagnoses, has led to a surge of interest in the availability of personalized risk estimates in the clinical setting and hence the development of cancer risk assessment models used to generate these estimates. Cancer risks may be presented in both relative and absolute terms and may define risk for a discrete period of time or over a lifetime. A wide variety of statistical methods exist to estimate cancer risk, with the most fully developed existing in the area of breast and ovarian cancer. Although the concept of risk assessment is not new to the fields of medicine or genetics, the use of detailed genetic information on a large population-based scale is, with all the associated difficulties of presentation and interpretability.

The field of cancer epidemiology is an exciting scientific discipline, which is able to adapt well to new information and technology. New developments in the field include the integration of biomarkers into exposure data, the inclusion of both molecular genetics and environmental risk factor data into study designs in an effort to explore the complex interaction between genotype and the environment, the creation of international data-bases via the Internet, and the merging of large data-bases that combine risk factor information with cancer incidence and mortality data. All of these advances should continue to assist scientists and health care-professionals in the identification of individuals at increased risk of developing cancer so that screening and prevention regimes as well as treatment plans may be developed.

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Epidermal Growth Factor Receptor

Definition

The epidermal growth factor receptor, also known as ERBB, EGFR, ERBB1 and HER-1, is a protein of 1210 aa and 134 kD that mediates the biological signal of → epidermal growth factor (EGF), also of transforming growth factor [→ transforming growth factor β] (TGF). The binding of EGF to EGFR leads to the internalization of the EGF-EGFR complex, induction of the tyrosine kinase activity asociated with EGFR, stimulation of DNA synthesis and cell proliferation. The gene maps to 7p12, and is amplified or rearranged in → glioblastoma multiforme and prostate carcinoma.

Epigenetic

Definition

Epigenetic changes influence or modify the phenotype in addition to the developmental program directed by the genotype. They are phenotypic changes that occur without changes in the genome, i.e. changes in gene expression that are not due to mutation.

Epigenetic Modification

Definition

Epigenetic modifications are inheritable changes that affect the phenotype but not the genotype. → DNA methylation is one example in which the DNA is modified in a way that does not change the DNA sequence but is also inheritable.

Epipodophyllotoxins

Definition

Epipodophyllotoxins are compounds or semi-synthetic derivatives of compounds extracted from the mayapple plant. VM-26 and VP-16 belong to this chemical class of compounds and inhibit topoisomerase II.

Epistaxis

Definition

Epistaxis is profuse bleeding from the nose.

Epithelial Cells

Definition

Epithelial cells form continous sheets covering the surface of the body and lining the internal organs.

EPO

Definition

→ Erythropoietin.

Epoxide Hydrolase

Definition

Epoxide hydrolase is an enzyme that catalyses the *trans*-addition of water to alkene epoxides and arene oxides.

EPR-1

Definition

→ Effector cell protease receptor-1.

Epstein-Barr Virus

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Synonyms

- human Herpes virus 4 (HHV4)

Definition

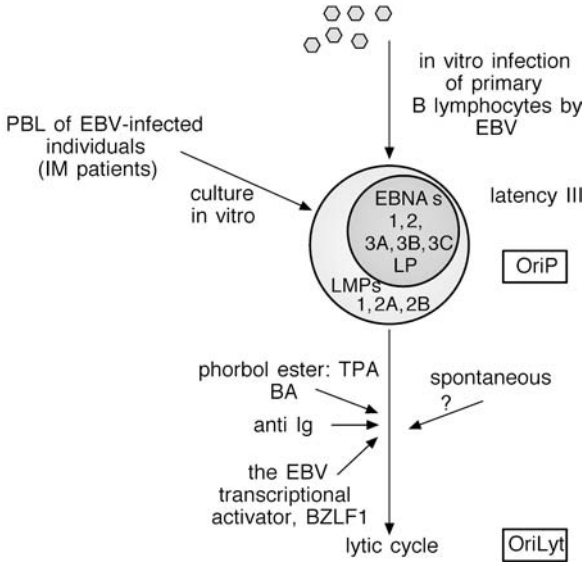
Epstein-Barr Virus (EBV) was the first virus isolated from a human tumour, the Burkitt lymphoma (BL). EBV is a lymphotropic γ-herpesvirus widely spread in the human population: 90 to 95% of adults have antibodies against the virus. In the majority of cases, the primary infection occurs within the first 3 years of life and is asymptomatic. When EBV infection occurs later in life, usually during adolescence, it results in the symptomatic illness known as infectious mononucleosis (IM). Infected individuals carry the virus all their life in a very low number of B lymphoid cells (probably resting B cells), in their peripheral blood and lymphatic organs. Intermittent viral shedding occurs into the saliva due to viral replication in the oropharyngeal lymphoid or epithelial tissues: saliva is the main transmission route of the virus. Since its first discovery in 1964 in a Burkitt's lymphoma tumour, EBV has been found to be associated with several other human maligancies including the undifferentiated nasopharyngeal carcinoma (NPC), Hodgkin disease, rare nasal T cell lymphomas, gastric carcinomas, breast carcinomas and B and T cell lymphomas in immuno-compromised individuals. A characteristic unique to EBV is its capacity to induce the indefinite proliferation or immortalization of quiescent B lymphocytes upon their infection *in vitro*.

Characteristics

The usual consequence of primary infection with EBV *in vivo* is the establishment of a persistent infection. *In vitro*, infection of B cells by EBV is not productive but results in outgrowth of lymphoblastoid cell lines (LCL) carrying EBV. Such cell lines can also be obtained by culture of peripheral blood lymphocytes (PBL) from naturally infected individuals (Fig. 1). The phenotype of LCLs (i.e. morphology and cell surface markers) is very similar to that of antigen activated B cells, and a limited set of viral gene products is expressed: six nuclear proteins (EBNA-1, -2, -3A (or -3), -3B (or -4), -3C (or -6) and -LP (or -5), three membrane proteins (LMP-1, -2A (or TP-1) and -2B (or TP-2) and two small non-polyadenylated nuclear RNAs (EBER-1 and EBER-2). This expression profile defines what is called latency III. In such immortalized cell lines, the viral DNA is maintained as a circular multi-copy episome in the nucleus. Some of these proliferating EBV-infected cells produce infectious virus, but the number of such cells is very low. However, the proportion of cells in which productive replication is occuring can be increased by treatments with various agents such as the phorbol ester TPA, butyric acid (BA) or cross-linking of surface immunoglobulin (Fig. 1). A key mediator of entry into the productive cycle is the viral-encoded transcription factor BZLF1 (also called EB1 and Zta) that activates both transcription of all the EBV early genes and replication from the replication origins (Ori_{lyt}) active during the lytic cycle.

Molecular mechanisms of B cell immortalization by EBV

The EBV genome is a 172 kb double-stranded DNA molecule. During latency III only four transcriptional units are active giving rise to nine viral proteins (Fig. 2). Among these nine proteins only six (EBNA-1, -2, -3A, -3C, -LP and LMP-1) are essential for efficient transformation of B-lymphocytes *in vitro*. These six

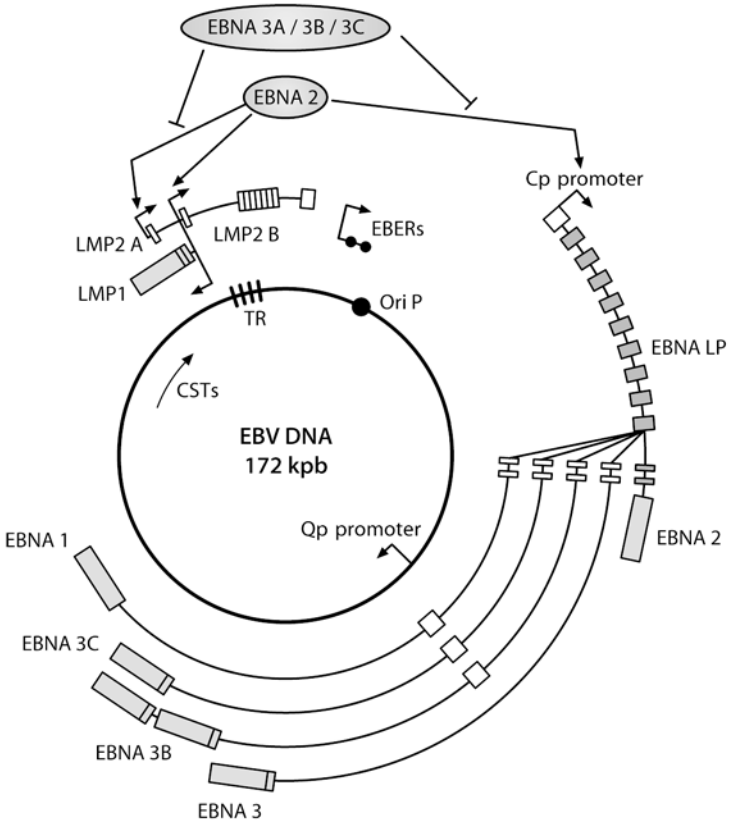


Epstein-Barr Virus. Fig. 1 – Immortalization of B cells by EBV. LCLs can be established by *in vitro* infection of primary B cells with purified virions or by culturing PBL from individuals, infected by EBV (mostly IM patients). These cell lines usually express a set of nine proteins plus two non-polyadenylated RNAs (EBERs) that define latency III. During this latency, the EBV genome is replicated by cellular proteins, together with the cellular genome through a replication origin oriP. A small subpopulation of the cells (which, according to the cell line, vary in number) is permissive to the EBV lytic replication program. This lytic replication depends on viral proteins and is initiated at replication origins (orilyt) that differ from those used during latency. This lytic replication program leads to the amplification of the viral genome, the synthesis of structural viral proteins and the assembly of infectious virus particles. The number of cells, permissive to the lytic replication, can be increased by treating the cells with various chemicals, by crosslinking of the surface immunoglobulins or when expressing the EBV transcriptional activator BZLF1 by transfecting the cells with expression plasmids that carry the *BZLF1* gene. The BZLF1 protein activates both the expression of all the EBV early genes and the replication at the orilyt replication origins.

proteins are thought to cooperate for the initiation and maintenance of B cell proliferation.

- EBNA-1 is a sequence-specific DNA-binding protein that binds to the EBV origin of replication (OriP). This protein is essential for equilibrated segregation of the EBV episomes following replication and thus for the maintenance of a constant viral genome copy number throughout cell divisions. EBNA-1 may have other roles in EBV-induced oncogenesis: EBNA-1 transgenic mice display an increased incidence of B-cell lymphoma.
- EBNA-2 is a transcriptional activator that regulates the expression of all the genes expressed in latency III (Fig. 2) as well as certain cellular genes including *CD21*, *CD23* and *cfgr* (Fig. 3). EBNA-2 does not bind DNA directly but is recruited to EBNA2-responsive elements by the cellular sequence-specific DNA-binding factor RBP-Jκ (also called CBF-1). As RBP-Jκ is part of the Notch signalling pathway, EBNA-2 may mimic part of Notch signal transduction. Although the exact function of EBNA-LP is still unknown, this nuclear factor has been found to cooperate with EBNA-2 for the transcriptional activation of the LMP-1 gene. Furthermore, coexpression of EBNA-2 and EBNA-LP in primary resting B cells previously activated through binding of the EBV glycoprotein gp350 to the EBV receptor CD21, induces Cyclin D2 expression and drives resting B lymphocytes into the G1 phase of the cell cycle.
- EBNA-3A, -3B and -3C are related proteins but only -3A and -3C are essential for B-cell immortalisation by EBV *in vitro*. However, these proteins have at least one common function, repressing EBNA2-activated transcription by directly contacting RBP-Jκ and inhibiting its binding to DNA. Furthermore, EBNA-3C is able to cooperate with activated (Ha)-ras to induce the proliferation of primary rat fibroblasts.

LMP-1 is an integral membrane protein with a short N-terminal cytoplasmic domain, six membrane spanning hydrophobic segments and a long cytoplasmic C-terminal domain. LMP-1 transforms Rat-1 fibroblasts in culture and induces several lymphocyte activation markers. LMP-1 acts as a constitutively active receptor that influences mitogenic signal transduction pathways (Fig. 3). It activates NFκB transcription factor activity through a pathway



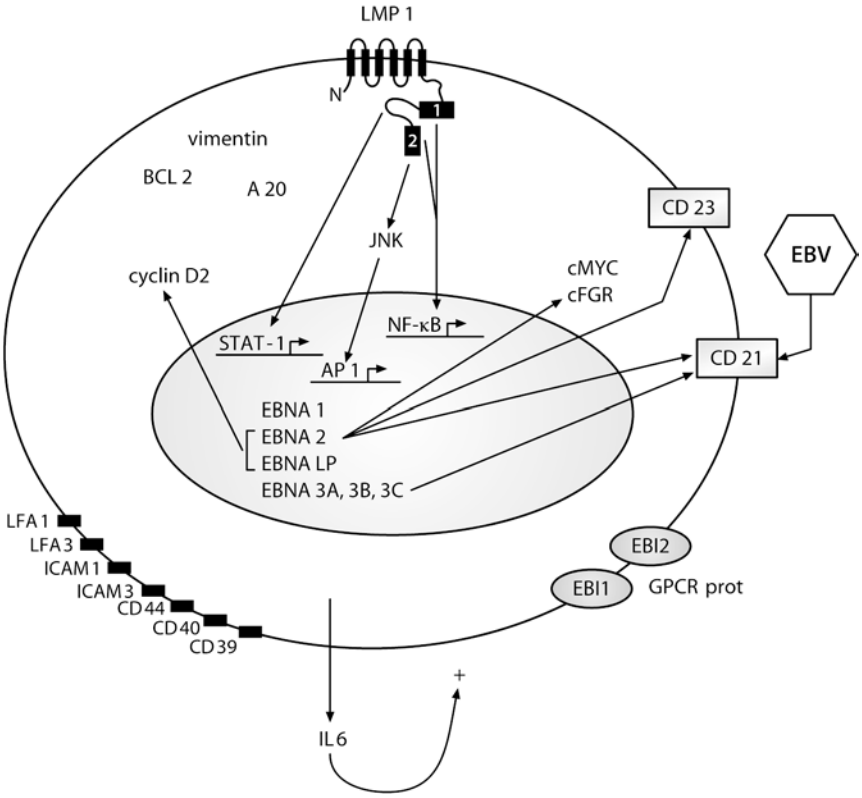
Epstein-Barr Virus. Fig. 2 – Schematic representation of the EBV RNAs expressed in B lymphocytes during latency III. Several precursor RNAs (transcribed by RNA-polymerase II) are initiated at the three different promoters, Cp, LMP-2A and the bidirectional promoter LMP-1/-2B (transcription initiation sites are indicated by arrows). From the precursor RNA, initiated at the Cp promoter, the mRNAs for EBNA-2, -LP, -3A, -3B, -3C and -1 are generated by facultative splicing and all the proteins can be expressed (exons are represented by boxes, introns by thin lines; coding exons are grey shadow boxes, dotted boxes are for EBNA-LP). EBNA-2 then activates the LMP-2A and LMP-1/LMP-2B promoters as well as its own promoter Cp. This activation is counterbalanced by the EBNA-3s, which interfere with EBNA-2 activation, creating an autoregulation loop. The Qp promoter, which is used for expressing EBNA1 in latencies I and II, and antisense Complementary Strand Transcripts (CSTs) are shown. OriP= replication origin used during latency; TR= terminal repeats, a repetitive sequence found at both ends of the linear EBV genome through which the genome is circularized. The EBER genes are transcribed by RNA-polymerase III, their RNAs are nucleolar and are non-polyadenylated.

that involves the recruitment of TNF-RI receptor-associated factors (TRAFs). It also induces AP-1 transcription factor activity via triggering of the c-jun N-terminal kinase (JNK). The STAT-1 transcription factor has also been shown to be a target of LMP1, which induces STAT-1 phosphorylation and thus its subsequent transfer to the nucleus.

The observation that there is an autoregulation loop for EBV genes expressed in infected-B cells, with EBNA-2 stimulating expression from all the latency genes and the EBNA-3s exerting a negative feedback control (Fig. 2), suggests that a very strict regulation on the level of latency III proteins may be essential for the survival of the infected cells and the maintenance of the immortalized phenotype.

Clinical relevance

EBV is associated with several human malignancies both in immuno-compromised and immuno-competent individuals. In immuno-depressed individuals - post-transplant pa-



Epstein-Barr Virus. Fig. 3 – Cellular genes activated by EBV. EBV enters the resting B cell via its receptor, CD21. Virusbinding to the receptor is sufficiant to induce rapid morphological changes and the decondensation of the chromatin. The consecutive expression of the latency EBV genes induces indefinite proliferation of the B lymphocytes *in vitro* and the expression of various cell molecules. The latter include adhesion molecules and activation markers. LMP-1 has an essential role in the induction of many such molecules (ICAM-1, LFA-1, LFA-3, HLA-II, CD21, CD23, CD39, CD40, CD44, etc.) as well as in the down-regulation of CD10. The LMP-1 molecule has six transmembrane domains. The C-terminal domain of LMP-1 is essential for the protein function and contains two effector domains, CTAR1 and CTAR2, indicated in the above figure as 1 and 2. CTAR1 contacts TRAF-1, TRAF-2 and TRAF-3, thus initiating a kinase cascade that mediates the activation of the transcription factor NFκB. CTAR2 induces activation of NFκB and AP-1 (AP-1 being a family of transcription factors which bind DNA as homo- or hetero-dimers). A third pathway, involving a different domain of LMP-1, induces the activation of the STAT-1 transcription factor. Cellular factors such as this one, activate transcription of specific targets. The EBNAs are in the cell nucleus and can activate specific cellular genes either on their own or in cooperation with LMP-1.

tients or AIDS (Acquired Immunodeficiency Syndrome) patients - EBV is probably directly involved in the appearance of immunoblastic B-lymphomas due to the loss of normal cytotoxic T cell surveillance. These lymphomas are monoclonals or polyclonals and the cells usually express the full set of EBV genes found in lymphoblastoid cells proliferating in culture (latency III phenotype).

In immuno-competent individuals, EBV is associated with several cancers. The endemic Burkitt's lymphoma (BL) is found in certain parts of Africa and South America where malaria, which appears to act as a cofactor, is endemic and affects mainly children from 7 to 9 years old. In these regions, BL is associated with EBV in more than 90% of cases. In lower incidence regions the association is only found in 20 to 30% of cases. BL is a monoclonal tumour characterized by a translocation of the c-myc gene to one of the immunoglobulin loci that results in altered regulation of c-myc. The expression of EBV in the tumour cells is limited to EBNA-1 (expressed from a different promoter,

Qp, to that used in latency III, Cp; cf Fig. 2), the EBER RNAs plus several transcripts from the BamHIA region of EBV, also called Complementary Strand Transcripts (CST) (Fig. 2). This profile of expression is defined as latency I. Burkitt's lymphoma cell lines can be readily established from tumour biopsies. Contrary to LCLs, these cells are tumorigenic in nude mice. However, after several passages of these cells in culture the expression profile of the EBV genes has been shown to derive towards a latency III profile.

The undifferentiated nasopharyngeal carcinoma (NPC) is found essentially in restricted areas (particularly Southeast Asia). The viral genome is detected in 100% of these NPC cases. EBV gene expression in NPC epithelial cells consists of EBNA-1, the EBER RNAs and LMP-1, LMP-2A/-2B (in 65% of cases) plus the CSTs. This profile of expression is defined as latency II. Several factors suggest that a reactivation of EBV (i.e. entry into the lytic cycle) precedes or accompanies the development of NPC.

In Hodgkin disease, EBV is present in the Reed-Sternberg cells in about 40% of cases, mostly of the mixed cellularity type. Expression of EBV in Hodgkin disease is also characteristic of latency II. This latency II profile of expression is also found in several other malignancies associated with EBV such as gastric carcinoma, T cell lymphoma and breast carcinoma.

The exact role of EBV in the development of these different tumours is not yet understood and both environmental and genetic cofactors also contribute. However, the fact that the EBV genome is present in the great majority of the cells in the EBV-associated malignancies and the demonstration that the virus was present in the tumour cells at a very early stage, argue for a causative role for EBV in these cancers.

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ERBA2

Definition

→ THRB.

ERBB

Definition

→ Epidermal growth factor receptor.

ERBB1

Definition

→ Epidermal growth factor receptor.

ERBB2

Definition

→ HER-2/neu.

ERBB3

Definition

ERBB3, also known as HER-3, v-erb-B2 avian erythroblastic leukemia viral homolog 3, is a tyrosine kinase-type cell surface receptor of 1342 aa and 148 kD. It belongs to the epidermal

growth factor receptor EGFR family and the gene maps to 12q13.

ERBB4

Definition

ERRBB4, also known as HER-4, is a homolog of the avian erythroblastosis leukemia viral (v-erb-b2) oncogene, protein p180. It is a tyrosine kinase-type cell surface receptor, belonging to the epidermal growth factor receptor family EGFR. The gene maps to 2q33-34.

ERG

Definition

Ets [→ ETS transcription factors] related gene (ERG) is a member of the Ets gene family encoding sequence specific transcription factors. It is the closest relative to the → *Fli-1* gene, and is rearranged with → *EWS* by chromosomal translocation in about 10% of Ewing sarcoma [→ Ewing sarcoma] family tumors.

ERK

Definition

Extracellular signal-regulated kinase. Isoforms are ERK 1 and 2 (→ MAP kinase 1 and 2, or p42 and p44, respectively) and ERK5. ERK 1/2 are phosphorylated at threonine and tyrosine residues by their upstream activator → MEK1/2. Once phosphorylated they can translocate into the nucleus, where they can induce activation of transcription factors, such as → CREB.

ERM Proteins

Definition

ERM refers to the cytoskeletal protein family named after their prototypic members, ezrin, radixin, and moesin. These proteins are thought cross-link the actin cytoskeleton, and to link it to membrane receptors for extracellular matrix molecules in the substratum.

Erythroid Colony Stimulating Factor

Definition

Erythroid colony stimulating factor (ECSA); → erythropoietin.

Erythropoiesis Stimulating Factor

Definition

Erythropoiesis stimulating factor (ESF); → erythropoietin.

Erythropoietin

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Synonyms

- ESF (erythropoiesis stimulating factor)
- ECSA (erythroid colony stimulating activity)

Definition

Erythropoietin (from Greek erythro for red, and poietin to make) is a small glycoprotein hormone that is essential for the production of red blood cells. Erytropoietin (Epo) promotes the survival, proliferation and differentiation of erythroid progenitor cells (CFU-E, BFU-E) to mature erythrocytes and initiates hemoglobin synthesis. Cells responsive to Epo have been identified in adult bone marrow, fetal liver and adult spleen.

Characteristics

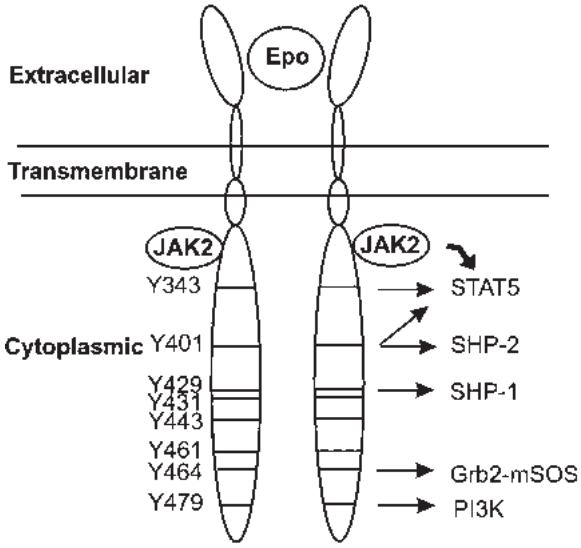
Epo is produced and secreted primarily in adult kidney and fetal liver cells. It is an acidic hormone with a molecular weight of 34-37 kD. Epo is synthesized as a 193 amino acid precursor that is cleaved to yield an active protein of 166 amino acids. It is relatively heat- and pH-stable (pI=4.5), is N-glycosylated at asparagine residues 24, 36 and 83 and O-glycosylated at serine 126. Epo is also sialylated and contains two disulfide bonds at positions 7/161 and 29/33. Glycosylation, which comprises approximately 40% of the molecular mass of Epo, is important to the pharmacokinetic behavior of the protein *in vivo*; non-glycosylated Epo has a very short biological half-life. The DNA sequence of monkey and mouse shows identity at about 90% and 80% to human Epo, respectively.

Cellular and Molecular Regulation

The *Epo* gene, which contains at least five exons, resides on chromosome 7q21-q22 in humans and chromosome 5 in mice. The synthesis of Epo in liver and kidney is induced by anemia, decrease in arterial oxygen tension (hypoxia), hepatic viral infection and exposure to hepatotoxic substances. Transcriptional response of the *Epo* gene to hypoxia is mediated partly by promoter sequences but mainly by a 24 base pair hypoxia-response element located 3′ to *STET*, the human *Epo* gene.

The biological activity of Epo is mediated by specific receptors present at 300 to 3000 copies per cell that undergo phosphorylation in response to Epo. Although pluripotent embryonic stem cells and multipotent hematopoietic cells express the Epo receptor, cells that are committed to non-erythroid lineage cease to express the receptor. The mouse Epo receptor consists of 507 amino acids with an extracellular domain, a single hydrophobic transmembrane domain and a cytoplasmic domain. The human Epo receptor is a 66 kD protein comprised of 508 amino acids. It consists of 8 exons spanning some 6 kb on human chromosome 19p13.3. A point mutation at position 129 of the mouse *Epo* receptor gene results in constitutive activation of the receptor without stimulation with Epo. Mice infected with a retrovirus expressing this aberrant receptor develop erythroleukemia and splenomegaly.

The interaction of Epo with its receptor, a member of the cytokine super-family of receptors, results in the formation of a homodimer and its subsequent internalization (Fig.). Dimerization of the receptor results in the autophosphorylation of Janus kinase 2 (JAK2), a protein kinase that is tightly associated with the Epo-receptor. Once activated, JAK2 phosphorylates eight tyrosine residues located in the cytoplasmic domain of the Epo receptor. Phosphorylation of the Epo-receptor leads to the recruitment and phosphorylation of a number of signal transduction proteins. One such protein is STAT5, a transcription factor that binds to tyrosine 343 and 401 of the Epo-receptor. Once phophorylated, STAT5 translocates to the nucleus to activate the expression of several genes. Binding of Epo to its receptor triggers the activation of several signaling cascades. Examples include phosphatidylinositol 3-kinase (PI3K) that binds to tyrosine 479 and is involved in erythroblast survival, and Grb2 that binds to tyrosine 464 and is involved in the activation of the ras pathway. The Epo-receptor mediated activation of phospholipase A2 and C also leads to the release of membrane phospholipids, the synthesis of diacylglycerol and increase in intracellular calcium levels and pH. Since phosphorylation of the Epo-receptor by Epo is diminished after 30 minutes of stimulation, a number of tyrosine phosphatases have been identified that are involved in attenuating the signal. The tyrosine phosphatase SHP-2 binds to tyrosine 401 of the Epo-receptor and



Erythropoietin. Fig. – Schematic diagram of the Epo Receptor depicting the positions of tyrosine (Y) residues in the cytoplasmic domain and attachment sites of signal transduction proteins such as STAT5, SHP-1 and SHP-2. Binding of Epo to its receptor results in the autophosphorylation and activation of JAK2, which in turn phosphorylates eight tyrosine residues in the cytoplasmic domain of the Epo receptor.

stimulates erythroid proliferation, while SHP-1 binds to tyrosine 429 and inhibits proliferation.

Abnormalities of the Epo receptor do not appear to play a role in the pathogenesis of hematological diseases. However, prolonged activation of STAT5 has been observed in cells transfected with mutant (tyrosine 429) Epo receptor suggesting that → STAT5 DNA binding activity may play a role in the pathogenesis of erythrocytosis.

Clinical relevance

The synthesis of Epo is subject to a complex circuit that links bone marrow and kidney in a feedback loop. Epo synthesis is increased under hypoxic conditions, where the oxygen sensors in the kidney are believed to be a heme protein. The production of Epo is also influenced by other factors such as testosterone, thyroid hormone and growth hormone. The pathophysiological excess of Epo leads to erythrocytosis that is accompanied by increased blood viscosity and may cause heart failure and pulmonary hypertension. Chronic kidney disease causes the destruction of Epo-producing cells resulting in hyporegenerative normochrome normocytic anemias. Epo is therefore clinically used for the treatment of patients with severe kidney insufficiency (hematocrit below 0.3) occurring in approximately 50% of dialysis patients. In uremic patients, prolonged bleeding times have been shown to be improved by Epo treatments, and hemodialysis treatment with recombinant Epo also improves platelet adhesion and aggregation. Hypertony is an important complication in the treatment of renal anemia with Epo. The main reason for insufficient response to recombinant Epo therapy is iron deficiency, which can be overcome by concomitant intravenous iron administration. An important application of Epo is the pre-surgical activation of erythropoiesis allowing for the collection of autologous donor blood. Epo is also used to treat non-renal forms of anemia caused by chronic infections, inflammation, radiation therapy and cytostatic drug treatment.

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ES

Definition

→ Ewing sarcoma is a largely undifferentiated small-round-cell tumor of bone and soft tissue in children and young adults.

ESF

Definition

→ Erythropoiesis stimulating factor.

Esophageal Cancer

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Definition

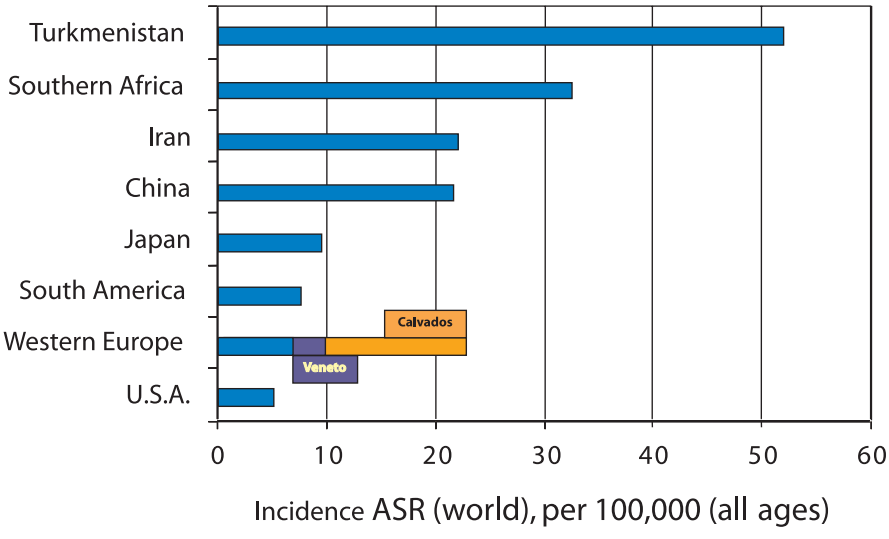
Esophageal cancer comprises two main types of malignant epithelial neoplasms: squamous cell carcinoma, originating from the lining squamous epithelium of the esophagus and adenocarcinoma (also called Barrett's adenocarcinoma), originating from metaplastic columnar epithelium in the lower part of the esophagus.

Characteristics

Esophageal cancer is the sixth most frequent cancer worldwide. In 1996, the estimated number of deaths due to esophageal cancer amounted to about 450 000 out of a total of 7.1 million cancer deaths. Of those, more than 80% occured in developing countries, the majority being squamous cell carcinomas. The occurrence of this cancer varies greatly in different part of the world, with areas of high mortality rate per year in regions of South Africa, North east of Iran and China (30 or more per 100 000 in males and 10 per 100 000 in females). In Europe or USA the age standardised annual mortality of squamous cell carcinoma is no more than 5 in males and 1 in females per 100 000. There are however, areas in Europe, namely in Normandy and Brittany in France and North east of Italy, where the mortality rates, at least in males, are as high as those observed in China.

Adenocarcinoma of the esophagus is less frequent and occurs mainly in industrial countries. However, recent epidemiological data show increasing numbers of adenocarcinoma cases and this type of cancer accounts for more than 50% of all oesophageal cancer in USA.

The 5-year survival rate for patients with squamous cell carcinoma and adenocarcinoma of the esophagus is similarly poor (~10%), with no difference between industrial and develop-



Esophageal Cancer. Fig. – Incidence of esophageal cancer in males by selected world regions [from Parkin et al. (1999)].

ing countries. This is mainly due to their late detection and the poor therapy efficacy. No reliable prognostic markers are available.

Epidemiological studies have clearly shown that tobacco smoke (→ tobacco carcinogenesis) and alcohol, together with a low intake of fresh fruit, vegetables and meat, is causally associated with squamous cell carcinoma. It is estimated that in industrial countries approximately 90% of this cancer is attributable to tobacco and alcohol consumption. Other risk factors are chewing of betel in South East Asia and the consumption of pickled vegetables in China and hot mate drink in South America.

Adenocarcinoma of the esophagus arises from Barrett's esophagus, a condition in which the normal squamous epithelium is replaced by metaplastic columnar epithelium. This condition is frequently present in patients with chronic gastro-esophageal reflux and these patients have a more than 100-fold higher risk than the general population to develop adenocarcinoma.

Squamous cell carcinoma and adenocarcinoma of the esophagus show multiple genetic alterations (point mutations, allelic loss and gene amplification) of several oncogenes and tumour suppressor genes. The most interesting observation in both cancer types, is the high prevalence of mutations (up to 80%) of the tumour suppressor gene p53. In addition a distinct pattern of p53 mutations, namely a high prevalence of G >A transitions at CpG sites in adenocarcinoma and a higher prevalence of G >T tranversions and mutations at A:T base pairs in squamous cell carcinoma. There is good evidence that the mutations in squamous cancer types are attributable to carcinogens present in tobacco smoke. In both types of cancers, p53 mutations occur very early and are followed by the accumulation of other genetic alterations during the process of esophageal carcinogenesis. It is evident that these genetic alterations are relevant not only in the understanding of the multifocal monoclonal origin of this cancer but also to the elucidation of his multifactorial etiology.

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ESR1

Definition

→ Estrogen receptor 1.

EST

Definition

An expressed sequence tag (EST) is a sampling of sequence from a cDNA.

Established Cell Lines

Definition

Established cell lines consist of eukaryotic cells that have been adapted to indefinite growth in culture (they are referred to as being immortalized). Established cells from a cell line can be perpetuated indefinitely. Changes may occur during the process of adaptation to culture, therefore results involving established cell lines may not necessarily mimic the in vivo situation in the organism.

Estrogen Receptor 1

Definition

Estrogen receptor 1 (ESR1), also known as ESR is an intracellular protein with which the estrogenic hormone associates to activate gene expression. Steroids and their receptors are involved in the regulation of eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues; steroid regulation [→ steroid sex hormones]. The estrogen receptor 1 is a nuclear protein of 595 amino acids and 66 kD. The hormone-receptor complex appears to recognize DNA motifs upstream of transcriptional start sites of genes. The gene maps to 6q25.

Estrogenic Hormone

Definition

Estrogenic hormones play a role in both in normal development and the genesis and treatment of several types of cancer. In general, estrogen-related tumors are those involving tissues of the female reproductive tract, although estrogens produce liver cancers in the hamster and are probably a factor in their occurrence in humans; → estrogenic hormones and cancer.

Estrogenic Hormones and Cancer

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Definition

Estrogens are steroid sex hormones produced chiefly in the ovary and responsible for development and function of the female reproductive tissues such as uterus and mammary gland. Smaller amounts are also are produced by the testis in the male and the adrenal gland in both sexes, and contribute to maintenance of bone density and function of cardiovascular and neurological tissues. Estrogenic hormones play a role in both the genesis and treatment of several types of cancer. In general, estrogen-related tumors are those involving tissues of the female reproductive tract, although estrogens produce liver cancers in the hamster and are probably a factor in their occurrence in humans.

Characteristics

Etiology

Carcinogenesis is known to be a multistep process (→ multistep development), involving both initiation (alteration of DNA) and promotion (proliferation of the altered cells). It is generally agreed that the principal effect of estrogenic hormones is on the promotion stage, especially in tissues where growth and function are normally regulated by estrogen. It has been controversial whether estrogens, especially in physiological amounts, also cause genetic changes in a manner similar to the action of chemical carcinogens such as dimethylbenzanthracene or nitrosourea.

Breast cancer. The human malignancy most studied in relation to estrogen is carcinoma of the breast, both because of its high incidence and because its involvement with estrogens is especially striking. Much evidence indicates that estrogenic hormones play an important role in the appearance of mammary cancer, both in experimental animals and in the human. It has long been known that early menarche and/or late menopause increases the risk of breast cancer and that artificial menopause induced by ovariectomy or radiation reduces the risk, suggesting a cumulative effect of the number of ovulatory cycles on the incidence of the disease. It is also known that full-term pregnancy before the age of 20 years confers a significant protective effect, whereas nulliparous women have an increased susceptibility to breast cancer, but the basis of this phenomenon is not clear. The effect of → hormone-replacement therapy has been the subject of much in-

vestigation and some controversy, but from the most recent studies it appears that for breast cancer the risk from unopposed estrogen is small, and the addition of progestin to the regimen makes little difference.

The putative involvement of estrogens in the genesis of → breast cancer has afforded an approach to its prevention that is currently under investigation. The → antiestrogen tamoxifen was shown to prevent both the induction of mammary tumors by dimethylbenzanthracene in the rat and the appearance of cancer in the contralateral breast after → mastectomy in the human. Following this, a large clinical trial has demonstrated that this agent, and related antiestrogens, can lower the incidence of breast cancer in women who are at high risk for developing this disease.

Uterine, cervical and ovarian cancer. Exposure to estrogen, unopposed by progestin, is a major factor in the occurrence of cancer of the uterine → endometrium. Unopposed estrogen-replacement therapy for more than five years results in an elevated risk of endometrial cancer, which persists for several years after the medication has been discontinued. The addition of progestin to the estrogen-replacement regimen substantially reduces this risk. In comparison with cancers of the breast and uterus, involvement of estrogens in the etiology of cervical neoplasia is less clear. Most studies have been of the effect of oral contraceptives and generally have shown some correlation between the prolonged use of these agents and the incidence of cervical cancer. As in the case of breast cancer, late menopause, which gives rise to a longer period of ovulatory activity, results in an increased risk of ovarian cancer. Similarly, pregnancy and the use of oral contraceptives, which decrease the number of ovulatory cycles, are protective.

Vaginal adenocarcinoma. During the period 1945 to 1955, large doses of estrogenic hormones were often administered to pregnant women with a history of miscarriage in the belief that this would protect against spontaneous abortion. Because orally-active steroidal hormones were not available at that time, a synthetic estrogen called diethylstilbestrol (DES) was used. In the early 1970s, a previously rare cancer, clear cell → adenocarcinoma of the vagina, began to appear in the daughters born to the DES-treated mothers, leading to the impression that estrogens in general, and DES in particular, are carcinogens and should not be used for human medication. However, the amounts administered (0.5-1.0 g) were 5,000-10,000 times the hormonally active dose, and the cancers produced were not in those persons receiving the estrogen but in their offspring, indicating that this is an in utero phenomenon and the action of the hormone is better described as → teratogenic than carcinogenic. Longer folllow-up has demonstrated a slightly increased incidence of breast cancer among the DES-treated mothers, but not what might be expected from such high doses of a true carcinogen. Genital abnormalities were produced in the male offspring, in keeping with a teratogenic phenomenon.

Hepatoma. In certain animal species such as the hamster, liver cancer can be induced by the simple administration of estrogens. In the western world, primary liver cancer in humans is a relatively rare phenomenon, except for individuals with cirrhosis. However, it is a major cause of death in Asia and South Africa. The introduction of oral contraceptives has led to an increased incidence of liver tumors after long-term use of preparations containing substantial amounts of estrogen. It has been reported that hepatomas can arise from the use of prolonged DES therapy for prostatic cancer.

Therapy

When cancer occurs in tissues where growth and function depends on estrogenic hormones, in some instances the malignant cells retain their hormone dependency while others lose the need for continued stimulation. It is not clearly established what is the exact basis for hormone dependency, and whether escape from this regulation takes place on neoplastic transformation or during subsequent tumor progression. For cancers that retain hormone dependency, depriving them of estrogen provides an effective palliative treatment, less traumatic than → cytotoxic chemotherapy, which is

the only recourse for the majority of non-hormone-dependent → metastatic cancers.

Breast cancer. Hormone-dependent mammary tumors can be deprived of supporting estrogen either by removing the organs in which the hormone is produced, administration of substances that inhibit estrogen → biosynthesis, or by giving a so-called antiestrogen that prevents the hormone from exerting its growth-stimulating effect in the cancer cells. More than a century ago, before it was known what estrogens are or that they are produced in the ovary, it was found that removal of the ovaries from young women with advanced breast cancer caused remission of the disease in some patients. But the majority of breast cancers occur in post-menopausal women, where the ovaries are no longer functional, and it was long suspected that in the older patient the adrenal glands are the source of supporting estrogen. When cortisone became available, first for the treatment of inflammatory diseases, it became possible to remove the adrenal glands or the pituitary gland which controls them and maintain the patient on → glucocorticoid replacement therapy. Subsequent clinical experience showed that about one-third of all the patients have mammary tumors that undergo remission when deprived of supporting hormone by any of these procedures, and → endocrine ablation became first-line therapy for advanced breast cancer, especially after methods were developed to predict which patients will or will not respond to endocrine manipulation.

When it was demonstrated that estrogens, like steroid hormones in general, exert their physiological actions in combination with specific receptor proteins, it was established that patients whose tumors contain low or neglible amounts of estrogen receptor (ER) rarely respond to any kind of endocrine therapy, whereas most, but not all, patients with ER-rich cancers benefit from such treatment. Determination of → estrogen receptor on excised breast cancer specimens, either by immunological or hormone-binding procedures, is now standard clinical practice.

As an alternative to endocrine ablation, hormone deprivation can be effected by inhibiting the enzymes involved in estrogen biosynthesis. This approach has the advantage that it eliminates not only estrogen arising from the ovary or adrenal gland, but also that which, in some cases, appears to be produced by the tumor itself. The first successful agent for this purpose was aminoglutethimide, which inhibits the key enzyme, → aromatase, but its clinical utility has been limited by undesirable side effects. Several improved compounds have been developed recently including fadrozole, letrozole, vorozole and arimidex, which show promise of increased activity with reduced toxicity.

With the advent of tamoxifen, the first antiestrogen to be tolerated on prolonged administration, this reversible treatment has largely replaced the irreversible endocrine ablation as first-line therapy for ER-rich breast cancers. Although there are side effects from prolonged treatment, as well as a slightly increased risk of endometrial cancer, the benefits greatly outweigh the drawbacks. Tamoxifen and related non-steroidal compounds such as torimifene, raloxifene and droloxifene, show curious pharmacology in that depending on species, tissue and dose they can act either as stimulators or inhibitors. A limitation of tamoxifen therapy is the development in many patients of an 'acquired tamoxifen resistance' in which the medication no longer inhibits but actually stimulates the growth of the cancer. More recently, steroidal antiestrogens such as faslodex (ICI 182,780) and RU 58668 have been developed, which show only inhibitory (antagonist) but not stimulatory (agonist) action.

Uterine and cervical cancer. Because growth and development of the uterus are stimulated by estrogen, attempts have been made to treat endometrial cancer with tamoxifen in a manner analagous to mammary cancer, but the response rate is low and variable. The most widely used hormonal therapy for this malignancy is treatment with progestins. Cervical cancer is especially sensitive to radiation and does not metastasize agressively and so surgery and/or radiotherapy are the usual therapeutic procedures, and endocrine therapy has found little application.

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ET

Synonyms

- Ewing sarcoma family tumor (EFT)

Definition

Ewing tumor (ET), a generic term for → Ewing sarcoma, is a peripheral primitive neuroectodermal tumor and → Askin tumor.

Etiology

Definition

Etiology is the cause of disease.

ETO

Definition

Eight-twenty-one (ETO), also known as myeloid tumor gene-8 is a component of one or more co-repression complexes.

Etoposide

Definition

VP-16; → epipodophyllotoxin.

ETS Transcription Factors

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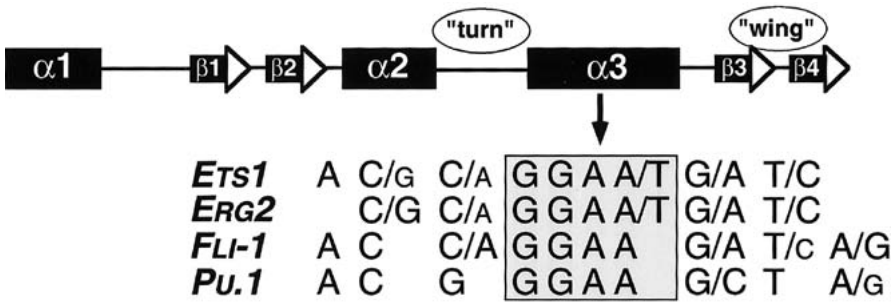
Definition

Ets transcription factors are defined by a unique DNA binding domain, the ETS domain, which specifically interacts with an approximately 10bp long DNA sequence containing a 5′-GGAA/T-3′ core motif. Ets stands for **E**26 **t**ransformation **s**pecific or **E** **t**wenty **s**ix, as the Ets sequence (v-ets) was first identified in the genome of the avian retrovirus E26. c-Ets1, closely related to v-Ets, was the first cellular Ets protein to be discovered. Currently more than 30 different Ets proteins are known, found throughout the metazoan world including mammals, sea urchins, worms and insects. The Ets family is subdivided into subfamilies based on homology within the ETS domain.

Characteristics

Ets proteins bind to DNA as monomers. While the majority of Ets proteins are transcriptional activators, some Ets members (ERF, NET, Tel, Drosophila YAN, Caenorhabditis lin-1) act as repressors. Proteins of the Ets family can interact synergistically with each other or with members of other transcription factor families. Synergism with a particular transcription factor is usually restricted to a subset of Ets proteins that share a specific protein-interaction domain. For example, only the Ets factors of the TCF subfamily and Fli-1 can cooperate with SRF due to the B-domain. Ets1, Ets2 and Drosophila Pointed are the only Ets pro-



ETS Transcription Factors. Fig. 1 – The ETS domain. This winged helix-turn-helix domain binds DNA by a loop-helix-loop scaffold, composed of the helix(α2)-turn-helix(α3) motif and the loops between α2 and α3 ('turn') and between the β strands β3 and β4 ('wing'). All direct contacts with specific bases of the DNA are made by residues in the α3 recognition helix while residues of the two loops contact the phosphate backbone. The resulting neutralization of the phosphate charges is likely to induce DNA bending, as observed in Ets protein-DNA complexes. In contrast to the helices, the loops are not strictly conserved among members of the Ets family. They may, therefore, be responsible for the preference of an individual Ets protein for the sequences flanking the conserved GGAA/T binding motif.

teins that mediate Ras-dependent activation through AP1/Ets composite enhancer elements, as these Ets proteins carry the Pointed domain plus a flanking Ras-dependent MAPK phosphorylation site. Selective partnership provides a means to limit the number of Ets proteins that can regulate a given Ets-responsive gene.

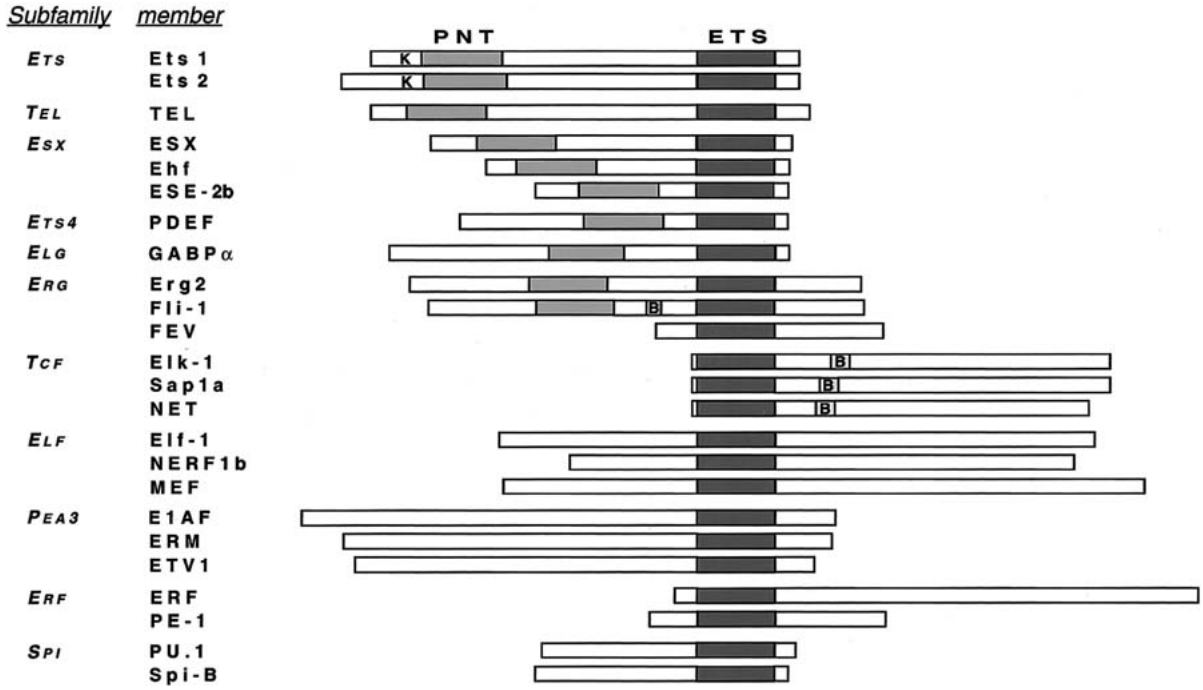
Ets proteins play an important role in transcriptional regulation. Many eukaryotic genes contain Ets DNA binding sites and are responsive to Ets proteins. Ets-responsive genes are found among critical genes that regulate fundamental cellular processes such as proliferation, differentiation, invasion and adhesion. However, in most cases, it is still unknown which member(s) of the Ets family and to what extent Ets proteins contribute to the expression of a given Ets-responsive gene *in vivo*. One of the best-studied Ets proteins is PU.1, which has been demonstrated to be essential for the development of myeloid cells by regulating certain cytokine receptor genes.

Ets factors and development

Some Ets factors, including Ets2 and Tel, are essential for embryonic development. Disruption of the *ets2* or *tel* gene in mice results in early death of the embryo. Ets2-deficient embryos show defects in extraembryonic tissue function that may partly be due to a decreased expression of the Ets2-responsive MMP-9 gene. *Tel* (-/-) mutant embryos fail to develop a vascular network in the yolk sac.

Regulation of Ets protein activities

The activities of Ets proteins are controlled transcriptionally and post-transcriptionally. The expression of many Ets proteins are restricted to certain cell types and/or can be induced by specific extracellular stimuli. Ets proteins are targets of signalling pathways. Many Ets proteins including Ets1, Ets2, TCFs, PEA3 and ERF are phosphorylated in response to Ras resulting either in activation (Ets1, Ets2) or repression (ERF). Calcium- or c-Jun N-terminal kinase kinase-dependent phosphorylation also affect activities of Ets proteins (Ets1, NET). The mechanisms by which the repressors ERF and NET are inhibited by phosphorylation involve nuclear exclusion of these proteins. A unique DNA-binding regulating module has been discovered in the Ets1 protein. A key element of this auto-inhibitory module is a short domain whose structure can be changed from a random coil to an α-helix and vice versa, thereby turning DNA binding on or off. By arresting this molecular switch in either the 'on' or 'off' position Ets1-specific kinases or other Ets1 interacting proteins are able to modulate Ets1 activity.



ETS Transcription Factors. Fig. 2 – Members of the Ets family of transcription factors in humans. The SRF-interacting B-domain, the Pointed (PNT) domain and the Ras-dependent MAPK phosphorylation sites (K) in the Ets1 and Ets2 proteins are marked. Note that although some Ets proteins have several different names, only one name is used here. Splicing variants of the different Ets proteins are not listed. Tel= translocation, Ets, Leukaemia, Esx= epithelial-restricted with serine box, Ehf= Ets homologous factor, ESE= epithelium-specific Ets, PDEF= prostate derived Ets factor, ELG= ets like gene , GABP= GA-binding protein, Erg= ets-related gene, Fli= Friend leukaemia integration, FEV= Fifth Ewing variant, TCF= ternary complex factor, Elk = ets like gene, Sap= SRF accessory protein, NET= new ets transcription factor, Elf= E74-like factor, NERF= new ets related factor, MEF= myeloid elf-1 like factor, PEA3 = polyoma enhancer A3, E1AF= adenovirus E1A factor, ERM= Ets related molecule, ETV= Ets translocation variant, ER81= Ets related clone 81, ERF= Ets2 repressor factor, PE= PU-Ets-related, Spi= SFFV provirus integration site, PU= recognizes purine-rich sequences.

Clinical Relevance

There are several lines of evidence suggesting that Ets proteins contribute to tumorigenesis. First, when over-expressed, some Ets proteins (Ets1, Ets2, Fli-1, Erg) are able to transform murine cells. Of these, Ets1 and Ets2 are targets of Ras-dependent signalling pathways, which may be of significant importance for tumorigenesis as the proto-oncogene Ras is constitutively active in a high percentage of tumors. Second, many genes (e.g. *met*, *upa*, several *mmps*, *pthrp*, *tgf*β-receptor II, *vegf*-receptor I) known to be involved in tumor development and tumor-induced neo-angiogenesis are Ets-responsive. Third, certain Ets proteins are often found over-expressed in tumors, e.g. Ets1 in many invasive tumors. Fourth, Ewing tumors produce Ets fusion proteins (e.g. EWS-Fli-1) that show transforming activity and are therefore suggested to be involved in the development of these tumors. Fifth, certain haematopoietic malignancies express Tel fusion proteins, which are capable of self-associating through the Pointed domain of Tel. In the case of Tel-tyrosine kinase chimeric proteins this oligomerization can lead to a constitutively active protein tyrosine kinase. Tumorigenesis is often accompanied by activation of a tyrosine kinase.

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ETV6

Definition

ETS-variant gene 6, → TEL.

Euchromatin

Definition

Euchromatin comprises all of the genome in the interphase nucleus except for the heterochromatin.

EVI-1

Definition

Ecotropic viral integration site 1 (Evi-1) protein is a nuclear zinc-finger protein involved in leukaemic transformation of hematopoietic cells.

Ewing Sarcoma

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Synonyms

Ewing sarcoma (→ ES) and peripheral primitive neuroectodermal tumor (→ pPNET), also called → neuroepithelioma are currently defined as biologically closely related tumors along a gradient of limited neuroglial differentiation. Askin tumor is the historical designation of Ewing sarcoma of the chest wall. Today, all these neoplasms are summarized as Ewing tumors (→ ET), or Ewing sarcoma family tumors (→ EFT). Although first described in 1866 and 1890 by Lücke and Hildebrand, respectively, the disease carries the name of the American pathologist James Ewing who, in 1921, was the first to recognize the tumor as a separate entity. Despite the historical designation 'sarcoma', there is no evidence that it actually arises from mesenchymal tissue.

Definition

Aggressive small-round-cell tumor affecting bone and soft tissue in children and young adults. The exact histogenesis is not known. However, the current view is that the disease originates from a very primitive, ectodermally derived, migrating cell from the neuroepithelium. On the genetic level, Ewing sarcoma family tumors are defined by the consistent presence of a reciprocal → chromosomal translocation between the long arms of chromosome 22 and either chromosome 11 (85%) or 21 (10%). In rare cases, alternative rearrangements of chromosome 22 with either chromosome 7, 17 or 2 have been reported. These aberrations result in a gene fusion that serves as a diagnostic criterion allowing to discriminate Ewing sarcoma family tumors from osteomyelitis and childhood malignancies with a similar small-round-cell phenotype, including → neuroblastoma, → rhabdomyosarcoma, non-

Hodgkin lymphoma and small cell osteosarcoma. Immunohistochemically, Ewing sarcoma family tumor cells are defined by the abundant presence of the cell surface marker → CD99.

Characteristics

Ewing sarcoma family tumors comprise about 10 to 15% of malignant bone tumors with a yearly incidence of 0.6 per million in the caucasian population. The disease is rarely observed among black Africans and Chinese people. It typically occurs in adolescence (average age at diagnosis is 13.5 years) with a slight male prevalence. Nevertheless, infants less than 5 years of age as well as adults up to 60 years of age, have been diagnosed with Ewing sarcoma family tumors. The tumor usually presents as a painful swelling, rapidly increasing in size. The most frequent localizations are pelvis, the long bones of the extremities, the ribs, the scapula and the vertebrae. Less frequently, Ewing sarcoma family tumors arise from extraosseous locations.

About 20 to 25% of Ewing sarcoma patients present at diagnosis with gross, clinically detectable metastases in the lung and/or in bone and/or bone marrow. In contrast to patients with localized disease, cure of this group of patients is very difficult to achieve.

By means of immunohistochemistry, the tumor cells occasionally stain positive for neuroglial markers, such as neuron specific enolase, S100 protein, chromogranin A and B or the gene product PGP9.5. This is in addition to CD99, which is highly expressed in Ewing sarcoma family tumors with consistency. The inclusion of glycogen can frequently be observed in the tumor cells.

Cytogenetics and gene alterations

Cytogenetically, trisomy 8 and 12 accompany the characteristic rearrangement of chromosome 22 in about 44% and 12% of tumors, respectively. The most consistent marker of Ewing sarcoma family tumors, however, is the rearrangement of the Ewing sarcoma gene → *EWS* on chromosome 22 (band q12) with a gene encoding for an → Ets transcription factor. These proteins are characterized by a unique structure of their DNA binding domain determining target gene specificity. In the majority (85%) of Ewing sarcoma family tumors *EWS* is rearranged with → *Fli-1*, which is located on chromosome 11 (band q24). The second most frequent translocation partner of EWS in this disease is the Ets family member → *ERG* on chromosome 21 (band q22) (10%). These gene rearrangements are currently monitored for diagnostic purposes, either on chromosomal level using fluorescent *in situ* hybridization (FISH) or on RNA level using reverse transcriptase polymerase chain reaction [→ PCR] (→ RT-PCR). The latter method also allows for high sensitivity detection of minimally disseminated disease in blood, bone marrow and peripheral blood progenitor cell (PBPC) collections. The prognostic impact of → RT-PCR detectable tumor cells in these samples is currently under prospective evaluation in several clinical studies.

As a result of the gene fusion a potent novel transcription factor with altered structural and functional features is expressed in the tumor cells. EWS-Fli-1 and EWS-ERG fusion proteins have been shown to render mouse fibroblast cell lines tumorigenic in animal models. Using antagonistic agents, generated by means of gene technology, involvement of these aberrant gene products in Ewing sarcoma tumor cell proliferation has experimentally been demonstrated. It is commonly assumed that the EWS-Ets chimeric transcription factors mediate their transforming properties by inappropriately activating other genes that remain to be defined. Due to variable chromosomal breakpoint locations in the individual tumors the translocation products vary in size. A prognostic impact of certain *EWS* gene fusion types for localized disease has been suggested recently and is currently under prospective evaluation.

Otherwise, no useful genetic indicators of prognosis have been identified for Ewing sarcoma family tumors. Genetic aberrations associated with unfavorable disease in many human malignancies such as mutations of the tumor suppressor gene *p53* and of the *Ras* oncogene are infrequent in Ewing sarcoma family tumors.

Besides the *EWS-Ets* gene rearrangement the only molecularly defined genomic alteration in this group of neoplasms is the homzygous loss of the → *INK4A* gene, occurring in about 30% of primary tumors. The prognostic impact of this aberration remains to be clarified. Thus to date, the extent of disease at the time of diagnosis and the response to initial chemotherapy, constitute the only significant prognostic indicators for the disease.

Aetiology and therapy

The aetiology of Ewing sarcoma family tumors is not known. Neither is there evidence for genetic predisposition nor for a role of environmental exposure. Due to its tight association with the disease, the *EWS-Ets* gene rearrangement is considered the primary event during Ewing sarcoma pathogenesis. No specific recombinogenic activity has been identified as responsible for this aberration and although involvement of a viral agent in generating the chromosomal translocation has been suggested recently, it has not been confirmed.

Patients with Ewing sarcoma family tumors are currently treated by multi-modal therapeutic regimens including radiotherapy and chemotherapy (combinations of vincristin, actinomycin D, cyclophosphamid, doxorubicin, ifosfamide, etoposide) as well as surgical resection wherever possible. By using this treatment strategy together with optimized schedules and dose intensities, the results for patients with localized disease was improved to an overall survival rate of 60 – 70% in recent years. In contrast, the management of primary metastatic disease and early relapse remains a clinical challenge that is currently assessed by myeloablative approaches, combining high-dose chemotherapy and total-body irradiation with stem cell reinfusion. The efficacy of this therapeutic approach for high-risk Ewing sarcoma patients remains to be established.

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EWS

Definition

→ Ewing sarcoma protein, a nuclear RNA-binding protein of unknown function, interacts with calmodulin. The Ewing sarcoma gene *EWS*, identified as a gene on chromosome 22, is consistently disrupted by chromosomal translocation in Ewing sarcoma family tumors.

EWS-FLI (ets) Fusion Transcripts

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Definition

The *EWS-FLI1* fusion transcript is the result of a balanced reciprocal chromosomal translocation between chromosomes 11 and 22, which fuses the *EWS* gene in chromosome 22 to the *FLI1* gene in chromosome 11. This fusion transcript is detected in the Ewing family of tumors (→ Ewing sarcoma) and is considered a tumor-specific molecular rearrangement, therefore useful for diagnosis, prognosis and presumably

for specific therapeutics. The FLI gene is one member of the family of → ETS transcription factors.

Characteristics

Structure of the fusion

Chromosomal translocations result in the genesis of chimeric genes, encoding hybrid transcripts and novel fusion proteins. Many fusion proteins contain juxtaposed functional domains usually found in separate proteins. The EWS-FLI1 fusion protein contains the amino-terminal domain of EWS and the carboxy-terminal region of FLI1. EWS is an RNA binding protein, which is believed to mediate mRNA transcription probably through its interaction with RNA polymerase II complex. Several forms (at least 12 types) of EWS-FLI1 exist because of variations in the location of the EWS and FLI1 genomic breakpoints. They contain different combinations of exons from EWS and FLI1, the most frequent being the fusion of EWS exons 1-7 to FLI1 exon 6 to 9 (type 1) and fusion of EWS exons 1-7 to FLI 1 exon 5 to 9 (type 2).

Properties of the *EWS-FLI1* fusion transcript

EWS is widely expressed in most tissues, and because of the genomic structure of the fusion, the *EWS* promoter drives the expression of *EWS-FLI1*. The amino-terminal domain of EWS, which is included in the fusion, has strong transactivating properties. The *FLI1* gene encodes a member of the → *ETS* transcription factors and its expression is highly restricted to hematopoietic, endothelial and mesodermal cells as well as to neural crest cells. The ETS DNA-binding domain of FLI1 is included in the fusion. The resulting EWS-FLI1 protein is therefore an aberrant transcription factor. The chimeric product EWS-FLI1 can transform some cell lines in culture and can inhibit or activate diverse cellular pathways. For example, EWS-FLI1 protein can suppress transcription of transforming growth factor beta type II receptor gene leading to TGF-β resistance, can activate MFNG, a member of fringe family related to somatic development, and other genes. The action of EWS-FLI1 as a transcription factor is probably related to the cell context in which this fusion is detected. This cellular context is probably influenced by cell type, stage of differentiation and microenvironment, and could include expression of several growth factors, like for example IGF1 and its receptor. On the other hand it is likely that *FLI1* gene is developmentally regulated, being expressed at certain times and places, and this can confer some site and tissue specificity to the transcriptional activity generated by the EWS-FLI1 fusion protein.

EWS and other specific human translocations

EWS can fuse after chromosomal translocation to other genes, including several members of the ETS transcription factor family. In an analogous way to *FLI1*, *EWS* can fuse with the:

- *ERG* gene through a t(21;22),
- ETV gene through a t(7;22),
- E1A-F gene through a t(17;22),
- FEV gene through a t(2;22) chromosomal translocation.

All of these *EWS-ETS* gene fusion transcripts can confer a common tumorigenic phenotype of small round cells and can be found in the Ewing family of tumors. But *EWS* can fuse to other genes and be detected in other tumor types. For example, in desmoplastic small round cell tumor, *EWS* is fused to the tumor suppressor gene → *WT1* through a t(11;22). In clear cell sarcoma of soft tissue *EWS* is fused to *ATF1* through a t(12;22), in myxoid and round cell liposarcoma can be fused to the *CHOP* gene, and in extraskeletal myxoid chondrosarcoma is fused to the *CHN* gene, located in chromosome 9.

Genesis of the translocation

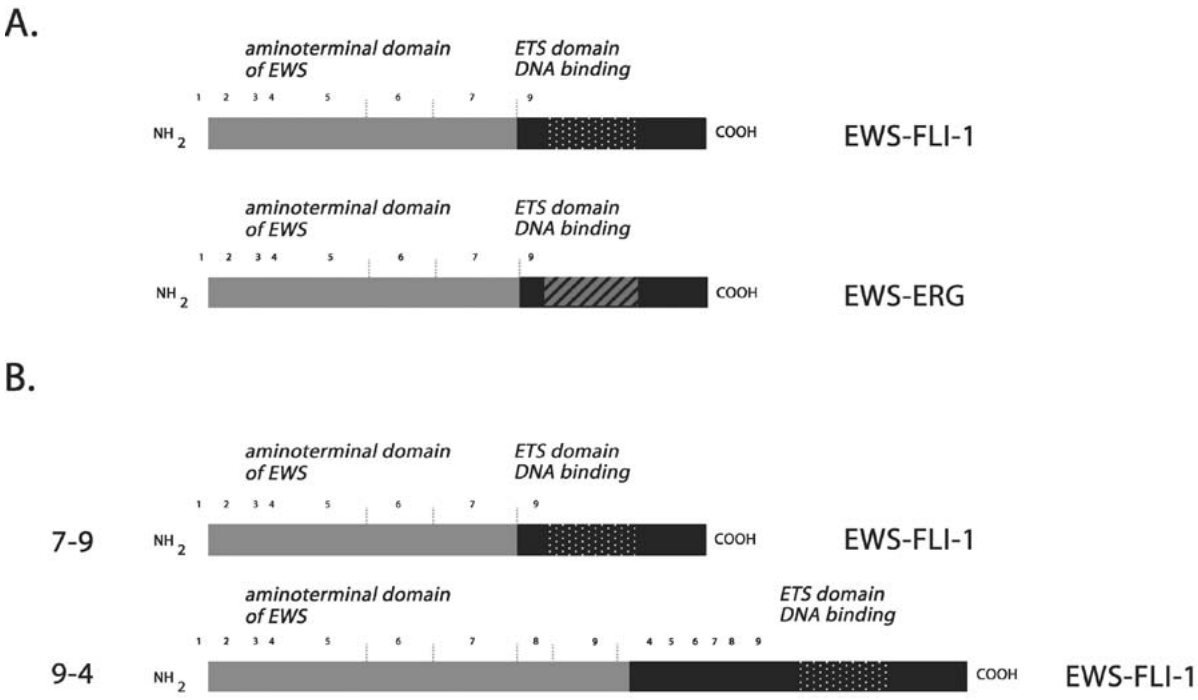
Why do the breakpoints always occur in the same introns? Is this a random event or are there certain areas of these particular genes particularly prone to recombine? There is some evidence that several chromosomal translocations may not be random events, but may

be specifically promoted by the presence of certain DNA sequence motifs at or around certain target genes. A number of recombinogenic sequences have been described, like topoisomerase binding sequences in leukemias or lymphomas, and translin sequences in alveolar rhabdomyosarcoma and in myxoid liposarcoma. Furthermore, it has been suggested that mobile elements or endogenous retroviruses may take part in gene rearrangements. In Ewing tumors, in which illegitimate recombination has been reported to occur, recombinogenic sequences have not been described in a large study of genomic breakpoints. Expression of → adenovirus *E1A* gene in several human cell lines has been associated with the induction of the *EWS-FLI1* transcript, by a mechanism still unknown. Moreover, adenoviral sequences have been found in clinical samples of Ewing tumors.

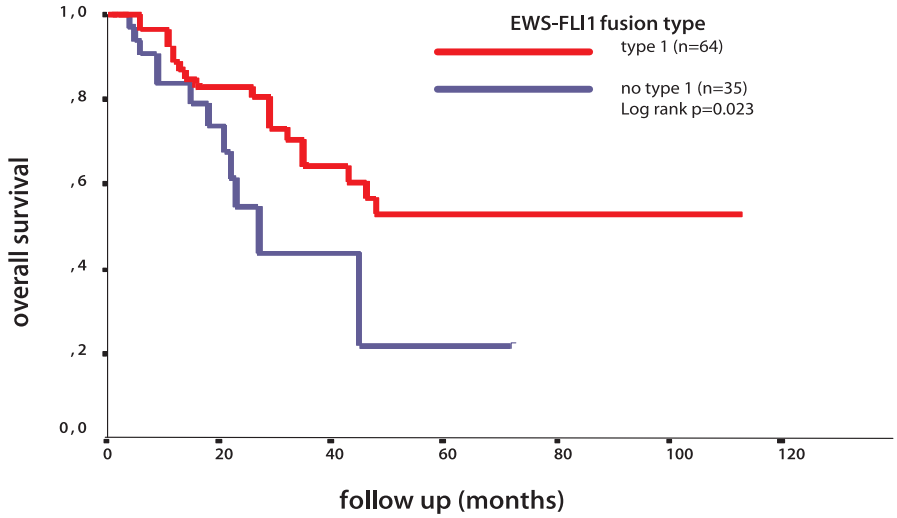
Clinical aspects

EWS-FLI1 (ets) in diagnosis. The detection of the chimeric protein EWS-FLI1 in the morphologic and immunophenotypic context is diagnostic of a Ewing family tumor. The fused product can be studied by → RT-PCR in very small samples of tissue. Frozen tissue is the preferred source of RNA, which can also be extracted from formalin-fixed paraffin-embedded tissue with variable success. EWS-FLI1 detection is particularly useful in cases in which a Ewing family tumor arises in unusual locations (kidney, skin, lung, ovary, pancreas), or in patients over 30 years or shows atypical features (epithelial differentiation, no CD99/MIC2 expression).

EWS-FLI1 (ets) fusion type and prognosis. Ewing tumors display a great molecular heterogeneity (Fig. 1). The variability in the chimeric transcript structure may help to define clinically distinct risk groups of Ewing tumors. In



EWS-FLI (ets) Fusion Transcripts. Fig. 1 – a) EWS-FLI1 or EWS-ERG chimeric protein contains the aminoterminal domain of EWS (gray), joined in-frame to the carboxyl-terminal domain of FLI1 or ERG (in black). The latter contains the DNA-binding ETS domain (white dotted in FLI1, diagonal stripes in ERG). Small size figures represent the exons participating in the fusion. Variability of gene fusions in Ewing tumors depends first of all on the EWS partner. b) For any given fusion gene (EWS-FLI1 fusion is represented here) several possibilities exist depending on the number of exons from both genes that participate in the fusion. The upper part shows the shortest (EWS ex.7-FLI1 ex. 9), and the longest is represented in the lower part (EWS ex.9-FLI1 ex.4).



EWS-FLI (ets) Fusion Transcripts. Fig. 2 – Survival curve of Ewing tumor patients with localized disease (n = 99). Significant differences are seen between patients having tumors with EWS-FLI1 Type 1 fusions (red line) with respect to those with other fusions (blue line) (Log rank test p = 0.023).

fact, two independent groups have found that the type 1 *EWS-FLI1* fusion transcript is associated with less aggressive clinical behavior than the patients carrying tumors with other *EWS-FLI1* fusion types (Fig. 2) regardless stage at diagnosis, tumor location or tumor volume. A recent study has shown that this particular gene fusion (Type 1) encodes for a chimeric protein that functions as a weaker transcription factor than chimeric proteins encoded by other fusion types. Tumors with *EWS-FLI1* type 1 fusions have a lower proliferative rate than their counterparts with other fusion types. So far, no clinical differences have been seen between patients with tumors bearing *EWS-FLI* or *EWS-ERG* fusions.

EWS-FLI1 (ets) and detection of minimal residual disease. EWS-ETS fusion transcripts can be detected by RT-PCR in peripheral blood and bone marrow. This may contribute to patient management and staging, although clinical relevance is still unclear.

EWS-FLI1 (ets) and therapeutics. Efforts are focused on directly inhibiting chimeric proteins (or their downstream targets) and on immunotherapy directed at tumor cell specific epitopes derived from chimeric products.

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Excision Repair

Definition

In excision repair a damaged base is removed by a glycosylase, leaving a 'baseless sugar' or apurinic/apyrimidinic (AP)-site that is acted on by an AP-endonuclease.

Exons

Definition

Exons are the protein coding sequences of genes. Exons only comprise about 10% of the human genome; → introns.

Exonucleases

Definition

Exonucleases cleave nucleotides, one at a time, from the end of a polynucleotide chain. They may be specific for either the 5′ or 3′ end of DNA or RNA.

Expression Vector

Definition

An expression vector is a cloning vector designed so that a coding sequence inserted at a particular site will be transcribed and translated into protein.

Expressivity

Definition

Expressivity is the degree to which an expressed gene produces its effects in an organism.

External Granular Layer

Definition

The external granular layer (EGL) is a secondary transient germinal matrix zone from the rhombic lip, containing vast numbers of densely packed neurons. The EGL disappears in humans during the first two years of life when all external granular cells have migrated inwardly towards the internal germinal layer.

Extracellular Matrix

Definition

The extracellular matrix (ECM) is a meshwork of glycoproteins, proteoglycans and glycosaminoglycans that serves as support for adhesion and growth of cells in coherent tissues. It is the connective tissue that fills up the space between cells, consisting of a network of protein fibers in a polysaccharide matrix. The compounds making up the extracellular matrix are mainly secreted by fibroblasts. The ECM serves as a structural element of tissues to which cells attach through cell-substrate adhesion molecules. Interaction of cells with the extracellular matrix is important for a variety of processes such as adherence, cell migration, differentiation and the regulation of inflammation.

Extracellular Signal Regulated Kinase

Definition

Within the Ras/→ MAP kinase pathway, the extracellular signal regulated kinases 1 and 2 (ERK1/2, MAP kinase1/2 or p42/p44, respectively) can both be phosphorylated at threonine and tyrosine residues by their upstream activator MAP kinase kinase (MEK). Phosphorylated ERKs can translocate to the nucleus where they can induce activation of transcription factors. This can happen either directly (i.e. Elk-1) or indirectly (i.e. → CREB), via the phosphorylation of the MAPKAP kinase RSK2, respectively.

Extramedullary

Definition

Extramedullary means located or taking place outside the bone marrow.

Ezrin

Definition

Ezrin belongs to the family of ezrin-radixin-moiesin (ERM) membrane proteins that mediates attachment of membrane proteins, such as CD44 to the cortical cytoskeleton. It is involved in Rho [→ RHO family proteins] and Rac (small → G proteins) signaling pathways.

F

FADD

Definition

Fas-associating protein with death domain (FADD) is an intracellular mediator of lymphocyte → apoptosis. It is an adaptor molecule in apoptosis that recruits caspase 8 or caspase 10 to the activated → Fas or TNFR-1 receptors. The resulting aggregate is referred to as the death inducing signalling complex (disc), which performs caspase 8 proteolytic activation. Active caspase 8 triggers the subsequent cascade of caspases mediating apoptosis. It is a widely expressed protein of 208 aa and 28 kD, and the gene maps to 11q13.

FAK

Definition

Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase found in focal adhesions of cells. Focal adhesions are structures that anchor the actin cytoskeleton to extracellular matrix. Upon attachment of cells to extracellular matrix proteins, FAK is thought to associate with matrix receptors called integrins and becomes activated. Activation results in autophosphorylation, binding of → Src family members to FAK and tyrosine phosphorylation of multiple proteins contained within focal adhesions. Such phosphorylation regulates the ability of cells to adhere to substrata and migrate.

Familial Cancer

Definition

If a cancer is familiar, it is more common in relatives of an affected individual than in the general population. The level of aggregation is usually measured as a relative risk in affected families as compared to all families. A relative risk of 2.0 implies a two-times higher risk for the offspring of an affected parent.

Breast cancer, for example, is two-times more common in daughters whose mothers had breast cancer. The risks can be measured between parents and offspring, between siblings or between any first-degree relatives.

Familial aggregation is found in all types of cancer. The highest familial risks are observed for thyroid and testicular cancers, with familial risks between 5and 10. Higher than 3.0 are the risks for melanoma, prostate, endometrial and squamous cell skin cancer. The breast and colon cancer the risk is about 2.0.

The reasons for familial cancer are inherited susceptibility. A shared environment and common patterns of behaviour also play a role. Familial aggregation of lung and cervical cancer (relative risk about 2.0) can be partially explained by environmental factors. Inherited cancers are a subgroup of familial cancers where the genetic component is obvious. In many cases the underlying genes have been identified and gene tests are available for some cancer-related genes. Inherited cancers are often monogenic and confer a high risk in those family members who have inherited the defective gene. The frequency of disease is described by penetrance, which is 100% if all carriers of the defective gene contract can-

cer. Inherited cancers include cancers at many sites and syndromes at multiple sites.

FAMMM

Definition

Familial atypical multiple mole-melanoma syndrome (FAMMM); multiple naevi of atypical appearance; → mole; → melanoma.

Fanconi Anemia

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Synonyms

- Fanconi anaemia (British spelling)

Fanconi anemia is not synonymous with Fanconi syndrome, which is an autosomal dominant kidney disease.

Definition

Fanconi anemia (FA) is a hereditary disease characterized by diverse clinical features including multiple somatic abnormalities, stunted growth, reduced fertility and progressive bone marrow failure. FA patients have a markedly increased risk of developing specific malignancies, in particular acute myeloid leukemia and squamous cell carcinomas. Typical somatic abnormalities involve the skeleton (absent thumbs and radii), kidney and heart. There is considerable variability in these features, even among patients within a single family. The combination of spontaneous chromosome instability and proneness to cancer has led to the classification of FA as a 'caretaker gene disease', along with diseases such as → xeroderma pigmentosum, → Bloom syndrome, → Werner syndrome, → ataxia telangiectasia and hereditary non-polyposis colorectal cancer (→ HNPCC). Compared to these other diseases, where the basic molecular functions of the relevant genes is known, FA is exceptional in that the molecular process controlled by FA genes is unclear.

Characteristics

Diagnosis

Cells from FA patients exhibit increased chromosomal fragility and are highly responsive to chromosome breakage induced by a class of compounds known as DNA → cross-linkers (such as mitomycin C, diepoxybutane, and cyclophosphamide). Given the variability of clinical features, a clinically suspected FA diagnosis needs to be confirmed by a laboratory test in which peripheral blood lymphocytes or skin fibroblasts are examined for their sensitivity to chromosomal breakage by a cross-linking agent.

Course of the disease

Most FA patients are diagnosed between 5 and 10 years of age, when blood cell counts are dropping and patients present with anemia (low red cell counts) and/or thrombocytopenia (low platelets). Without treatment these features, which are thought to be due to dysfunctioning bone marrow stem cells, may become fatal. Treatment involves platelet and red cell transfusions as well as medications that stimulate bone marrow function, i.e. androgens and anabolic steroids. Transplantation with bone marrow from a HLA-identical sibling donor is currently the only curative treatment with a reasonable likelihood of success (75%), but because of a lack of suitable donors this option is not available for the majority of patients. Main causes of death for FA patients are bone marrow failure or leukemia (AML). Older patients frequently develop solid tumors (squamous cell carcinomas), particularly in the oral cavity.

Genetics

FA is a rare disease (estimated worldwide prevalence 1-5 per million) with an autosomal re-

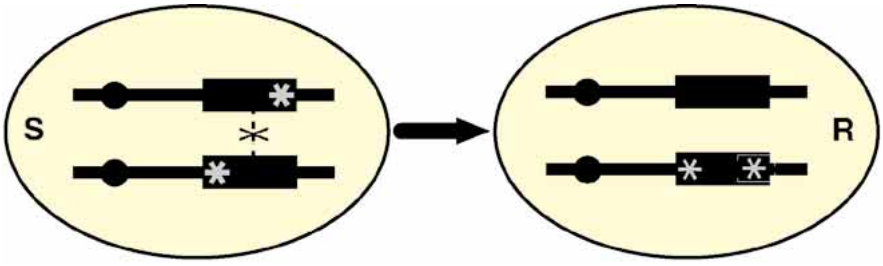
cessive mode of inheritance. This means that the disease gene is on one of the autosomes (non-sex chromosomes) and that both copies of the gene must have an inactivating mutation for the disease to be expressed. Patients inherit a mutated copy from each parent, who themselves are asymptomatic carriers. At least 8 different FA genes have been implicated on the basis of cell fusion studies, four of which have been identified so far (see below).

Cellular phenotype

The single unique feature that characterizes FA cells (by definition, since this is the basis of the FA diagnosis) is their hyper-responsiveness to chromosomal breakage and hypersensitivity to growth inhibition by DNA cross-linking agents. Even without the addition of cross-linkers, FA cells have significant 'spontaneous' chromosomal damage. Therefore FA is also known as a 'chromosomal instability disorder'. The excessive chromosomal damage implies that a cellular mechanism that normally helps to maintain chromosomal integrity is deficient in FA cells. Whether this deficiency is an immediate consequence of the primary molecular defect remains to be established. Many other features have been associated with the cellular FA phenotype, such as spontaneous arrest in the G2 phase of the cell cycle and an increased tendency to undergo apoptosis. All of these features must somehow relate to the primary FA defect, but the precise molecular connections are unknown.

Somatic mosaicism and gene therapy

Some 20% of FA patients are so-called → mosaics. Such patients have a proportion of lymphocytes that have lost their FA phenotype, that is, these lymphocytes behave normally in chromosomal breakage tests. This correction (or reversion) to normal, which must have occurred at some point during the patient's life time, is associated with the appearance of a functional FA gene. Different mechanisms are responsible for this phenomenon such as → mitotic recombination (Fig. 1), and errors made during the DNA replication process that can fortuitously correct minor sequence alterations such as frame-shift or missense mutations. The proportion of reverted lymphocytes in mosaic patients tends to increase over the years and can reach a point where the patient would no longer be diagnosed as FA, since essentially all T lymphocytes (which are used in the chromosomal breakage test) respond as normal. In some, but not all, mosaic FA patients the blood counts are normal or close to normal, suggesting that the genetic correction has occurred in a bone marrow stem cell that has subsequently taken over → hematopoiesis.



Fanconi Anemia. Fig. 1 – Reversion of the FA phenotype by mitotic recombination. The FA cell (MMC-sensitive, S) has two non-overlapping mutations in the *FA* gene. An intragenic DNA exchange between the two mutations results in a cell in which one gene allele has lost its mutation and the other has both mutations. Since one wild type allele is sufficient to correct the FA phenotype, this cell is reverted to normal (MMC-resistant, R). Nevertheless, this scheme is simplified because the mutation exchange occurs more likely during the S phase of the cell cycle when four copies of the gene are present and different genotypes can segregate into the daughter cells.

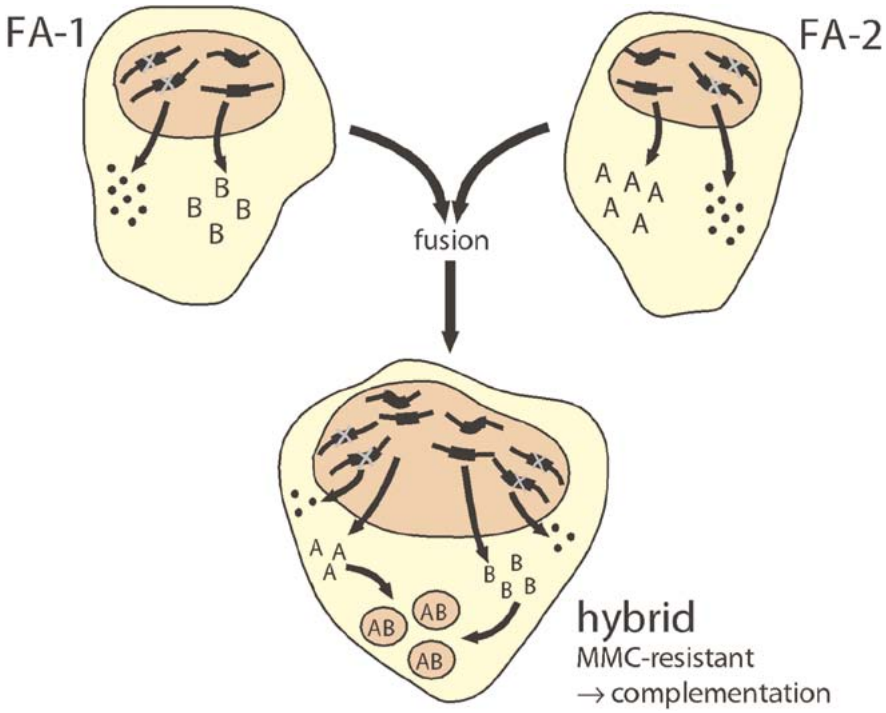
→ Mosaicism in FA may be considered an example of 'natural gene therapy'. Current → gene therapy trials are designed to deliver a functional copy of the defective FA gene into bone marrow stem cells, which are then presumed to have a proliferative advantage over the affected stem cells and eventually could take over proficient hematopoiesis. This situation seems to present itself spontaneously in some mosaic FA patients.

Genetic heterogeneity

Somatic cell hybridization studies have helped to resolve the question whether one or more genes can cause FA. In such experiments lymphoblastoid cell lines established from FA patients are fused to generate → tetraploid hybrid cell lines, which can be analyzed for sensitivity to the growth-inhibiting effect of cross-linking agents such as mitomycin C (MMC) (Fig. 2). If the hybrid cells still express the FA-like hypersensitive phenotype this result indicates that the two patients' cell lines are defective in the same gene. On the other hand, if the hybrid has a resistant (wild type) phenotype, this is taken to indicate that the two cell lines making up the hybrid have defects in different genes. Patients whose cell lines mutually correct (or 'complement') each others hypersensitive phenotype in fusion hybrids belong to different 'complementation groups'. Each complementation group is thought to be connected to a distinct FA gene. Using this type of analysis, 21 FA patients recruited at random from different European countries were grouped into 8 different complementation groups (A-H), predicting the existence of at least 8 distinct FA genes (gene symbols *FANCA* - *FANCH*). Thus far, four of these genes have been identified; *FANCA*, *FANCC*, *FANCF* and *FANCG*. The major gene is *FANCA*, which is mutated in an estimated 65% of all FA patients worldwide. Relative prevalence of complementation groups show marked differences depending on ethnic background. For example, over 80% of FA cases in the Ashkenazi Jewish population are in complementation group C and homozygous for a particular splice site mutation (IVS4+4A>T) in the *FANCC* gene. In The Netherlands most FA patients are also group C, but these patients typically are homozygous for the 322delG frame-shift mutation. In Germany all known complementation groups are represented (group A accounting for 50 – 60%), whereas in Italy only group A patients have so far been found. The high degree of genetic heterogeneity in FA makes molecular diagnosis in individual patients tedious. Moreover, most mutations found in the *FANCA* gene are 'private' (not found in any other patient) and more or less evenly distributed over the 43 exons that make up the coding sequence of the gene.

Fanconi Anemia. Table – The FA genes.

gene	chromosomal location	protein: amino acids	protein: Mol weight [kD]
FANCA	16q24.3	1455	163
FANCB	?	?	?
FANCC	9q22.3	558	63
FANCD	3p22-26	?	?
FANCE	6p21-22	?	?
FANCF	11p15	374	42
FANCG	9p13	622	70
FANCH	?	?	?



Fanconi Anemia. Fig. 2 – Complementation analysis in FA. MMC-sensitive lymphoblastoid cell lines from patients FA-1 and FA-2 are fused to form a tetraploid hybrid cell line. In this example healthy (MMC-resistant) cells are supposed to depend on at least two proteins, A and B, which act in a functional AB complex. Absence of either protein causes an FA phenotype. FA-1 cells have no protein A (but have B), whereas FA-2 cells fail to produce B, but make A. After cell fusion the hybrid cell has both A and B and therefore is 'complemented' for the FA phenotype. In this example patients FA-1 and FA-2 belong to different complementation groups.

The genes

The 4 known FA genes have all been identified by complementation cloning, although FANCA has also been found independently by → positional cloning. Complementation cloning relies on the capacity of a cDNA to correct the MMC-hypersensitive phenotype of a FA cell line that is defective in that particular gene. After → transfection of a FA cell line of defined complementation group with a cDNA expression library and selection for corrected (MMC-resistant) transfectants, the cDNAs recovered are 'candidates' to represent the defective gene. Proof of identity is obtained by demonstrating pathogenic mutations in the 'candidate' gene in the cell line initially used for the transfection and other cell lines assigned to that complementation group. The current situation with respect to the mapping and cloning of the FA genes corresponding to the different complementation groups is shown in the Table.

Genotype-phenotype correlations

An important question is whether differences in clinical phenotype might be correlated with the specific FA gene that is mutated. Although this issue still awaits careful evaluation, it is already known that even within a complementation group patients may present with either mild or severe manifestations of the disease. The type of mutation found in a particular FA gene might therefore have a more important influence on the clinical phenotype than the specific gene affected. Furthermore, ethnic background can affect severity of mutations. For example, the splice site muta-

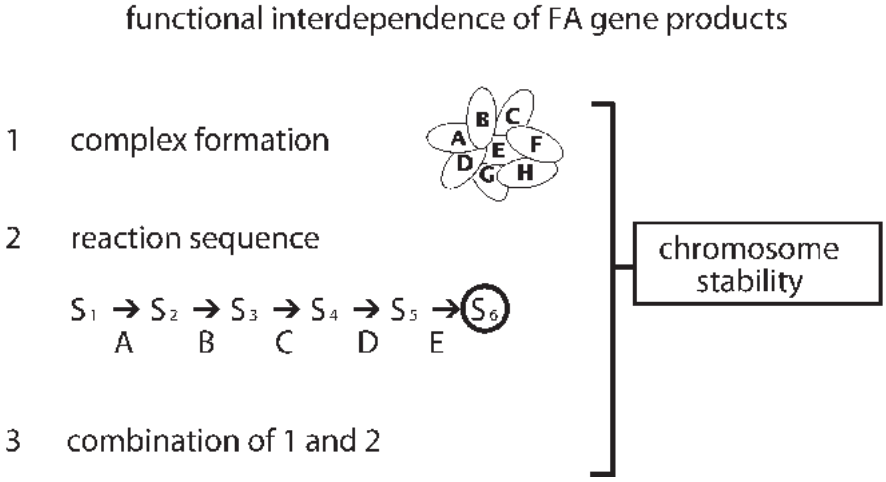
tion IVS4+4A>T in FANCC causes a more severe clinical phenotype in Ashkenazi Jewish FA patients than in Japanese patients.

Knockout mice

Mice have been generated with a targeted disruption of the gene that is homologous to the human *FANCC* gene. These mice, which do not express any functional protein for this gene, are perfectly viable and have no FA-like symptoms except for a marked reduction in fertility. However, FA-like anemia can be induced in these mice by injections with MMC at dosages that do not affect normal mice.

The gene products

FA genes serve an important function in healthy humans, since defects in any of these genes disturb normal development and growth of the individual, affect hematopoiesis, destabilize the genome and facilitate the formation of malignancies. It is somewhat surprising that the proteins encoded by the known FA genes lack homology to any other known proteins, as well as the absence of domains or sequence motifs that could suggest a possible function. Because FA patients share a unique clinical and cellular phenotype, it is plausible that the different FA proteins act in concert to control a specific cellular process and that each individual protein is essential for this process to take place. Mutual interdependence of the FA proteins may involve the formation of functional multi-protein complexes or cascade reactions such as signal transduction, or a combination of these (Fig. 3). Indeed, at least two FA proteins (FANCA and FANCG) are known to form a functional molecular complex. At least some of the other FA proteins are suspected to also participate in this complex formation. Studies using cell fractionation and immunofluorescence have shown that the FANCA and FANCG proteins are predominantly found in the nuclear compartment of cells, while FANCC seems to be present in both the cytoplasm and nucleus. Localization in the nucleus strongly suggests a molecular function for FA proteins that may depend on a direct interaction with DNA or



Fanconi Anemia. Fig. 3 – Ways to envisage 'collaboration' among FA proteins. In view of the similarity of clinical and cellular phenotypes in FA patients of the different complementation groups, the different FA gene products are assumed to function *in concert* to control an integrated pathway that ensures chromosomal stability and MMC resistance. The absence of a single FA protein is sufficient to inactivate the whole pathway. This interdependence of FA proteins may reflect e.g. the formation of a functional multi-protein complex, or a reaction sequence in which one protein catalyzes the formation of a product which is a substrate for another protein in the pathway; interruption of the sequence due to a defect in any of the participating proteins would result in the absence of an essential end product. Although these considerations are largely theoretical, complex formation between the FANCA and FANCG proteins has been demonstrated experimentally.

chromatin. On the other hand, in the absence of any further knowledge, a function of some FA proteins in the cytoplasmic compartment can not be excluded.

The future

FA research faces many challenges, both on the clinical and the fundamental sides. On the clinical side, existing treatment options need to be optimized such as transplantation with bone marrow from unrelated donors, while new methods such as gene therapy are to be developed and implemented. Successfully transplanted FA patients now reach relatively old ages at which the development of solid tumors may become a next life-threatening complication. Mice carrying disrupted FA genes (knockout mice) should be further exploited to serve as experimental models to answer important questions related to pathogenesis and treatment.

The role of FA genes in the origin of specific malignancies both in FA patients and non-FA individuals is poorly understood. This question deserves rigorous investigation, as new clues may be obtained about how such malignancies should be managed more effectively. For an ultimate understanding of the FA defect, the functions of the individual FA proteins and their role in 'the FA pathway' need to be defined in molecular terms. The cloning and characterization of the remaining FA genes undoubtedly will help to unravel this intriguing pathway.

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Farnesyltransferase Inhibitors

Definition

Farnesyltransferase inhibitors have been developed as antagonists of the cytoplasmic farnesyltransferase enzyme. Farnesyltransferase catalyses the addition of the C15-farnesyl isoprenoid lipid onto a variety of proteins such as the → Ras oncoprotein. The inhibition of this enzymatic activity prevents this key posttranslational modification of Ras proteins, rendering the proteins completely inactive. These inhibitors, originally developed as anti-Ras drugs, have shown potent anti-tumor activity in preclinical animal models and are currently being evaluated as anti-cancer drugs.

FAS

Definition

Fas, also known as APT1, tumor necrosis factor receptor superfamily member 6 (TNFRSF6), CD95, APO-1 and FAS1 is a type I membrane protein of about 40 kD. It was originally identified by mouse monoclonal antibody called anti-Fas or anti-APO-1. In the 6th Workshop and conference on Human Leukocyte Differentiation Antigens (Kobe, 1996), Fas was designated as CD95. The gene maps to 10q24 and is mutated in → Autoimmune Lymphoproliferative Syndrome. The protein mediates → apoptosis through sequential activation of → ICE-like caspases (Casp 4, Casp 5, Casp 3) and may also be involved in autoimmune diabetes.

FAS1

Definition

→ Fas.

FAST

Definition

Forkhead activin signal transducer (FAST) is a transcription factor that interacts with a specific DNA sequence called activin responsive element (ARE) through a winged helix motif. This factor needs to interact with the Smad2/Smad4 complex to be fully active.

Fatty Acid Synthase

Definition

Fatty acid synthase (ec 2.3.1.85) is a fatty acid ethyl ester synthase 1 of 2504 amino acids, 273 kD, which is NADPH-dependent. The human FASN or FAS gene locus maps at 17q25 and the mouse fasn locus at chromosome 11 (72.00 cM).

F-box

Definition

F-box proteins are components of → SCF complexes. The F-box proteins are distinguished by the presence of an F-box motif, which mediates their binding to the cullin subunit of the SCF. F-box proteins are the main substrate specificity determinants in SCF complexes. The F stands for cyclin F, where the F-box motif has been originally found, subsequently it was detected in tens of other proteins; → ubiquitination.

FERM Domain

Definition

FERM is name of the protein 4.1 family domain and stands for F (four point one) E (ezrin) R (radixin) M (moesin). It is a unique module involved in the linkage of cytoplasmic proteins to the membrane.

Fermentation Products

Definition

Dietary fibre including resistant starches, complex carbohydrates and cellulose, have been put forward as cancer protective food components. The theory is that dietary fibre may protect against colon cancer through absorption of risk factors and especially through secondary events resulting from the fermentation of carbohydrates by the microflora. These will lead to faecal bulking, increased speed of colon transit, increase of nitrogen metabolism, increased bacterial load in the colon, acidification and finally to the production of short chain fatty acids (SCFA). Butyrate, one of the major SCFA, is considered to be beneficial due to its trophic effects as an essential nutrient to the colon epithelium. Suggested mechanisms of cancer prevention at a cellular level have been reported to be the promotion of differentiation, induction of apoptosis and inhibition of proliferation in colon tumour cell lines. These mechanisms are classified as suppressing activities of cancer preventing agents.

FGF

Definition

→ Fibroblast growth factor.

FHIT

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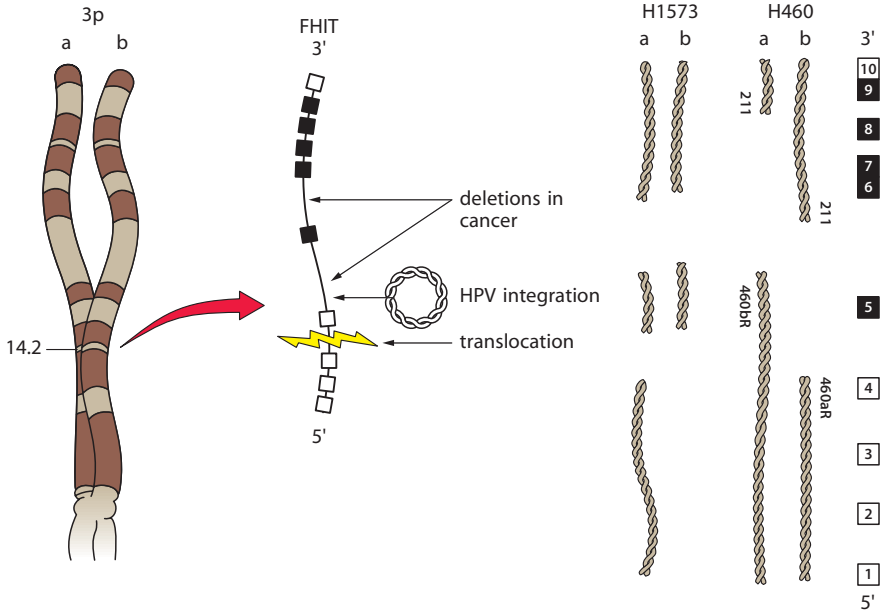
Definition

Fragile Histidine Triad (*FHIT*), a gene at human chromosome arm 3p14.2 that encompasses a familial clear cell → renal cancer translocation breakpoint and the most active chromosomal constitutive → fragile site. The gene is inactivated in a large fraction of most types of human cancers, frequently through biallelic intragenic deletions, probably as a result of carcinogen damage to the fragile region *FRA3B*.

Characteristics

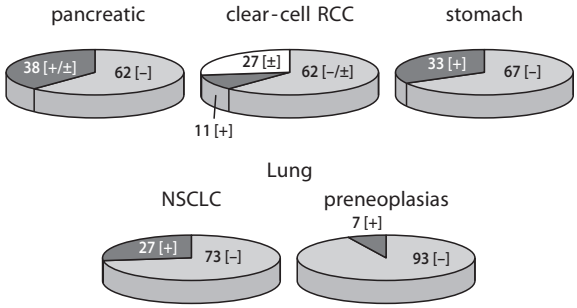
The *FHIT* genomic locus spans more than a megabase of DNA, while the *FHIT* cDNA is 1.1 kb. The amino acid coding region begins in exon 5, ends in exon 9 (Fig. 1) and encodes a protein of 147 amino acids. The Fhit protein is a member of a large Histidine Triad (HIT) gene family of nucleotide binding proteins with four conserved histidine residues, three of which occur in a histidine triad, HxHxHxx (where 'x' is hydrophobic). There are more than thirty HIT genes in species representing all branches of life. Fhit and its orthologs form a closely related subfamily of HIT proteins, found only in eukaryotes, that bind and hydrolyse dinucleoside oligophosphates, especially diadenosine tri or tetraphosphates (Ap3A, Ap4A), to produce a nucleoside monophosphate plus a nucleoside di- or tri-phosphate. Site-directed mutagenesis of the four conserved Fhit histidine residues to asparagine results in decreased enzymatic activity for each mutation, with the largest change occurring with replacement of the central histidine, H96. Thus, H96 is essential for hydrolysis of the diadenosine tri- or tetra-phosphate.

The *FHIT* gene is hemi- or homozygously deleted in the majority of lung, stomach, eso-



FHIT. Fig. 1 – The *FHIT* gene at chromosome region 3p14.2. The gene encompasses *FRA3B* where numerous chromosomal alterations are observed in cancer cells. This locus is highly susceptible in all individuals to carcinogen-induced breakage. As a consequence chromosomal alterations including deletions, insertions and translocations occur. The chromosome alterations frequently lead to loss of parts of the Fhit protein-coding sequence and the consequent loss of Fhit protein expression (on the right are examples of alleles in lung cancer-derived cells).

phageal and kidney cancers and in a large fraction of many other cancers; point mutations are almost never observed. Fhit protein is absent or reduced in lung, stomach, kidney, pancreatic (summarized in Fig. 2) and other cancers, consistent with a role as a tumor suppressor and fulfilling the prediction that fragile site alterations contribute to the neoplastic process.



FHIT. Fig. 2 – Reduction or loss of Fhit protein expression in a high percentage of many human cancer types. The numbers shown in the pie charts represent percentages of primary tumors negative or positive for Fhit protein.

Gene

Fig. 1 illustrates the structure of the gene and the positions of fragile region landmarks such as the familial renal cancer translocation and the → human papillomavirus integration site. The right side of Fig. 1 illustrates the types of biallelic deletions observed in this locus in lung cancer [→ tobacco carcinogenesis] and other cancer-derived cell lines. Sequencing of nearly a megabase of the gene and isolation of cancer cell deletion endpoints has revealed that many deletion endpoints are within recombined LINE-1 repeats, suggesting that carcinogen damage to the fragile region is repaired through recombination between LINE-1 [→ LINE element] sequences flanking the damaged region, with concomitant loss of portions of the *FHIT* gene.

The *FHIT* gene is expressed in most tissues. The protein is cytoplasmic, and is easily visualized by immunohistochemistry in epithelial cells of most organs. The protein is lost early in the development of lung cancer with most dysplastic lesions being negative, as are most non-small cell lung cancers (Fig. 2 and 3).

The mouse *Fhit* locus is also fragile and the mouse Fhit protein is more than 90% identical to human Fhit. One *Fhit* allele was inactivated in murine embryonic stem (ES) cells to create *Fhit* knockout mice in which the gene is functionally inactive. Mice heterozygous and homozygous for the mutant *Fhit* locus are fertile and long-lived.

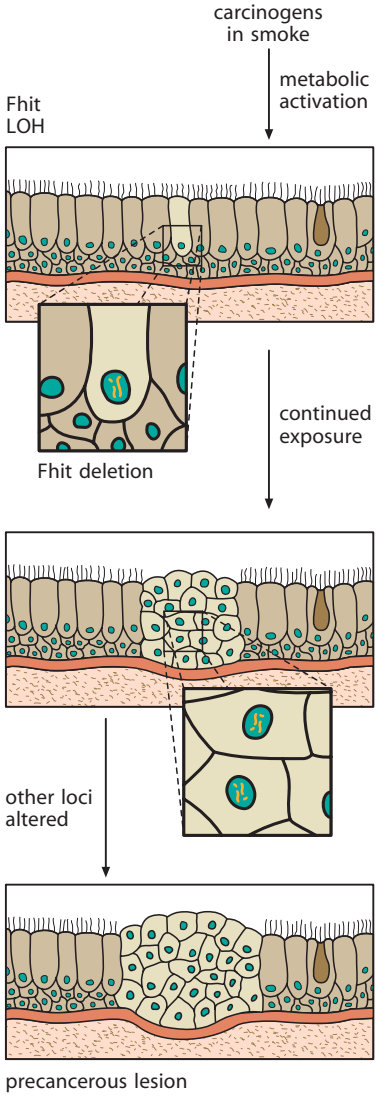
Bioactivity

The biological function of Fhit has been studied in human cancer cell lines stably over-expressing exogenous Fhit. Tumorigenicity was suppressed in some gastric, lung and renal cancer lines but not suppressed in some cervical cancer lines. Similarly, cancer cell lines infected with → adenovirus carrying the *FHIT* gene were killed by → apoptosis. The most likely hypothesis is that Fhit sends a signal for apoptosis in response to various types of cellular stress.

The *Fhit* knockout mice are currently being assessed for susceptibility to spontaneous tumors, but it is already known that mice hemizygous for the mutant *Fhit* (i.e. mice carrying only one wild type *Fhit* allele) are much more susceptible to carcinogen induction of forestomach cancer than wild type litter-mates.

Clinical Relevance

A large fraction of kidney, lung, gastric, colon, pancreatic, breast, cervical and other cancers show absence of expression of Fhit protein, suggesting that its inactivation by carcinogens is critical in the progression of many human cancers, especially cancers of organs directly exposed to environmental carcinogenic agents. Studies of large panels of lung cancers have suggested that Fhit loss is a very early step in lung carcinogenesis (see Fig. 2 and 3). Some studies have suggested that absence of Fhit may correlate with poor outcome in specific types of cancers. But studies of very large tumor series will be important to confirm diagnostic or prognostic indications correlated with Fhit loss. In addition, the *Fhit* knockout mouse strains will be useful in understanding the interaction of fra-



FHIT. Fig. 3 – *FHIT* inactivation in lung epithelial cells. Possible scenario for *FHIT* inactivation early in carcinogenesis as a result of exposure to tobacco smoke is shown. As the initial event in a normal lung epithelial cell, one *FHIT* locus is broken and misrepaired, as observed by the loss of one *FHIT* allele. This leads to the reduction of Fhit protein (indicated by the light brown cells with one broken chromosome 3). The cell with only one active *FHIT* gene has presumably a slight growth advantage compared to surrounding cells. This will result in a clone of dividing, normal-looking lung epithelial cells that are susceptible to breakage of the other *FHIT* locus. When both *FHIT* genes are damaged and inactivated, Fhit protein is completely absent, the cells grow better and proceed to a preneoplastic stage. Such lesions are uniformly negative for Fhit protein expression.

gile regions with carcinogens and in the development of tumor models for mechanistic and therapeutic studies.

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Fibroblast Growth Factor

Definition

Fibroblast growth factor (FGF) is an extracellular polypeptide that binds to and activates cell surface receptors and thereby stimulates cells of mesoderm and neuroectoderm origin to grow, proliferate and/or differentiate. It belongs to a family of angiogenic growth factors that bind to heparin and heparan sulfate.

Fibroids

Definition

→ Uterine leiomyoma.

Fibromyoma

Definition

Leiomyoma [→ uterine leiomyoma, cellular and genetic pathology].

Fibronectin

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Definition

Fibronectin is a large adhesive glycoprotein and a normal constituent of extracellular fluids, extracellular matrices, most basement membranes and certain cell types. It is implicated in a variety of different biological phenomena such as cell adhesion, establishment and maintenance of normal cell morphology, cell migration, differentiation, transformation, haemostasis, thrombosis, wound healing, oncogenic transformation and ontogeny (1, 6).

Characteristics

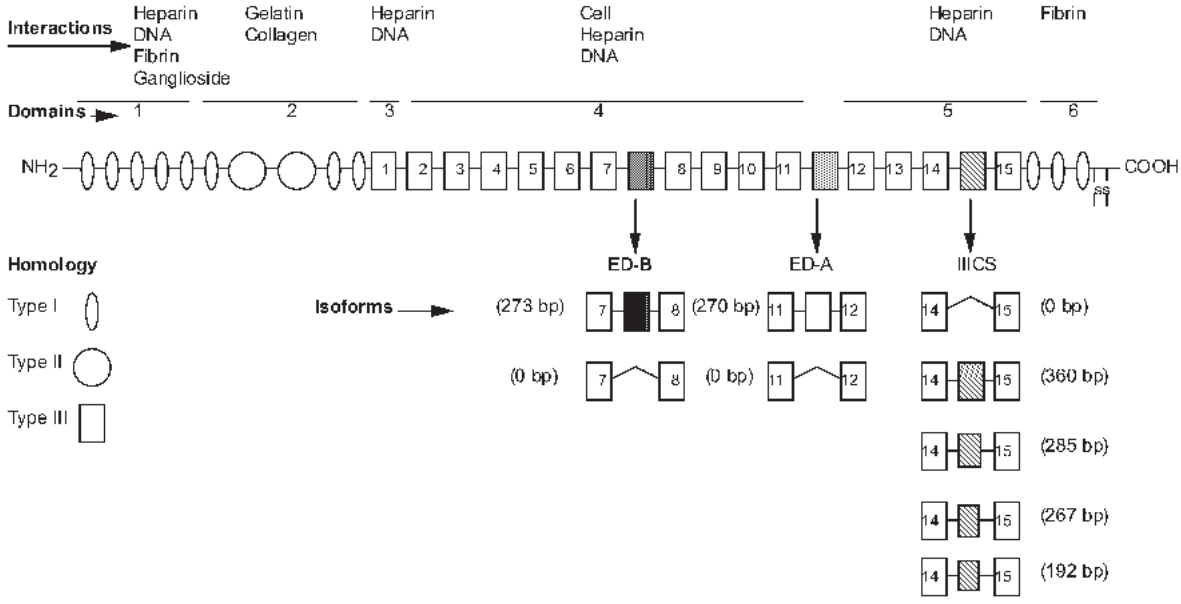
The molecule consists of two similar but non-identical polypeptide subunits of 235-220 kD and 215-210 kD, which are disulphide-linked at the carboxyl terminus. The longer α-chain and shorter β-chain each contain two free SH-groups and 60 cysteine residues, many of which appear to be involved in intrachain disulphide loops. Fibronectin is glycosylated at about 4-6 sites within the protein via arginine (N-linked), which account for 4-5% of the molecular weight of the molecule. Major sugar residues in glycosylation units are mannose, galactose, glucosamine and sialic acid (6).

Both α and β chains consist of similarly ordered globular domains:

- (1) amino-terminus
- (2) gelatin-binding domain
- (3) cell adhesive/attachment domain
- (4) heparin-binding domain
- (5) carboxy-terminus (Fig. 1)

Forms of fibronectin

Cellular fibronectins are secreted by various cell types, such as fibroblasts, epithelial and endothelial cells, chondrocytes, macrophages and platelets, which organise them into extracellular matrices. Cellular fibronectins appear to be more interesting, in the context of neoplastic disease, than plasma fibronectin because their



Fibronectin. Fig. 1 – Fibronectin molecule. Diagrammatic representation of the domain structure of fibronectin. Shown are homology repeats and different sites of alternative splicing (ED-B, ED-A and IIICS), giving rise to different isoforms of fibronectin.

expression is affected by oncogenic transformation.

Functions

Fibronectin is implicated in a variety of different biological phenomena such as cell adhesion via its receptors (Fig. 2) and the establishment and maintenance of normal cell morphology as a component of the extracellular matrix (ECM).

Fibronectin is also implicated in haemostasis and thrombosis. Fibronectin contains binding domains for cells, gelatin (collagen), heparin and some other proteoglycans, DNA, hyaluronic acid and fibrin (6).

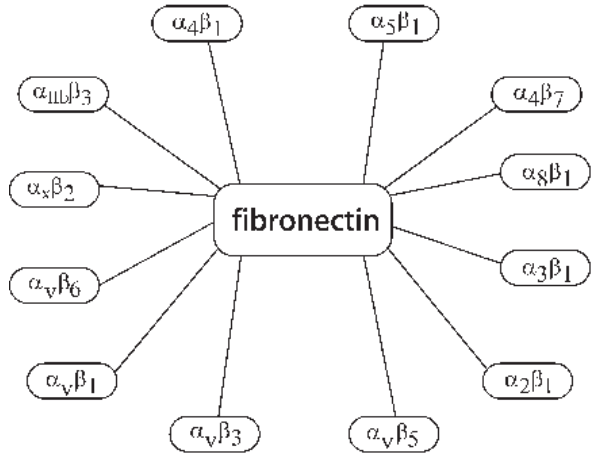
Structure

Each polypeptide chain consists of internal repeats of three types of homologies termed type I, II, and III repeat. There are 12 type I repeats, 2 type II repeats and 15-18 type III repeats (Fig. 1). Each exon of the single gene encodes one type I or type II repeat. Most type III repeats are encoded generally by two exons, but this pattern is altered at three positions that exist as a single exon, and where alternative splicing occurs. Type I repeats are ~40 amino acids long, type II are ~60 and type III are ~90, as originally shown in bovine plasma fibronectin (10).

Fibronectin polymorphism

The diversity observed among fibronectins is due to both alternative splicing of the single primary transcript (12) and due to post-translational modifications.

Post-translational modifications. Fibronectin undergoes glycosylation that appears to stabilise fibronectin molecules against proteolysis. In addition, the degree of glycosylation may influence the interaction of the protein with cells; for instance, higher amounts of glycosylation decrease the avidity for collagen. Other post-transcriptional modifications of fibronectin include phosphorylation and sulphation. Fibronectin is phosphorylated on a serine residue near the carboxy terminus. Certain fibronectin variants are found to be tyrosine-linked sulphated.



Fibronectin. Fig. 2 – Fibronectin interactions with integrins. Different integrins that interact with fibronectin.

Alternative splicing. Alternative splicing is a widespread gene regulation mechanism able to generate diversity in a reversible way and without requiring the expression of new genes. Some eukaryotic genes, such as the gene encoding for tenascin, produce multiple isoforms by alternative mRNA splicing. In general the exon changes caused by alternative mRNA splicing do not produce radically different proteins. Rather, they produce a set of similar proteins called protein isoforms.

Alternative splicing in fibronectin occurs in three regions of the primary transcript and generates 20 different isoforms that differ in the number of internal repeats (12). The regions of variation are: type III constant region (IIICS), extra domain A (ED-A) and extra domain B (ED-B). The same regions in rats are known as V, EIIIA and EIIIB, respectively.

Alternative splicing of fibronectin mRNA is regulated in a cell-, tissue- and developmentally-specific manner. Furthermore, it has been demonstrated that the splicing pattern of fibronectin mRNA is deregulated in transformed cells and in malignancies (2). The fibronectin isoforms containing the IIICS, ED-A and ED-B sequences are expressed more in transformed cells and in tumour tissues than in their normal counterparts. Because of the preferential expression of ED-B and *de novo* glycosylated fibronectin variants in foetal and tumour

tissues, these fibronectin isoforms are known as oncofoetal or embryonic fibronectins.

- IIICS region is situated between the last two type III homology repeats. This region of fibronectin undergoes complex patterns of alternative splicing; it may be totally included, partially included or excluded and may yield up to five different isoforms. IIICS domain is also subject to glycosylation. This fibronectin variant results from the addition of an α-N-acetyl-galactosamine to a threonine residue in a hexapeptide segment (Val-Thr-His-Pro-Gly-Tyr) located in the IIICS domain (8). *De novo* glycosylation of fibronectin is associated with cellular immaturity, cancer formation and the malignancy of breast, gastric and oral carcinomas.
- ED-A is situated between 11^{th} and 12^{th} type III homology repeats and it is the second region of alternative splicing. It is a single exon that is either included or omitted from the mature mRNA (12). This variation is tissue specific, and ED-A is not found in the mRNA of liver where plasma fibronectin is synthesised. In human adult tissues, ED-A fibronectin has a restricted expression in certain tissues such as renal and colonic mucosa (11). ED-A is also present in normal adult myocardium where it is deposited as spots in the interstitium.
- The third region of variation is ED-B that is localised in the middle of the cell binding domain 4, between 7^{th} and 8^{th} type III homology repeats. ED-B is itself a complete type III homology repeat composed of 91 amino acids and coded by a single exon, which is either included or omitted from the mature mRNA (12). ED-B is the most conserved fibronectin region with 100% and 96% homology with rat and chicken fibronectin, respectively. ED-B containing fibronectin, with very few exceptions (superficial synovial cells, intima of some vessels and areas of interstitium of ovary, functional layer of endometrium during the proliferative phase and isolated areas of basement membrane of celomic epithelium), is reported to be absent in normal tissues (12). It has a greater expression in the intima of vessels of foetal brain cortex, stomach, jejunum, thymus and lungs of 8-12 week old foetuses and tumour tissues as established by immunohistochemistry using the monoclonal antibody BC-1 (specific for ED-B containing fibronectin). In tissues from older foetuses (20-22 week old) only basal portions of gastric and duodenal glands were found positive for ED-B fibronectin (12). This suggests that ED-B fibronectin undergoes a programmed expression during ontogenesis.

Biological significance of ED-B

The biological significance of ED-B is unknown. Its proximity to the cell-binding region suggests a role in cell adhesion and migration. This is also supported by the expression of ED-B in foetal tissues and during angiogenesis. In both the cases, cell migration and interactions are required. So far, no binding peptide is located to the ED-B repeat. But the ED-B domain seems to enhance cell adhesion and spreading (5). This adhesion effect of the ED-B may be mediated via conformational changes. It has been reported that insertion of the ED-B in fibronectin causes conformational changes in the molecule (3). The conformational changes induced by the ED-B in the downstream segment of the fibronectin may improve the access to the integrin binding sites in the 9^{th} and 10^{th} type III repeats. It has been shown that ED-B domain with its neighbouring type III repeats is important in promoting cell adhesion(4).

Fibronectin isoforms in disease

Expression of fibronectin isoforms differs in disease. Although the expression of ED-A, ED-B and *de novo* glycosylated fibronectin is not disease specific, it is correlated with tissue modulation processes and particularly with connective tissue formation (fibroplasia).

Dilated cardiomyopathy (DCM) is a heart muscle disease in which alterations to cardiomyocytes result in changes in the composition of the ECM, including increase in the deposition of fibronectin, laminin and collagens in the intercellular spaces and in the vicinity of blood vessels. In patients with DCM, ED-A is

detected as spots in the interstitium like in normal hearts. In contrast, ED-B and *de novo* glycosylated fibronectin, which are not seen in normal adult myocardium, are expressed in DCM. Re-expression of the ED-B and *de novo* glycosylated fibronectin in the adult heart was also found in myocardial hypertrophy in the rat. ED-A is synthesised by the synovial lining fibroblast-like (type B) cells. The expression of ED-A seems to be correlated with activated or transformed states of synovium. *De novo* glycosylated fibronectin was present in the synovial fluid of patients with rheumatoid arthritis. Patients with osteoarthritis also produce the same variant of fibronectin although at lower levels. *De novo* glycosylated fibronectin was also proposed as a predictor of preterm birth. High levels of this fibronectin variant were detected in the cervicovaginal secretion of women during the second and third trimesters of pregnancy.

Fibronectin isoforms and cancer

Fibronectin isoforms are detectable at higher levels in tumours, whereas they are least or not at all expressed in normal adult tissues. In pituitary adenomas the presence of ED-A and ED-B has been reported. They were localised in the adenoma neovasculature, especially in the endothelium and smooth-muscle layers of the vessel walls. Expression of ED-A and ED-B fibronectins was also shown in human colorectal carcinomas and in tumours derived from rat colon carcinoma (11). ED-A labelling was detected in all the samples of human carcinomas in the stroma separating the tumour glands but also in normal colon stroma. Apart from ED-A and ED-B, the glycosylated isoform of fibronectin is also expressed in colorectal cancer but is absent from the normal tissue. Increased expression of ED-B fibronectin mRNA was observed in different types of lung cancer including adenocarcinoma, squamous cell carcinoma, small cell carcinoma and large cell carcinoma. These results suggest that in lung cancer alternative splicing of fibronectin mRNA in ED-B domain occurs irrespective of type of cancer or degree of differentiation. In liver cancer both ED-A and ED-B mRNAs are increased in malignant tumours whereas ED-B mRNA was also detected in benign neoplasms. Natali and co-workers tested primary and metastatic melanomas for ED-A and ED-B fibronectins (9). While both isoforms were detected in a small portion of primary tumours, the metastases were consistently expressing only ED-A.

In 97 cases of invasive ductal carcinomas of breast studied by Kaczmarek and colleagues (7), 93% and 99% were expressing ED-B and the glycosylated isoforms of fibronectin, respectively. The two fibronectin variants were localised in the tumour stroma, tumour vessels and in connective tissue but their distribution differed. In blood vessels ED-B predominated whereas the glycosylated isoform was mostly detected in the tumour stroma.

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Field Cancerization

Definition

Field cancerization is where a large field of oral epithelium undergoes preneoplastic changes as a result of prolonged exposure to mutagenic agents. This fundamental general mucosal disturbance may lead to the eventual occurrence of multiple, independent tumor foci.

Field Change

Definition

In the concept of a 'field change', e.g. in bladder cancer, it is postulated that the entire epithelium - not just the cancerous part - of the bladder has a 'defect' in patients which get the disease. This defect is the reason why the cancer recurs with a high frequency in these patients when their bladders are not removed.

FIGO

Definition

International Federation of Gynecology and Obstetrics (FIGO); www. figo.org.

Filter Hybridization

Definition

Filter hybridization is performed by incubating a denatured DNA preparation immobilized on a nitrocellulose filter with a solution of radioactively labeled RNA or DNA.

FISH

Definition

Fluorescence *in situ* hybridization (FISH) is a molecular cytogenetic technique where fluorescently labeled small DNA or RNA probes are hybridized to chromosomes on interphase or metaphase spreads on slides. It enables microscopic visualization of genes to define their number and chromosomal localization.

Fish Melanoma

Definition

→ Xiphophorus.

FITC

Definition

Fluoresceine-isothyocynate is a fluorescence dye, used to label biomolecules (proteins, nucleic acids). FITC can be used to determine the subcellular localization of proteins by fluor-

escence microscopy. FITC excitation is at 492 nm, the excited FITC molecule emits light of 520 nm.

FKHR

Definition

Forkhead Drosophila homolog 1 rhabdomyosarcoma, *FKH1* is a nuclear protein of 655 aa and 69 kD. The gene maps to 13q14 and is involved in → rhabdomyosarcoma in chromosomal translocation t(2;13)(q35;q14) with *PAX3*, the resulting fusion protein is a transcriptional activator. Sometimes *PAX7* is involved in the translocation; new nomenclature: → FOXO1A.

FL

Definition

→ Follicular lymphoma.

FLI-1

Definition

The *ETS* oncogene *Fli-1* (*Friend leukemia virus integration 1*) is involved in the induction of erythroleukemia in mice by Friend murine leukemia virus. *Fli-1* is activated by the insertion of Friend leukemia virus. In humans, the → *EWS*/*FLI-1* fusion gene, resulting from a t(11;22)(q24;q12) translocation, plays a key role in the pathogenesis of a large proportion (approximately 85%) of → Ewing sarcoma. EWS is an RNA-binding protein. The EWS/FLI fusion protein functions as a transcription factor, since the C-terminal RNA binding domains of EWS are replaced by the DNA-binding domain of FLI-1 (DNA recognition site: 5′-C(CA)GGAAGT-3′). EWS and EWS/FLI can associate with the RNA polymerase II holoenzyme as well as with the essential splicing factor SF1. Although it is known that *EWS/FLI-1* alters the expression of various genes, the precise mechanism by which *EWS/FLI-1* acts as an oncogene remains to be defined.→ Ets transcription factors.

FLICE

Definition

FLICE stands for → FADD (→ FAS-associated death domain)-like interleukin-1β-converting enzyme that is also known as → caspase 8.

FLIP

Definition

FLIP is a family of cellular and viral proteins capable of inhibiting death receptor mediated → apoptosis by suppressing the activation of → FLICE/→ caspase 8. It protects cells from → CD95/→ APO-1/→ FAS-induced apoptosis. For example, the → Kaposi sarcoma-associated herpesvirus FLIP is expressed by human herpesvirus 8, which is associated with malignancies such as Kaposi sarcoma certain types of → malignant lymphoma.

FLP/FRT System

Definition

FLP/FRT system is a site specific recombination system found in yeast. FLP is the site-specific recombinase and the FRT is the target recognition site. The system has been adapted for use in the *Drosophila* system. When FRT sites are located at the same cytological location on a pair of homologous chromosomes, introducing a source of FLP recombinase can be used to efficiently and reliably induce → mitotic recombination.

Fluid Phase Endocytosis

Definition

→ Endocytosis.

Fluorescence Diagnostics

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Synonyms

- tissue spectroscopy
- laser induced fluorescence diagnosis (LIFD)
- photodynamic diagnostics

Definition

Fluorescence diagnostics are procedures designed to detect neoplastic tissues based upon spectroscopic recognition of tissue-specific accumulations of natural or artificial fluorescing molecules (fluorophores).

Description

Certain fluorescing dyes selectively accumulate in cancer cells and may, since fluorescing molecules are very rare in the biological environment, serve for sensitive detection of early cancerous lesions in human tissues without interfering with tissue integrity. Some of the dyes used for → photodynamic therapy present a strong fluorescence, and since they localize to malignant tumors, early attempts were made to use these dyes for tumor diagnosis. Polyporphimer sodium (Photofrin®) for example, is a more effective purified fraction of the classical hematoporphyrinderivate (HpD), which has now obtained clinical approval for various indications in several countries. Polyporphimer sodium has to be applied intravenously and reaches a peak tumor:normal-tissue ratio 48-72 hours after injection. The possible wavelengths for stimulation are 405 nm (Soret), 505, 525, 565 and 630 nm (Q-bands). The main advantage of this substance lies in the large clinical experience with its use for Photodynamic Therapy. The disadvantages are a long-lasting → photosensitivity of the skin, a still unsatisfactory tumor selectivity, and the relatively unfavorable longer activation wavelenght of 630 nm. New dyes have entered the field, one of which is → 5-aminolevulinic acid (→ ALA). This substance is unique in the group of photodiagnostic agents, since it is not fluorescent by itself and has to be transformed by a cellular enzymatic reaction to the natural cellular fluorophore protoporphyrin IX. Since ALA can be absorbed after topical application, it is possible to locally apply by endoscopic spraying. The fast photobleaching kinetics of ALA is a disadvantage, however, this substance has actually gained importance as a photodiagnostic drug.

The autofluorescence features of certain tumors may also allow sensitive detection of early cancers. Background subtracting technology has contributed to sensitivity and specificity of point measuring and imaging systems. However, to date, there are few organ sites where such diagnostic modality is truly established.

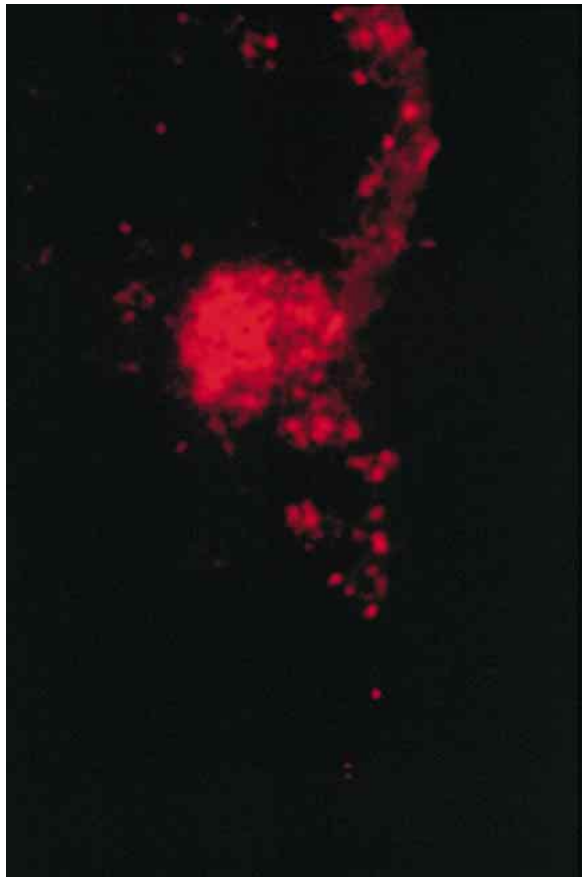
Detection of early lung cancer by fluorescence bronchoscopy. The bronchoscopic detection of severe dysplasia and early invasive lung cancer generally requires the expert analysis. Hematoporphyrin mediated specific fluorescence was early investigated to improve diagnostic sensitivity during bronchoscopy. A group in Vancouver, trying to implement background subtracting techniques realized that the autofluorescence background differed significantly between tumorous and healthy mucosa. Malignant tumors are characterized by a reduction in elastin- and connective tissue-mediated autofluorescence. Thus, instead of using an exogenous tracer, they developed an imaging system based on autofluorescence characteristics only. This system was commercialized in the mean time under the name → light-induced fluorescence endoscopy (LIFE) and is gaining rather widespread acceptance. In a recently published multicenter evaluation (2), the relative sensitivity of white light bronchoscopy enhanced by LIFE versus white light broncho-

scopy alone was 6.3 for intraepithelial neoplastic lesions and still 2.7 for invasive cancers. The positive predictive value was 0.33. The authors conclude in that report that autofluorescence bronchoscopy, when used as an adjunct to standard white light bronchoscopy, enhances the ability of the bronchoscopist to localize small neoplastic lesions, especially intraepithelial lesions that may have significant implication in the management of lung cancer in the future (2).

Urinary bladder. If the success of autofluorescence detection originates from the bronchi, the success of ALA-induced fluorescence detection originates from the urinary bladder. While early attempts had been undertaken with hematoporphyrin fluorescence imaging, the topical use of ALA to induce protoporphyrin IX fluorescence has rapidly set a standard. In their latest assessment, a group from Germany reported in 208 consecutive patients with superficial bladder cancer a best estimate for the sensitivity of ALA-induced fluorescence endoscopy of 93.4% (95% confidence intervals 90 to 97.3) compared with a value of 46.7% (95% confidence intervals 39.4 to 54.3) for white light endoscopy (3). Some autofluorescence based approaches do not find the same acceptance as yet.

*Other organ sites.*The sensitive detection of premalignant conditions or early tumor stages may be considered as the general aim of fluorescence enhanced endoscopic approaches. Based on endoscopic accessability and upon the presence of a risk group with sufficient incidence of dysplastic or cancerous lesions, research was directed towards patients with → Barrett esophagus or with → ulcerative colitis. However, both predisposing conditions are associated with an extended, inhomogenous chronic inflammatory process that seems to interfere with the homogeneity of the background fluorescence. Neither autofluorescence nor ALA-enhanced fluorescence techniques have been reported to be a worthwhile adjunct to conventional diagnostics. The differentiation of small colonic polyps may be possible by autofluorescence, it is, however, questionable, whether the clinical benefit will justify the effort.

The procedures discussed so far have all dealt with the tissue surface. If microscopic tumor deposits are to be detected at a certain depth in the tissue, more sophisticated techniques are required to isolate the specific fluorescence signal from the abundant autofluorescence originating from the interpositioned normal tissue. Single point measurements may be advantageous over imaging techniques. Using a single point background subtracting measuring device with an excitation wavelength in the red (630 nm), Mang et al. were able to detect very small tumor deposits in the skin of breast cancer patients and lymphatic micrometastases (Fig.) in a rat model (1).



Fluorescence Diagnostics. Fig. – Fluorescence micrograph of a lymph node micrometastases after systemic application of polyhematoporphyrin as photodiagnostic agent. The fluorescence intensity demonstrates the strong accumulation of the photodiagnostic agent within the metastatic deposit [image courtesy to T.S. Mang, Buffalo, USA].

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Fluorescence *in situ* Hybridization

Definition

Fluorescence in situ hybridization is a method in which chromosomal regions are evaluated using DNA probes that are labeled with a fluor, e.g. Texas Red, or with a non-fluorescent protein and then detected with a secondary fluor; → FISH.

Fluorophore

Definition

A fluorophore is a fluorescing molecule that is used, for instance, in → interphase cytogenetics, → FISH or → laser diagnostics.

FMR-1

Definition

Fragile X mental retardation (FMR-1), also known as → FRAXA (fragile site X first type of fragile sites identified on the X-chromosome) is expressed as a → fragile site at chromosome Xq27.3. It is the gene involved in fragile X syndrome, a common genetic disease with a prevalence of one in 2000 children. The disease is characterized by moderate to severe mental retardation, enlargement of the testicles and large ears. The disease results from an amplification of a CGG trinucleotide repeat directly in front of the coding region. The protein (632 aa, 71 kD) binds to RNA and might be involved in the transport of mRNA from the nucleus into the cytoplasm.

Follicular Lymphoma

Definition

Follicular lymphoma (FL) is a common low grade B-cell lymphoma, which accounts for ca. 40% of B-cell lymphomas. Typically these lymphomas carry a t(14;18) translocation, which brings the rearranged BCL-2 gene (chromosome 18) under the control of the immunoglobulin enhancer (chromosome 14). Tumours usually express B-cell markers and surface immunoglobulin. Many patients do not require treatment for a long time. A characteristic feature is a spontaneous 'waxing and waning' of the enlarged lymph nodes, which may reflect residual but inefficient attempts of the immune system to eliminate the cancer. FL is not considered curable, except for patients in very early stages of disease. Often this lymphoma transforms into a more aggressive form of lymphoma, diffuse large B-cell lymphoma, which carries a poor prognosis; → B-cell tumours.

Footprinting

Definition

Footprinting is a technique for identifying the site on DNA bound by some protein by virtue of the protection of bonds in this region against attack by nucleases.

FOS

Synonyms

- c-Fos

Definition

Fos, a *v-fos* FBJ murine osteosarcoma viral oncogene homolog, is a leucine-zipper protein of 380 amino acids and 40 kD. The human FOS gene locus maps at 14q24.3 and the mouse fos gene locus at chromosome 12 (40.00 cM). *FOS* is a protooncogene and c-Fos is a nuclear phosphoprotein that forms a tight but noncovalently linked complex with c-Jun to the → AP-1 transcription factor. c-Fos expression increases rapidly upon growth factor stimulation or wounding of cultured cells.

FOXO1A

Definition

FOXO1A is the acronym for forkhead box O1A (rhabdomyosarcoma), also known as → FKHR.

FRA3B

Definition

The constitutive → fragile site at 3p14.2 (FRA3B) is the most frequently activated constitutive fragile site, with up to 50% of normal lymphocyte derived metaphases showing the characteristic gaps or decondensation at 3p14.2 after aphidicolin treatment. Common or constitutive fragile sites are now known to be fragile regions; i.e. the position of the gaps can occur at many different sites within a fragile region, which may extend up to a megabase in size; → FHIT.

Fragile Histidine Triad

Definition

Fragile histidine triad (→ FHIT).

Fragile Site

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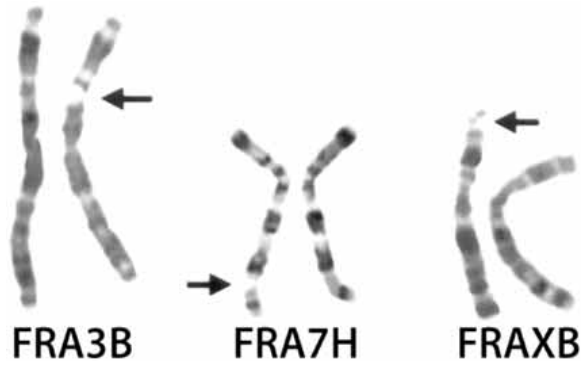
Definition

Fragile sites are specific chromosomal loci or regions that are especially prone to forming chromosome gaps, breaks or rearrangements in metaphase chromsomes following partial inhibition of DNA replication.

Characteristics

Fragile sites are generally seen as gaps on metaphase chromosome preparations, but they can lead to chromosome breaks and rearrangements. They are not normally seen on chromosomes, but rather appear and become detectable after culture of cells under conditions that partially inhibit DNA replication or with chemical compounds that bind to specific



Fragile Site. Fig. – Examples of common fragile sites on human chromosomes at bands 3p14.2, 7q32 and Xp22.3. Shown are homologs with and without expressed fragile sites. Fragile sites were induced by treatment of cells with aphidicolin.

DNA sequences. Such agents may be part of environmetal exposure, such as tobacco smoke. Their appearance at specific sites is thought to represent an underlying unusual aspect of chromosome structure or function.

Two broad classes of fragile sites are recognized based on their relative frequency of occurrence and means of induction:

- Rare Fragile Sites: The 30 or so rare fragile sites are seen in less than 5% of the population and segregate in individual families. Most are 'folate senstivie fragile sites' induced by inhibition of folic acid metabolism and leading to perturbation of DNA synthesis. Others are induced by compounds such as distamycin-A that bind to AT-rich DNA sequences.
- Common Fragile Sites: Common fragile sites are seen on chromosomes of all individuals and are thought to be a manifestation of underlying normal, but unusual, chromosome structure or function. They are also seen following growth under conditions of folic acid stress, but to a greater extent following treatment of growing cells with → aphidicolin. Over 80 common fragile sites have been described with one or more seen on most human chromosomes. Common fragile sites display a number of characteristics of unstable and recombinogenic DNA *in vitro* and *in vivo*. In addition to forming gaps and breaks on metaphase chromosomes, common fragile sites are hot spots for sister chromatid exchanges (SCE), chromosomal translocations, integration of transfected and possibly viral DNA sequences, and chromosome breaks associated with initiation of gene → amplification.

Cellular & Molecular Regulation

All rare fragile sites studied at the molecular level are caused by expansion of repeat DNA sequences. Most, such as the folate-sensitive fragile site FRAXA associated with the → Fragile-X Syndrome, are due to an expansion of (CGG)n → trinucleotide repeats. Others, such as FRA16B, are associated with an expanded short AT-rich sequence.

Less is currently known about the molecular basis of common fragile sites, and only a few have been studies on the molecular level. While similar in appearance to the rare fragile sites at the chromosomal level, common fragile sites do not appear to be caused by expansion of trinucleotide or other simple repeat sequences. The common fragile sites are large, encompassing hundreds of kilobases of DNA. The FRA3B site at 3p14.2, two fragile sites on chromosome 7, FRA7G and FRA7H, the FRAXB site at Xp22.3 and FRA16O at 16q23 have been studied at the molecular level. All are AT-rich, contain numerous → LINE elements or other long repeat elements. They contain clusters of sequences with a potential for high flexibility and low stability and unusual DNA structures that could impede DNA replication. Late or incomplete DNA replication may be a common characteristic.

Clinical Relevance

Rare fragile sites.

The FRAXA fragile sites at Xq28 is a cytogenetic manifestation of the expanded CGG repeat mutation in the promoter region of the → *FMR-1* gene associated with the → Fragile X syndrome, a leading cause of mental retardation in humans. A similar fragile site on the X chromosome, FRAXE, is also associated with mild mental retardation in some families. FRA11B on the long arm of chromosome 11 is believed to lead to terminal deletions of chromosome 11 in a small number of cases of Jacobsen syndrome. It is likely that other rare fragile sites will be found to be associated with specific genes and possibly genetic diseases.

Common fragile sites.

Based on their location at or near chromosome breakpoints seen in cancer cells, and their instability, a hypothesis has been forwarded that common fragile sites may play a mechanistic role in chromosome breakage and rearrangements in cancer. Direct testing for this hypothesis is provided by the molecular cloning of specific common fragile sites, and evidence to support this hypothesis has come from analysis of the FRA3B site at 3p14.2.

The FRA3B site lies within the large → FHIT gene which has found to be frequently deleted and to show aberrant mRNA transcripts in a large number of tumor types including gastric, lung and esophageal tumors. Translocations within *FHIT* and FRA3B have also been noted. Based on these findings, the *FHIT* gene has been proposed to be a tumor suppressor gene that is easily mutated due to the presence of the fragile site. The functional role of the *FHIT* gene in tumorigenesis is currently under study in a number of laboratories. Fragile sites have also been implicated in other types of genomic instability associated with cancer, such as gene or DNA amplification. The question of whether other common fragile sites are also associated with genes and are particularly unstable in tumors is currently under study. Other questions of importance, which are now approachable on the molecular level, include the mechanism of instability and the genetic and environmental factors influencing instability, particularly in cancer cells.

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Fragile X Syndrome

Definition

Fragile X syndrome is an X-linked hereditary disorder characterized by mental retardation in affected males and mild mental retardation in some carrier females. The Fragile X syndrome is a leading cause of familial mental retardation in humans. It is caused by the expansion of a (CCG)n trinuleotide repeat sequence in the promotor region of the *FMR1* gene located at chromosomal region Xq27. The repeat is normally found in less than 50 copies in normal individuals but expands to hundreds of copies in in patients with fragile X syndrome. The expansion leads to the appearance of a rare folate-sensitive fragile site on metaphase chromosomes, a finding that first led to the identification of the *FMR-1* gene; → FMR-1.

Frameshift Mutations

Definition

Frameshift mutations arise by deletions or insertions that are not multiples of 3 bp. They change the frame in which triplet base pairs are translated into protein.

FRAXA

Definition

Fragile site X first type of fragile sites identified on the X-chromosome; also known as → FMR-1.

Functional Genomics

Definition

Functional genomics is the systematic analysis of gene activity in healthy and diseased tissues.

Fusion Protein

Definition

A fusion protein is one whose polypeptide sequence is composed by portions encoded by different genes, often juxtaposed following chromosome translocation rearrangements.

G

G_1

Definition

G_1 is the period of the eukaryotic cell cycle between the last mitosis and the start of DNA replication.

G_2

Definition

G_2 is the period of the eukaryotic cell cycle between the end of DNA replication and the start of the next mitosis.

GAB1

Definition

Gab1, also known as Grb2-associated binder, is a signaling protein with modular domains that interact with receptors (eg., c-→ Met) and other signaling molecules, including inositol triphosphate, the tyrosine phosphatase SHP2 and phosphatidylinositol-3-kinase. Gab1 is an essential intermediary for → scatter factor-induced epithelial morphogenesis; → multi-substrate docking proteins.

GAGE

Definition

GAGE stands for G antigen. → HUGO nomenclature symbol is GAGE1, for G antigen 1. The *GAGE* gene maps to chromosome Xp11, among normal tissues the encoded protein (138 aa 15 kD) is exclusively seen in testis. Due to de-→ methylation the gene is expressed in → melanoma, also in a number of other tumors. The GAGE antigen is recognised on melanoma cells by autologous cytolytic T lymphocytes. GAGE is used in vaccination for immunotherapy of melanoma. A related gene, *GAGE2*, also maps to Xp11. The GAGE2 protein has 116aa 12 kD and behaves similar to the GAGE1 protein.

Gain-of-function Mutation

Definition

A gain-of-function mutation is any mutation of a gene that causes increased function and/or activity of its encoded protein or of a protein that is directly or indirectly regulated by the mutated gene.

Gamete

Definition

A gamete is either type of reproductive (germ) cell - sperm or egg - with a haploid chromosome content.

Gammopathy

Definition

Pathological conditions characterized by a monoclonal immunoglobulin secretion are designated as gammopathies, such as monoclonal gammopathy of undetermined significance (MGUS), Waldenström macroglobulinemia or → multiple myeloma.

Ganciclovir

Definition

Ganciclovir is an acyclic analog of 2′-deoxyguanosine used in → suicide gene therapy; → HSV-TK/Ganciclovir mediated toxicity.

Ganglioneuroblastoma

Definition

Ganglioneuroblastoma is a tumor consisting of Schwannian stroma with individually distributed neuronal elements; → neuroblastoma.

Ganglioneuroma

Definition

Ganglioneuroma is a tumor consisting of intermingled microscopic foci of neuroblastic elements in an expanding Schwannian stroma, comprising more than 50% of the tumor volume; → neuroblastoma.

GAP

Definition

→ GTPase-activating protein.

GAP Junctional Intercellular Communication

Definition

Gap junctional intercellular communication is cell-cell communication of small molecules (<1000 daltons) using specialized membrane channels; → Gap junctions.

GAP Junctions

Definition

Gap junctions are protein channels present on the surface of some cells which, when docked with a similar channel on a neighboring cell, results in a direct connection of the intracellular environments of the two cells. Gap junction channels are formed by a hexameric arrangement of a connexin protein to form a connexon, which allows the exchange of small molecules smaller than 1000 daltons.

Gastric Adenocarcinoma

Definition

Gastric adenocarcinoma is a malignancy of the stomach derived from epithelial cells.

Gastrin

Definition

Gastrin is a hormone released into the blood; → gastrinoma.

Gastrinoma

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Synonyms

- Zollinger-Ellison syndrome
- islet cell tumor
- neuroendocrine tumor
- neuroendocrine carcinoma
- carcinoid

Definition

Gastrinomas are functioning gastrin-producing tumors of the neuroendocrine cells that are located in the duodenum, pancreas and/or 'gastrinoma triangle' and cause clinical Zollinger-Ellison syndrome.

Characteristics

Gastrinoma is a functioning → neuroendocrine tumor of high malignant potential that follows an indolent clinical course. Neuroendocrine tumor cells release gastrin into the blood stream causing clinical symptoms, i.e. Zollinger-Ellison syndrome (ZES).

Annual incidence of ZES in the United States is estimated at 1 in 2.5 million people. It may occur in sporadic and familial forms. Twenty to 25 percent of ZES cases are associated with familial → multiple endocrine neoplasia type 1 (MEN1; ZES-MEN1). Sporadic ZES can develop at any age, but the majority of cases are diagnosed between the fourth and sixth decade of life (mean age 50.5 years). ZES-MEN1 patients are younger than those with sporadic ZES. Sixty percent of the patients are men.

Diagnosis

The diagnosis of ZES is usually established clinically and then confirmed by demonstration of the specific tumor type (gastrin-positive neuroendocrine tumor) in a pathology specimen. Clinical diagnosis of ZES is based on the finding of concurrent → hypergastrinemia (increased serum gastrin produced by the tumor) and → hyperchlorhydria (increased gastric acid secretion by hyperplastic parietal cells secondary to the trophic action of gastrin) in patients with ulcer disease. Because ZES symptoms overlap with those of much more common peptic ulcer disease, the diagnosis of gastrinoma (ZES) may be delayed for many years. Multiplicity and unusual (low duodenal and intestinal) location of ulcerations, resistance to medical therapy, frequent and early recurrence of ulcers, prolonged and unexplained diarrhea or steatorrhea, enlarged gastric or duodenal folds, and negative tests for *Helicobacter pylori*, when they occur raise a suspicion for ZES. The diagnosis is established by positive results with two or more of the following: elevated fasting serum level of gastrin (>100 ng/l), abnormal secretin stimulation test (increment in fasting serum level of gastrin >200 ng/l after intravenous secretin) and an elevated basal level of gastric acid output (>10 mEq/hr).

Gastrinomas are thought to originate from gastrin-producing (G) cells that are normally dispersed in gastric antral and upper duodenal mucosa. However, there is no direct correlation between the distribution of G cells in the digestive system and frequency of gastrinoma location. The tumors commonly arise in ectopic sites such as pancreas (the second most common location) that is normally devoid of G cells. More than 50% of gastrinomas arise in the duodenum. Ninety percent of all gastrinomas are located in the 'gastrinoma triangle' (the anatomic junction of cystic and common bile ducts, second and third portion of the duodenum and neck and body of the pancreas). Solitary pancreatic or duodenal gastrinomas are characteristic for sporadic ZES cases. Location of the tumors in proximal duodenum and their multiplicity are common in ZES-MEN1 patients. Cases of gastrinoma located solely in periduodenal and peripancreatic lymph nodes have been reported, and clinical cure has been achieved following surgical excision of regional lymph nodes in some of such cases.

Microscopically, gastrinomas are composed of small, uniform cells with abundant eosinophilic cytoplasm and small uniform nuclei with inconspicuous nucleoli. Correlation between histologic features and malignant behavior is poor. Mitotic activity and nuclear pleomorphism, when rarely present, are unreliable predictors of prognosis in gastrinoma. Despite their small size and lack of invasion into adjacent organs, duodenal gastrinomas demonstrate high malignant potential with propensity for regional lymph node and distant metastases.

Because gastrinoma may be an initial manifestation of MEN1 in up to one third of familial ZES cases, it is currently recommended to evaluate all ZES patients for medical and family history of the endocrine neoplasms and assess their parathyroid and pituitary function. The differentiation of patients with familial ZES-MEN1 from patients with sporadic ZES is important because of the differences in natural history of ZES, need for family screening, difficulty in controlling acid hypersecretion and need for exploratory laparotomy for cure.

Gastrinomas are reported to be malignant in 60% to 90% of cases. About 34% of patients with ZES have metastatic disease at the time of diagnosis. Periduodenal and peripancreatic lymph nodes, liver and bone are the most common sites of metastases. Gastrinomas in MEN1-ZES and sporadic ZES patients have similar rates of metastases.

Therapy

Since ZES can be cured only by excision of a gastrinoma in the early stage of the disease, the exact tumor localization may be crucial in successful management of ZES patients. Duodenal gastrinomas are frequently small in size (less than 1.0 cm) and are difficult to find preoperatively. Tumor localization studies (CT, MRI and → octreotide scanning) are necessary for pre-operative localization. Exploratory laparotomy with intraoperative ultrasonography, transduodenal endoscopic illumination, duodenotomy and surgical resection of the tumor is currently recommended for patients with sporadic ZES patients. The usefulness of surgery varies for patients with ZES-MEN1 who usually have multiple tumors.

Treatment of patients with ZES is also directed at reducing gastric acid hypersecretion. Total gastrectomy or vagotomy have been used. However, over the past 15 years a highly potent anti-secretory proton pump inhibitor, → omeprazole, has been effective in suppression of gastric acid output and has significantly decreased the early mortality of patients due to complications of ulcer disease. With the increased ability to control hyperchlorhydria, the progression of gastrinoma and metastases are becoming the primary factor in long-term survival of patients with ZES. The clinical course of gastrinoma is indolent and the prognosis is directly related to the spread of the tumor. Patients with liver metastases have only a 20% to 30% chance of surviving for 5 years, whereas patients without liver metastases have an excellent long-term prognosis (> 90% 5-year survival rate).

Genetics

The role of the *MEN1* gene as an early event in gastrinoma tumorigenesis has been recently established in MEN1-associated tumors as well as in 33% of sporadic gastrinomas. In both familial and sporadic gastrinomas the *MEN1* gene, located on chromosome 11q13, is thought to act as a tumor suppressor based on the presence of inactivating mutations in the normal tissue/blood DNA, accompanied by the loss of the wild type allele in the tumor. Somatic genetic changes associated with the development of 67% sporadic gastrinomas and tumor progression are currently unknown.

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Gastrinoma Triangle

Definition

The gastrinoma triangle is the anatomical location in the abdomen defined by the junctions of the cystic and common bile ducts superiorly, second and third portion of the duodenum inferiorly, and the neck and body of the pancreas medially; → gastrinoma.

Gastritis

Definition

Gastritis is inflammation of the stomach.

Gastrulation

Definition

Gastrulation is the formation of a gastrula from a blastula during embryogenesis.

Gatekeeper

Definition

A gatekeeper is a subgroup of gene products, whose major cellular function is in the control of cell division, death or lifespan. Inactivation of gatekeeper genes favours, through a variety of mechanisms, the unrestrained growth typical of cancer cells. Multiple gatekeeper mutations may be required for the neoplastic transformation of a cell. Gatekeeper gene products often participate in the control of cell cycle (for example, the Rb gene product [→ retinoblastoma protein, cellular biochemistry]) or in the signals that regulate proliferation (for example, the → APC gene product). It is possible that large proteins with several functional domains (→ pleiotropic genes), such as BRCA1 and BRCA2 proteins originally described as gatekeepers may also have → caretaker functions.

G-banding

Definition

G-banding is a technique that generates a striated pattern metaphase chromosomes that distinguishes the members of a haploid set.

GC-MS

Definition

Gas chromatography-mass spectroscopy (GC-MS) has the advantage of unequivocally identifying the analytes in a mixture. It is used to identify → adducts to DNA or altered DNA bases, which result for instance from → oxidative DNA damage.

GC/post-GC Type B-cell Tumors

Definition

Since somatically mutated *V* genes are a hallmark of B-cells that have left the → germinal center (GC), studies of *V* genes in B-cell tumors provide information on their stage of maturation; post-GC type tumors that carry mutated *V* genes include → DLBCL, → Burkitt lymphoma, marginal zone lymphoma and classical Hodgkin lymphoma [→ Hodgkin disease]. The pattern of mutations in → follicular lymphoma reveals intraclonal heterogeneity, indicating ongoing mutation (GC-type); chronic lymphocytic leukemia and mantle cell lymphoma cells harbor unmutated *V* genes (pre-GC type).

GDS

Definition

→ Guanine nucleotide dissociation stimulator.

GEF

Definition

→ Guanine nucleotide exchange factor.

Gene

Definition

A gene is a specific DNA sequence that carries genetic information. This information is in most cases expressed as a protein. However, the functional end product synthesized from some genes can be RNA, e.g. ribosomal RNA. The human genome was estimated to contain 100,000 to 150,000 genes. With the establishment of the human genome sequence, the number now is estimated to be lower, between 30,000 and 60,000.

Gene (cistron)

Definition

A cistron of a gene is the segment of DNA involved in producing a polypeptide chain. It includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

Gene Dosage

Definition

Gene dosage is the number of copies of a particular gene in the genome.

Gene Family

Definition

A gene family consists of a set of genes whose exons are related; the members are derived by duplication and variation from an ancestral gene.

Gene Knockout

Definition

The ability to disrupt the expression of specific genes in embryos of experimental organisms (such as mice, rats, the fruitfly *Drosophila melanogaster* etc.) is a cornerstone of biomedical research and a key strategy to link genes to diseases.

Gene Therapy

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Definition

Directed introduction and expression of new genetic information in cells of an organism for therapeutic purposes. Only somatic gene therapy is presently permitted, the introduction of genetic material into germ cells is not allowed.

Characteristics

Gene therapy has its beginnings in the early 1980s following the identification of several disease-related genes and the development of technologies for gene isolation, purification and transfer to cells in culture. Many different human diseases represent theoretical targets for gene therapeutic intervention, and the strategies developed depend on the nature of the disease to be treated. Table 1 illustrates several classes of human disease and the respective treatment strategies currently being followed. It goes without saying that detailed knowledge of the molecular mechanisms of disease development and progression is a prerequisite for the development of gene therapeutic intervention strategies.

Transfer of genes

Many different methods have been developed to transfer therapeutic genes to patient cells. A consideration that is central to the choice of gene transfer vehicle (→ vector) is whether stable or only transient gene expression is required. Stable gene expression is necessary for the treatment of hereditory genetic defects, whereas transient gene expression is sufficient and may even be desirable when employing genes encoding toxic gene products e.g. in the treatment of cancer. Many gene transfer ve-

Gene Therapy. Table 1 – Examples of gene therapeutic strategies for different disease groups.

disease group	genetic basis of disease	strategy
heriditary monogenic disease e.g. haemophilia, cystic fibrosis	defective gene that results in a single gene product, which is non-functional or missing completely	introduction of the wild type version of the mutated gene (e.g. into liver or muscle cells for the production of a protein in the blood circulation, into lung cells in the case of cystic fibrosis)
multifactorial disease e.g. cardiac disease, diabetes	direct or indirect involvement of many genes and their products	at present too complex, although restenosis (tissue proliferation after surgical dilation of a blood vessal) can be treated by the local expression of genes that inhibit cell proliferation
cancer	somatic mutation of (several) cellular genes	1. induction of cell death employing genes, encoding toxic products; 2. stimulation of the immune system to recognise and destroy cancer cells; 3. inhibition of 'cancer genes'
infectious diseases e.g. AIDS	new genetic material from the infectious agent (e.g. virus) induces pathogenic processes	1. inhibition of expression of genes encoded by the infectious agent employing e.g. antisense or ribozyme genes; 2. vaccination employing genes encoded by naked DNA i.e. infection prevented or eliminated by the patients immune system

hicles employ components of viruses (viral vectors) since viruses have evolved to efficiently transfer their own genes to cells and to express the respective gene products at high levels. On the other hand, since it may not be possible to completely eliminate potential safety problems with viral vectors, non-viral gene transfer vehicles employing synthetic lipids or simply naked DNA have been developed in parallel. In general, the transfer efficiencies and gene expression levels achieved with non-viral vectors are poorer than with viral vectors. The commonly used gene transfer vectors, their advantages and their disadvantages are summarised in Table 2.

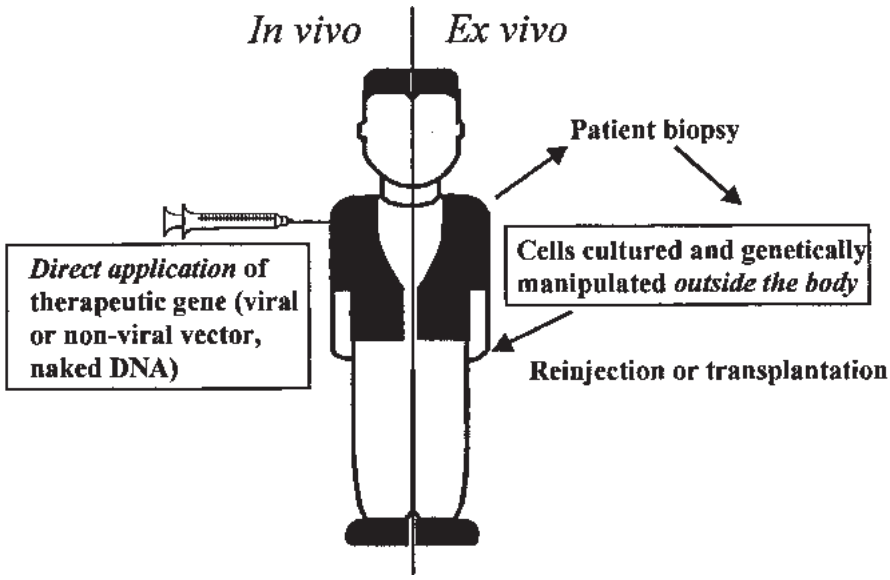
In many situations, in addition to high gene transfer efficiency, it is necessary that the transfer vector is selective i.e. mediates expression of the therapeutic gene exclusively in targeted diseased cells. Achieving selectivity is a major hurdle that is being approached in several ways. On the one hand, gene vectors are being manipulated (on the surface of the gene vector particle) such that targeted cells are exclusively accessed. On the other hand, selectivity can be achieved by ensuring that, even in the situation in which many cells have been accessed, expression of the therapeutic gene can only occur in specific targeted cells (e.g. by employing tissue-specific promoter/enhancer elements to control therapeutic gene expression).

Clinical aspects

Therapeutic genes can be introduced into patient cells either *ex vivo* or *in vivo* (see Fig.). In the *ex vivo* application, a patient biopsy is genetically manipulated outside the body and subsequently reinjected or transplanted. This procedure has the advantage that only the cells in the biopsy come into contact with the transfer vector. Furthermore, the circumstances in cell culture generally allow more efficient gene transfer than on *in vivo* application. In addition, in some cases it is possible to expand the cells in the biopsy and create a pool of genetically modified cells that can be reapplied to the patient as required. The main disadvantage of the *ex vivo* procedure is that it is technically cumbersome, time-consuming and very expensive. The most straightforward and desirable situation would be to apply the gene transfer vehicle *in vivo* either directly into specific tissues or organs (e. g. directly into tumour tissue) or

Gene Therapy. Table 2 – Gene transfer vehicles, their advantages and disadvantages.

gene vehicle	advantages	disadvantages
retrovirus vectors including those based on lentiviruses.	1. integration of vector into host genome leading to stable gene expression 2. no cellular toxicity 3. lentiviral vectors also infect (transduce) differentiated non-dividing cells.	1. titer not as high as e. g. adenoviral vectors 2. vector integration into host genome is potentially mutagenic although this has not been shown in animal or human experiments.
adenovirus vectors	1. very high titers (10^{13}/ml) 2. very high, but transient, gene expression.	1. only transient gene expression 2. immune response to vector and transgene.
adeno-associated virus (AAV) vectors	1. AAV is not pathogenic in humans 2. non-toxic 3. infects non-dividing cells.	biology not as well characterised as those of retroviruses and adenoviruses.
liposomes	no viral genes, no toxic effects.	mostly lower and transient gene expression.
naked DNA	no viral genes, no toxic effects.	mostly lower and transient gene expression.



Gene Therapy. Fig. – *In vivo* and *ex vivo* approaches to correct the effects of genetic damage in somatic cells.

by injection into the blood circulation. *In vivo* application requires that the transfer vector efficiently reaches the appropriate diseased cells and at present, this is often not the case. In fact, the major problems hampering gene therapeutic approaches today concern the efficiency and the selectivity of the transfer vectors when applied *in vivo*.

Permission for the first clinical gene therapy study was granted in 1989 and since then numerous clinical trials, for the most part with only small numbers of patients, have been carried out. For the reasons described above (lack of sufficient selectivity and efficiency of transfer as well as lack of stability of transgene expression) the results of these trials did not establish any statistically verified positive effects on disease progression or mortality. Several additional clinical studies are presently ongoing. Basic research is focused on improving gene vector properties and to gaining a better understanding of the interactions between the gene vector, the transgene and the patient́s immune system. It is to be anticipated that the combined knowledge gained from these efforts will allow gene therapeutic protocols to be developed that will represent valid treatments for diseases which have been difficult or impossible to therapy up until now.

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Genetic Disorders Associated with Cancer Predisposition and Chromosomal Instability

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Definition

Genomic instability is a universal property of tumour cells and one of the defining features of the transformed phenotype. A failure to suppress genomic instability is implicated as a cau-

sative factor in both tumourigenesis and tumour progression. Although this instability can take several different forms, characteristic chromosomal abnormalities such as aberrant → genetic recombination events and → aneuploidy are consistently observed in a wide range of tumour types. Perhaps not surprisingly, therefore, in those individuals in which there is chromosomal instability due to an inherited disorder in DNA metabolism, an elevated incidence of one or more types of cancer is seen.

Characteristics

There are several different chromosomal instability disorders of man. Basic principles underlying the relationship between genomic instability and cancer can be well illustrated in two types of disease:

- first, a disorder in which predisposition to the full spectrum of cancers is observed (Bloom syndrome);
- second, disorders in which hypersensitivity to radiation is a feature (xeroderma pigmentosum and → ataxia telangiectasia).

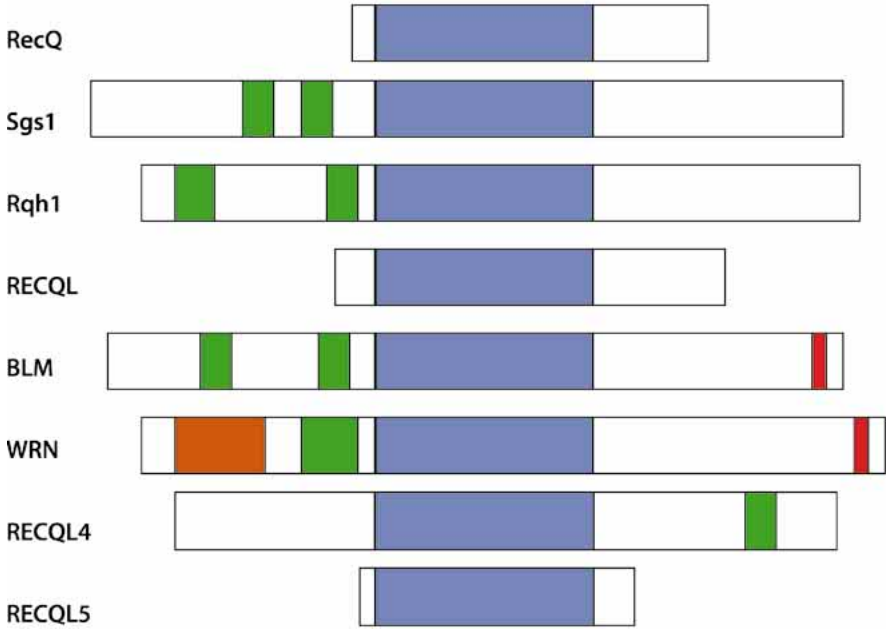
Bloom syndrome

Bloom syndrome (BS) is a rare, recessive disorder characterised by proportional dwarfism, sunlight sensitivity, immunodeficiency, male infertility and an increased incidence of cancer. The mean age at cancer diagnosis is approximately 24 years. Cells isolated from BS individuals show inherent genomic instability, with an increase in the frequency of several cytogenetic abnormalities; predominantly chromatid breaks, → quadriradial chromosomes and reciprocal exchanges between sister-chromatids (→ sister-chromatid exchanges; SCEs). This elevated rate of SCEs is pathognomonic of BS, and is used for diagnostic purposes. BS cells can also be viewed as having a mutator phenotype.

The gene mutated in BS, named BLM, was isolated in 1995 using an elegant procedure termed → somatic cross-over point mapping. Analysis of the 4.5 kb BLM cDNA revealed that it encoded a protein comprising 1417 amino acids with a predicted molecular mass of 159 kD. Confirmation that BLM is the BS gene came from the identification of mutations predicted to severely truncate the BLM protein in cells from BS individuals, and from the ability of the BLM cDNA to functionally complement the SCE defect in BS cells.

The predicted amino acid sequence of the BLM protein shows marked similarity to the sequences of a family of DNA → helicase enzymes of which the prototypical member is RecQ of E. coli. This so-called RecQ family (Fig.) contains the Sgs1 protein from the budding yeast *S. cerevisiae*, the Rqh1 protein from the fission yeast *S. pombe*, and currently 5 members from human cells; BLM, WRN, RECQL, RECQL4 and RECQL5. BLM is not the only member of this interesting family to be mutated in a human genetic instability disorder. The WRN gene is mutated in the premature ageing condition Werner's syndrome (WS), which is characterised by multi-organ accelerated ageing from the second decade of life until premature death around the age of 45-50. WS sufferers also display cancer predisposition, but generally this is limited to an increased frequency of non-epithelial cancers, particularly sarcomas. WS cells are genetically unstable and show, in particular, an increase in large chromosomal deletions. The RECQL4 gene is mutated in some cases of Rothmund-Thomson syndrome (RTS). RTS is a rare disorder associated with skin and skeletal abnormalities, as well as some features of premature ageing. Cells isolated from RTS sufferers show genomic instability, which appears to be associated with an elevated incidence of certain cancers.

As predicted from the deduced amino acid sequence, BLM is a DNA-stimulated ATPase and an ATP-dependent DNA helicase that shows 3′-5′ polarity. Using electron microscopy, it has been shown that active BLM has a ring-like structure, probably comprising 6 monomeric building blocks. Whether DNA feeds through the hole in the BLM ring during translocation along its substrates remains to be seen. The precise *in vivo* substrate for BLM is not known at this stage, but in line with data on the Sgs1 protein, it is known that BLM has a preference for → G-quadruplex DNA. This



Genetic Disorders Associated with Cancer Predisposition and Chromosomal Instability. Fig. – Alignment of the RecQ family of DNA helicases. The family members are from *E. coli* (RecQ, 610 residues), budding yeast (Sgs1, 1447 residues), fission yeast (Rqh1, 1328 residues), or human cells (RECQL, 649; BLM, 1417, WRN, 1432; RECQL4, 1208; RECQL5, 410 residues). Other splice variants of RECQL5 have been identified. BLM, WRN and RECQL4 are the proteins defective in Bloom, Werner, and Rothmund-Thomson syndromes, respectively. The proteins are aligned through their highly conserved central helicase domains, indicated in blue. The green boxes represent blocks of acidic amino acid residues. A nuclease motif in the *N*-terminal domain of WRN is shown as a brown box. Nuclear localisation signal sequences are shown as red boxes.

is a highly stable, atypical DNA structure that has yet to be shown conclusively to exist *in vivo*. It has been proposed that a failure to disrupt G-quadruplex structures in BS cells could be at least one trigger for genomic instability, perhaps as a result of their blocking progression of replication and/or transcription complexes.

BLM is likely to make specific contacts with several other cellular proteins. One well established partner for BLM is the α isoform of → topoisomerase III (topo IIIα). This interaction of a RecQ family helicase and topo III is conserved from yeast to man. Immunofluorescence analysis of human cells has revealed that BLM localises with topo IIIα (and a number of other proteins) in discrete nuclear foci. These foci correspond to one class of sub-nuclear domains, PML nuclear bodies. These bodies are disrupted in acute promyelocytic leukemia (APL) due to a chromosomal translocation that targets the PML gene, a growth and tumour suppressor. In PML-/- cells, BLM is mislocalized and these cells show an elevated frequency of SCEs. These findings implicate some aspects of the biology of → PML nuclear bodies in the maintenance of genome stability. Clearly, it is possible that BLM mislocalization in APL cells could play a role in the pathogenesis of this disorder.

Xeroderma pigmentosum/Ataxia telangiectasia

Although BS individuals display an abnormal, sunlight-induced erythema, cells isolated from them are not abnormally sensitive to killing by UV light or by ionizing radiation. However, cancer predisposition syndromes do exist in which sensitivity to one or other form of radiation is a key feature. The most extensively studied paradigms for the relationship between cancer predisposition and sensitivity to either UV light or ionizing radiation are xeroderma

pigmentosum (XP) and ataxia telangiectasia (AT), respectively.

XP is a rare disorder associated with acute photosensitivity upon exposure to UV light, leading to numerous abnormalities in skin pigmentation and to atrophy of the exposed skin. UV-induced skin cancers on sun-exposed parts of the body are common, frequently occurring in childhood. Unlike BS, which is caused by a defect in a single gene, XP can arise from mutation of any one of 8 genes, designated XP-A through G (classical forms) and XP-V (variant form) (Table 1). The classical forms of XP make up over 80% of cases and are associated with a defect in the major pathway for repair of UV-induced DNA lesions, which is known as the nucleotide excision repair (NER) pathway. Two inter-related NER pathways exist; one that deals primarily with repair of the transcribed strand of genes that are actively being transcribed into mRNA (transcription coupled repair; TCR) and one that deals with repair of the remainder of the bulk chromatin (global genome repair; GGR). Cells from most XP patients are defective to varying degrees in both TCR and GGR, but those from XP-C individuals are defective solely in GGR. This difference perhaps reflects the fact that the transcription machinery acts as an 'antenna' alerting the cell to damage in active genes, while the XP-C protein performs a related, lesion-recognition role in the genome overall. Analysis of human XP genes and their products, together with similar analyses on yeast homologues, has gone a long way towards defining the role played by each protein in the NER pathway (Table 1).

Of particular interest is the recent finding that the XP-V gene product acts quite differently from the XP-A to -G proteins in that it is not involved directly in NER. Instead, the XP-V gene encodes a DNA polymerase (polymerase eta) that is able to bypass the most common DNA adduct generated by UV light (pyrimidine dimers) and hence catalyses so-called 'translesion' DNA synthesis.

The most widely studied model in which defective responses to ionizing radiation are seen is AT. As with BS and XP, AT is relatively rare and genetically recessive. This disorder gets its name from the combination of symptoms commonly seen in AT patients; the ataxia is a result of Purkinje cell destruction in the cerebellum leading to severe loss of coordination, while the telangiectasia (dilated blood vessels, particularly in the eye) is of unknown origin. AT patients show a 250-fold elevated risk of lymphoma and a 70-fold elevated risk of T- and B- cell leukemias. A hallmark of AT is a dramatic radiosensitivity seen when patients are given standard doses of radiotherapy for cancer. This radiosensitivity is also observed with AT cells in culture. The key cytogenetic feature

Genetic Disorders Associated with Cancer Predisposition and Chromosomal Instability. Table 1 – Functions of the gene products corresponding to the XP complementation groups.

protein	budding yeast homologue	function
XP-A	Rad14	DNA damage recognition
XP-B/ERCC3	Rad25/Ss12	3′-5′ DNA helicase; subunit of transcription factor (TFIIH)
XP-C	Rad4	DNA damage recognition; could recruit repair machinery. Involved in genome overall repair.
XP-D/ERCC2	Rad 3	5′-3′ DNA helicase subunit of transcription factor (TFIIH)
XP-E	?	DNA damage recognition?
XP-F/ERCC4	Rad1	5′ endonuclease
XP-G	Rad2	3′ endonuclease
XP-V	Rad30	DNA polymerase (η) that can bypass DNA damage

of AT cells is striking genomic instability, with an increased frequency of chromosomal losses, gaps, breaks, translocations and telomere-telomere fusions [→ telomerase]. This karyotypic instability is seen in the absence of radiation, but a significant increase in the frequency of chromosomal breaks is evident post-irradiation.

AT cells show abnormalities in → cell-cycle checkpoint responses to DNA damage. Checkpoints normally regulate the orderly progression of cell cycle events to ensure, for example, that mitosis is not initiated until DNA replication is completed. There are several DNA damage checkpoints that operate at different points of the cell cycle; the G1-S boundary, within S-phase and at G2-M. In AT cells, the normal G1 phase arrest seen after irradiation is absent, with the result that these cells commence replication on damaged chromosomes having failed to effect DNA repair in G1. In addition, AT cells display so called radioresistant DNA synthesis (RDS), failing to slow the rate of DNA synthesis when irradiated in S-phase. It is not clear to what extent RDS is linked to radiosensitivity, since AT cell derivatives exist in which these features are separable.

The AT gene, → ATM, encodes a very large protein (~ 350 kD) that is frequently truncated by mutations found in AT cells. The sequence of the carboxyl terminal portion of the ATM protein is related to the sequences of several lipid kinases of the phosphoinositide-3-kinase family. However ATM is a protein kinase, with one of its major substrates being the *p53* tumour suppressor. ATM has also been shown to bind to and activate the c-Abl tyrosine kinase following DNA damage. Consistent with a cell-cycle checkpoint role for ATM, homologues of ATM found in both lower and higher eukaryotes play well established signalling roles during checkpoint responses to DNA damage, most notably Mec1p from *S. cerevisiae* and Rad3p from *S. pombe* (Table 2). It is possible that the ATM kinase also plays a role either in regulating DNA repair or in the repair process itself.

Clinical relevance

It is clear that loss of genome integrity is a vital component in the tumourigenesis process. The genetic disorders discussed above probably exhibit cancer predisposition by virtue of their elevated mutation frequency, which allows all of the necessary genetic changes required for tumour formation to occur prematurely. Whether mutations in BLM or ATM occur frequently in sporadic tumours in the general population is not clear at this stage, but there is at least some evidence that ATM mutations are seen in certain sporadic tumours. Conversely,

Genetic Disorders Associated with Cancer Predisposition and Chromosomal Instability. Table 2 – Functions of known ATM-related proteins.

homologue	species	function
Vps34	*S. cerevisiae*	vesicular trafficking
PI-3-kinase p110(cs)	mammals	lipid and protein kinase; signal transduction
Rad3	*S. pombe*	cell-cycle checkpoint roles at G2 and S-phase; responds to damaged or unreplicated DNA
Mec1/Esr1	*S. cerevisiae*	Cell-cycle checkpoint roles at G1/G2 and S-phase; responds to damaged or unreplicated DNA
Tel1	*S. cerevisiae*	→ telomere length regulation
MEI-41	*Drosophila*	→ cell-cycle checkpoint control in G2; DNA repair; meiotic recombination
DNA-PK(cs)	mammals	serine/threonine → protein kinase activated by DNA strand breaks; V(D)J recombination

the question of whether mutations in genes such as ATM are relevant to responses to chemotherapy and/or radiotherapy is only now being addressed. It is certainly possible that ATM gene malfunction is a contributory factor not only in the success or failure of radiotherapy but also in the unusually severe reactions to radiotherapy that is seen in a fraction of cancer patients.

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Genetic Epidemiology

Definition

Genetic epidemiology is the study of the role of genetics in the incidence and distribution of disease.

Genetic Polymorphism

Definition

Genetic polymorphism is the presence of multiple inheritable forms of a gene within the population; a genetic trait where the least common allele is found in approximately at least 1% of the population.

Genetic Recombination

Definition

Genetic recombination is the process whereby DNA from one chromosomal location is exchanged for, or replaces, another region of the genome.

Genome

Definition

The genome is the total genetic information possessed by an individual organism. Each cell contains a complete copy of the genome.

Genomic Imprinting

Definition

Genomic imprinting is a parent-of-origin-dependent allele inactivation process; the allele from either father or mother is partially or completely silenced through → imprinting. Imprinting can often be monitored by differential → methylation of one of the two parental alleles.

Genomic Imprinting and Cancer

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Definition

Genomic imprinting is an epigenetic alteration (i.e., not involving a change in base sequence) of a specific parental allele of a gene, or the chromosome on which it resides, in the gamete or zygote, leading to differential expression of the two alleles of the gene in somatic cells of the

offspring. Genomic imprinting challenges two assumptions of conventional Mendelian genetics applied to human disease: that the maternal and paternal alleles of a gene are equivalent, and that two functional copies of a gene always are associated with health.

Characteristics

Imprinted genes probably account for many examples of developmental malformations in humans, as uniparental disomy (UPD) of several chromosomes is associated with a variety of recognized defects, but UPD affecting most or all of a chromosome is rarely seen. Imprinting may have arisen in mammals as a result of an evolutionary conflict between maternal and paternal genomes. There is a strong and sometimes surprising relationship between imprinted genes and growth, including both prenatal growth and postnatal growth related to nurturing ability. Imprinting is also thought to underlie some quantitative trait loci for growth, with considerable potential commercial application, and imprinting may be a potential barrier to stem cell transplantation. For all these reasons, genomic imprinting has generated intense interest.

Imprinted Genes and their Regulation

Despite this interest, the identity of most imprinted genes remains unknown. At present there are approximately 40 known imprinted genes. Imprinted genes appear to be organized within genomic domains. Evidence for this idea comes from studies of the region of 15q11-13 involved in Prader-Willi and Angelman syndromes, which cause mental retardation and neurological problems. This region harbors at least 6 imprinted genes and the region of the *IGF2R* gene contains at least 3. Similarly, band 11p15 contains a domain of imprinted genes distributed over approximately 1 Mb. These genes play diverse roles including hormone-mediated growth stimulation (insulin-like growth factor II, or IGF2), control of the cell cycle ($p57^{KIP2}$) and ion trafficking (K_VLQT1). Both *cis*-acting and *trans*-acting factors appear to be important in the regulation of genomic imprinting. It is believed that cis-acting sequences can regulate imprinting over large distances. Evidence for this idea comes from the identification of patients with microdeletions involving upstream exons of the small nuclear ribonucleoprotein N gene (SNRPN), in the Prader-Willi region of chromosome 15. These deletions lead to disrupted imprinting extending over several megabases. This deletion site has therefore been termed an 'imprinting center' for this chromosomal region. However, the molecular basis for the function of the imprinting center is as yet unknown.

Imprinting and DNA Methylation. Cytosine DNA methylation also appears to be important in the regulation of genomic imprinting, as loss of the cytosine DNA methyltransferase I gene (Dnmt1) disrupts normal genomic imprinting in mice. DNA methylation is a covalent modification of DNA in which a methyl group is

Genomic Imprinting and Cancer. Table – Key ideas of genomic imprinting and cancer.

genomic imprinting	an epigenetic alteration in the gamete leading to differential allele expression in the offspring
examples of imprinting in disease	• UPD with birth defects • specific disorders (PWS, AS) • human cancer
imprinting in cancer	• LOI in childhood and adult tumors • both gene silencing and gene activation • link to cancer risk and MSI
mechanisms	• DNA methylation • conserved regulatory elements and genomic domains • modifier genes

transferred from S-adenosyl methionine to the C-5 position of cytosine. DNA methylation occurs almost exclusively at CpG dinucleotides. CpG islands, which are sequences unusually rich in CpG dinucleotides, are usually found in the vicinity of imprinted genes.

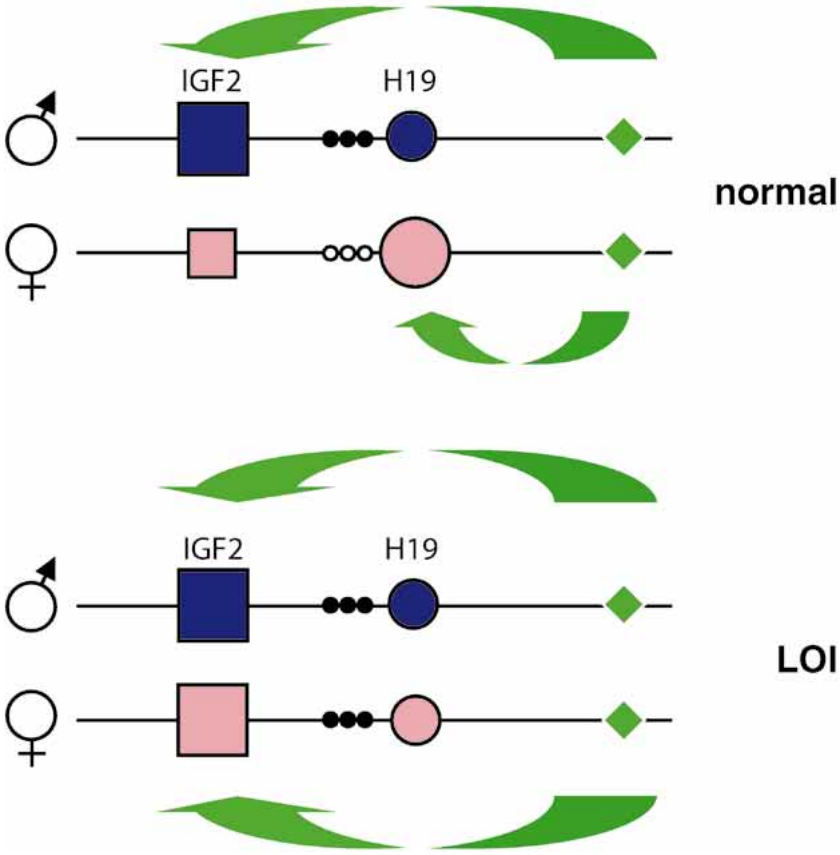
Recently, one mediator of imprinting was discovered. It is the CCCTC-binding factor (CTCF), a transcription factor that binds to an unmethylated, GC-rich sequence approximately 2 kb upstream of the H19 gene. The latter has previously been shown to be necessary for normal imprinting of H19 and IGF2. The same CTCF does not bind to methylated DNA. CTCF is known to be an insulator binding protein, and its binding prevents access by IGF2 to a shared enhancer 3′ to H19 (and about 200 kb telomeric to IGF2 itself).

Loss of Imprinting (LOI) in Cancer. In 1993, it was discovered that IGF2 can undergo loss of imprinting (LOI) in cancer, with abnormal expression of the normally silent maternal allele. LOI of IGF2 has been found in a wide variety of tumors, including embryonal tumors and adult malignancies. In the case of colon cancer, LOI was found not only in the tumors but in the normal tissues of patients with colon cancer. Furthermore, LOI was linked specifically to those tumors with microsatellite instability, a form of genetic instability more common in younger colon cancer patients or in those patients with tumors in the right colon, which are more difficult to detect by conventional screening tests (stool blood testing or sigmoidoscopy). Thus, tests for LOI may eventually prove useful for identifying patients at risk of cancer, thereby reducing overall cancer mortality.

In the case of embryonal tumors, this activation of the maternal allele of IGF2 is coupled to silencing of the maternal H19 allele and methylation of a normally unmethylated maternal H19 CpG island. However, LOI of IGF2 occurs independently of H19 in adult tumors, such as cervical and brain tumors. LOI of IGF2 is now recognized as one of the most common genetic alterations in human cancer. LOI of IGF2 is also found in about 20% of patients with → Beckwith-Wiedemann syndrome (BWS). BWS is a disorder of prenatal overgrowth, birth defects and cancer, which is transmitted as an autosomal dominant trait, although most cases arise sporadically. In addition, LOI of the LIT1 gene, also on 11p15, is found in about half of BWS patients. This gene is particularly interesting, as it is an antisense transcript normally expressed from the paternal allele, that lies entirely within the maternally expressed K_VLQT1 gene. A link between LOI and DNA methylation is also suggested by studies using the drug 5-aza-2′-deoxycytidine, which inhibits DNA methylation. This drug can restore a normal pattern of imprinting to tumor cells with LOI, suggesting that this or other pharmacological agents might eventually be used to treat cancers specifically with LOI or as chemopreventive agents in patients with LOI in their normal tissues (5).

Frequent loss of heterozygosity (LOH) of 11p15 has also been observed generally in embryonal tumors, including → Wilms tumor, → rhabdomyosarcoma and hepatoblastoma. Indeed, LOH of 11p15 is one of the most common genetic changes in cancer and is found in many adult malignancies, including those of the stomach bladder, ovary, breast and lung. Further strong support for the existence of an embryonal → tumor suppressor gene on 11p15 derives from genetic complementation experiments. Microcell-mediated transfer of a human chromosome 11 suppresses the growth of rhabdomyosarcoma cells *in vitro*. Furthermore, subchromosomal transferable fragments limited to 11p15 do suppress tumor cell growth. However, in the investigation of an 11p15 tumor suppressor gene it is puzzling that in virtually all cases of LOH of 11p15 in embryonal tumors it is the maternal allele that is lost, an observation made before the discovery of human imprinted genes or such genes on 11p15 in particular. To address this problem, Sapienza (7) argued that a gene that is not normally imprinted might become so aberrantly. According to Sapienza (7), a specific parental allele (in this case the paternal) would become silenced in some individuals. One or more of the maternally expressed genes on 11p15 might fulfill this role.

One of the most exciting frontiers in the study of genomic imprinting and its role in cancer is the identification of sequences that lie be-



Genomic Imprinting and Cancer. Fig. – Upper panel shows a normally imprinted IGF2 and H19 gene, with IGF2 expressed (drawn large) from the paternal allele, and H19 from the maternal allele. A shared enhancer (green) cannot cross an unmethylated CpG island (small open circles) upstream of the maternal H19 allele, presumably because of binding of CTCF to this island. The lower panel shows loss of imprinting (LOI) in cancer, with a switch of the paternal chromosome to a maternal epigenotype. Here the H19 CpG island is also methylated on the maternal chromosome, allowing the enhancer to interact with IGF2, with activation of the maternal IGF2 allele and silencing of the maternal H19 allele.

tween genes, which might serve a regulatory role in the maintenance of normal imprinting of large genomic domains, such as on 11p15. One recent approach to identifying such sequences is by comparative genomics, in which the mouse sequence is obtained and compared to human sequence, in order to identify species conserved orthologous elements. These elements can then be tested in functional assays and be analyzed for mutations or deletions in patients with cancer or BWS. Proteins that interact with such sequences could include as yet unidentified modifiers of DNA methylation and/or genomic imprinting. Thus, one of the most important implications of the study of imprinting is that the lessons learned may be applicable to understanding the regulation of genomic domains generally, and their dysregulation may provide novel insights into the mechanism of cancer.

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Genomic Instability

Definition

Genomic instability is the failure of tumor cells to maintain genomic integrity. It results from mutations in genes involved in DNA repair (such as → mismatch repair) or in maintaining chromosome structure.

Genomics

Definition

Genomics is the sequencing and characterization of the genome and the analysis of the relationship between gene activity and cell function.

Genotype

Definition

Genotype is the genetic constitution of an organism.

Germinal Center

Definition

The germinal centre (GC) is the secondary lymphoid follicle present in the lymph node cortex that serves as the site of B cell maturation to antibody producing cells. In the secondary lymphoid organs, B-cells are localized in follicles. When B-cells encounter their specific antigen and cooperating T-cells, they undergo intense proliferation to form a germinal center.

On microscopic examination the GC is divided into:

- the dark zone, where B-cell blasts (centroblasts) are proliferating, and
- the light zone, where small 'centrocytes' make contact with follicular dendritic cells.

The GC is a specialized microenvironment in which B-cell proliferation, somatic mutation and selection for antigen binding all occur. In contrast, neoplastic follicles consist of a single clone of B-cells that have centrocytic or centroblastic/centrocytic morphology.

Germinoma

Definition

Germinoma is an embryonal neoplasm derived from totipotent germ cells arising in the pituitary.

GGR

Definition

→ Global genome repair.

Gleason Grading System

Definition

Gleason grading system is a histological grading system for prostate cancer [→ prostate cancer, clinical oncology] that assigns scores to the two most predominant foci of adenocarcinoma based on glandular architecture. Gleason score is the most commonly used system of classifying histiologic characteristics of prostate adenocarcinomas, based on the sum of the grades of the two most predominant glandular architectures within the tumor.

Glioblastoma Multiforme

Definition

Glioblastoma is the most malignant of → astrocytic tumors. This → brain tumor is composed of poorly differentiated astrocytes. The tumor typically affects adults; may develop from low grade astrocytoma, but more frequently manisfests itself *de novo,* after a short clinical history without indication of a less malignant precursor lesion. Glioblastomas are the most frequent brain tumors with 2-3 new cases per 100 000 population per year. They can develop at any age, although incidences are peaking at 45 to 70 years of age. Glioblastomas are among the most malignant human cancers, with the average survival rate of less than 1 year for cases of primary glioblastomas.

Glioma

Definition

Glioma is a collective term for all stages of → brain tumors of astrocytic and oligodendrocytic origin.

Global Genome Repair

Definition

Global genome repair (GGR) is a subpathway of → nucleotide excision repair that removes lesions from nontranscribed DNA.

Glucocorticoid

Definition

Glucocorticoid is a steroid hormone produced in the adrenal gland that regulates glucose metabolism.

Glutathione S-transferase

Definition

Glutathione S-transferase is a superfamily of enzymes that catalyse the conjugation of reduced glutathione to compounds that possess an electrophilic carbon atom. Certain members of this family also possess glutathione peroxidase as well as steroid and prostaglandin isomerase activities; → detoxification.

Glycoprotein

Definition

A glycoprotein is a protein modified by the addition of relatively short sugar chains.

Glycosaminoglycan

Definition

A glycosaminoglycan is a long repetitive sugar chain attached to a protein core.

G_2/M Checkpoint

Definition

G2/M is a point in the G_2 phase of the cell cycle where cells become arrested in response to DNA damage. The G_2/M → checkpoint is regulated by proteins that modify or sequester the components of the Cyclin B/Cdc2 kinase complex, the principal enzymatic activity responsible for the initiation of mitosis. It is one of two major checkpoints (G_1/S and G_2/M) at which the transition from one phase of the cell cycle to the next can be stopped. The initiation of mitosis is subject to a number of checkpoint controls. These controls serve to arrest cell-cycle progression, if progression would otherwise result in progeny with incomplete or damaged genomes. To progress into mitosis the nuclear DNA of a cell has to be completely replicated and be in an undamaged state so it can condense into complete chromosomes. Cells in G_2 phase that sustain DNA damage will arrest prior to entering mitosis. This arrest continues until their DNA is sufficiently repaired. If damage cannot be repaired, apoptosis is induced. Other conditions such as separation of centrosomes to allow spindle pole formation are also necessary for a cell to begin the → G_2/M transition. As transformed cells typically lack some G_2/M checkpoint mechanisms and most studies have been carried out using such cells, it is likely that there exist additional checkpoint conditions yet to be identified.

G_2/M Cyclins

Definition

The cyclin family members can be divided into G_1/S and G_2/M subfamilies depending on when their associated cyclin-dependent kinase (CDK) activity peaks. Cyclins B1, B2 and A are all G_2/M cyclins. In humans these cyclins localise CDK1 to different parts of the cell as it enters mitosis. This probably confers some degree of substrate specificity and allows them to be differentially regulated, by keeping CDK1 away from or in contact with its respective regulators. Cyclin A/CDK2 is localised to the nucleus. Its role in inducing G_2/M events is unclear, however its activity peaks at this point of the cell cycle.

Cyclin B1/CDK1, translocated to the nucleus at G_2/M, can initiate → NEBD, inactivate the nuclearly localised inhibitory kinase wee1 and activate nuclearly localised phosphatase cdc25C. The abundance of this complex (cyclin B1/CDK1) enables it to efficiently phosphorylate many other substrates at the → G_2/M transition.

Cyclin B2CDK1, present at much lower amounts within the cell as compared to cyclin B1/CDK1, is localised to the Golgi apparatus and is involved in breaking up of this organelle during mitosis.

G_2/M Transition

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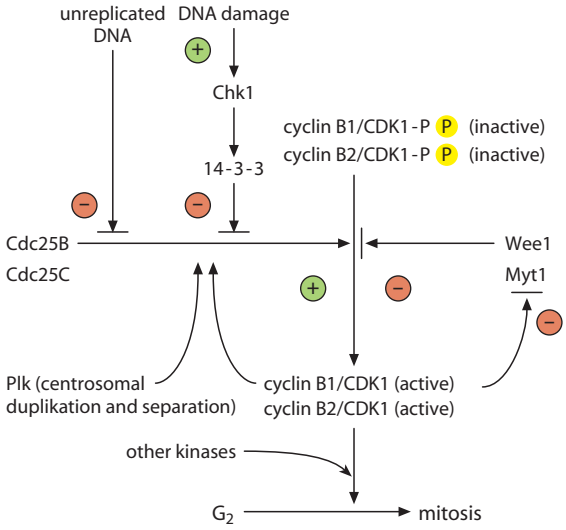
Definition

The G_2/M transition is a decisive point in a cells life cycle. The point at which, after successfully completing a second growth phase (G_2 phase) following the replication of its DNA (S phase), it begins mitosis (M phase), the phase during which it physically separates itself into two daughter cells.

Characteristics

To divide itself equally into two daughter cells a cell must fundamentally change almost all of its structures. These dramatic structural changes become apparent at the G_2/M transition. In addition to these structural changes, RNA processing stops and majority of mRNA translation is repressed (there are some specific mRNA sequences that can overcome this inhibition allowing them to be translated during mitosis). The G_2/M transition occurs because of a pivotal



G_2/M Transition. Fig. – Regulating the G2/M transition.

shift in the balance of kinase and phosphatase activities within the cell. A cell will not usually commit to such big changes unless a number of criteria have been met, such as accurate replication of its DNA or separation of its centrosomes. Such criteria are sensed and acted upon via → G_2/M checkpoint pathways.

Structural changes at the G_2v/M transition

One of the first visible signs that a cell is about to enter mitosis is the increasing compaction of its DNA. Cells stained with fluorescent DNA-binding dyes typically show a more granular nuclear staining pattern than is normally seen. As DNA becomes more condensed (a process known as → chromosome condensation), the nuclear envelope surrounding it disassembles (a process called → nuclear envelope breakdown or NEBD). NEBD effectively breaks the down nuclear-cytoplasmic compartmentalisation within the cell. The → nucleolus within the nucleus also disassembles causing RNA synthesis and processing to stop.

In the cytoplasm, membrane trafficking and sorting between the → endoplasmic reticulum, → Golgi apparatus and plasma membrane ceases. At the same time the endoplasmic reticulum and Golgi apparatus fragment, enabling these organelles to distribute themselves easily between the two daughter cells. Anchored cells grown in culture begin to round up at G_2/M; this is due to profound changes in the cytoskeleton seen in all cells as they enter mitosis. Actin and intermediate filament networks are reorganised. Microtubules undergo a dramatic change in their dynamic stability i.e. the rates at which they grow and shrink in length. The number of microtubules nucleated from each centrosome increases approximately ten-fold, but the length of these more abundant microtubules decreases by ten-fold. This change in microtubule dynamics together with the separation and moving apart of the two centrosomes allows the formation of a → spindle apparatus. The spindle apparatus functions to organise the chromosomes into an aligned configuration, a stage in mitosis known as metaphase. Following metaphase, the pairs of sister chromatids can separate from one another and migrate to the opposite poles of the spindle apparatus (a stage known as anaphase), allowing the final stage of mitosis (telophase) to proceed.

Triggering G_2/M transition

Many kinases and phosphatases are needed for a cell to complete its G_2/M transition (1, 2). The best characterised and most important of these being the cyclin B1/cyclin-dependent kinase (CDK1), sometimes called maturation promoting factor (MPF). Cells injected with active cyclin B1/CDK1 can be induced to enter mitosis. Cyclin B1/CDK1 activity, often taken as a measure of the mitotic state of a cell lysate, can be measured by its ability to phosphorylate exogenously added histone H1 as this is a very good *in vitro* substrate of cyclin B1/CDK1. The importance of cyclin B1/CDK1 in its activity for driving a cell into mitosis is reflected in the many ways in which its activity is regulated.

Members of the cyclin protein family each bind to a specific member of the cyclin-dependent kinase (CDK) family of serine/threonine kinases (3). CDK's alone are inactive, however when bound to a cyclin, the conformation of CDK changes to an active one. Thus the amount of cyclin within the cell regulates the amount of potential CDK activity. Cyclin B1 is the most

abundant → G_2/M cyclin protein and it associates with only one member of the CDK family, CDK1. It accumulates throughout S and G_2 phase, rapidly associating with CDK1 as it is synthesised (CDK protein levels are usually in excess of that of cyclin B1). This would cause a slow increase in CDK1 kinase activity as the cell progresses through S and G_2 phases, but CDK1 is rapidly made inactive by an inhibitory phosphorylation that blocks the active site of the CDK. This inhibitory phosphorylation is carried out by two kinases, wee1 and myt1, which are localised in the nucleus and cytoplasm, respectively. Wee1 and myt1 kinases allow the cyclin B1/CDK1 complex to stockpile in an inactive state. Removing the inhibitory phosphate masking the active site, rapidly activates accumulated cyclin B1/CDK1 causing the cell to enter mitosis. Cdc25C is the major phosphatase that removes the inhibitory phosphate on CDK1 and thus triggers cyclin B1/CDK1 activation. Cdc25B, another cdc25 family member, is also activated at G_2/M, although there is much less of this in the cell relative to Cdc25C. Positive and negative feedback loops reinforce the quick activation of cyclin B1/CDK1; cdc25 phosphatase activity and wee 1 kinase activity can be activated and inhibited respectively by phosphorylation by cyclin B1/CDK1. It is also likely that the timely destruction of the wee1 kinase helps to trigger and maintain activation of cyclin B1/CDK1.

Cells in G_2 phase that sustain DNA damage delay activation of their cyclin B1/CDK1 and thus their progression into mitosis. G_2/M checkpoint controls converge to a point where the phosphorylation state of CDK1 complexed to G_2/M cyclins is regulated. Cells expressing a mutated CDK1 that cannot be inhibited by Cdc25 can partially by-pass a DNA damage induced checkpoint arrest. Chk1 and cds protein kinases are two conserved nuclearly localised kinases needed for the DNA replication and DNA damage G_2/M checkpoints. When activated by the presence of inappropriate DNA breaks or incomplete DNA replication, both phosphorylate cdc25C phosphatase and in doing so create a → 14-3-3 protein-binding site. 14-3-3 protein bound to cdc25p blocks cyclinB1/CDK1 activation by inhibiting the phosphatase's activity and exports nuclearly localised cdc25C protein to the cytoplasm.

The dynamic localisation of key enzymes involved in the G_2/M transition is important in regulating entry into mitosis. During interphase cyclin B1 shuttles between the nucleus and the cytoplasm, but at steady state it is primarily cytoplasmically localised. A defining moment in the G_2/M transition is the rapid nuclear translocation of cyclin B1/CDK1. This is brought about both activating its rapid nuclear import and inhibiting its nuclear export. Shifting cyclin B1/CDK1 into the nucleus is thought to expose it to nuclearly localised activating cdc25 protein. Thus the movement of cdc25 protein out of the nucleus by 14-3-3 protein stops cyclin B1/CDK1 from being activated in the nucleus.

Although Cyclin B1/CDK is the most important kinase in triggering the G_2/M transition, many other kinases and phosphatases are active upon mitotic entry making it difficult to ascribe specific substrates to each kinase (4). Polo-like kinase (Plk) functions in multiple stages of mitosis. During G_2 phase of the cell cycle and early phases of mitosis it is localised to centrosomes and spindle poles where its activity is necessary for centrosome separation and bipolar spindle formation. It may also play a role upstream of cyclin B1/CDK1 activation by helping to activate the cdc25 phosphatase.

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GMP1

Synonyms

- → UBL1

Definition

GAP-modifying protein 1.

Golgi Apparatus

Definition

→ Endoplasmic reticulum.

Gonadotropin-releasing Hormones

Definition

Gonadotropin-releasing hormone (GbRH), also known as luteinizing hormone-releasing hormone (LHRH) is a peptide hormone secreted from the hypothalamus. GnRH stimulates the synthesis and release of LH (luteinizing hormone) and FSH (follicle-stimulating hormone).

Gorlin Syndrome

Definition

Gorlin syndrome was named after Robert J. Gorlin DDS, the first person to describe typical patients. Gorlin syndrome is a term used synomously with → basal cell nevus syndrome (BCNS), and is also called naevoid basal cell carcinoma syndrome (NBCCS).

GP170

Definition

→ P-glycoprotein.

GPCR

Definition

→ G-protein-coupled receptor.

G-protein-coupled Receptor

Definition

There are several hundred types, subtypes and isoforms of → G-protein-coupled receptors (GPCR), which are able to bind a huge variety of ligands have been identified. Since the structural hallmark of the GPCRs are seven transmembrane helical domains, they are also called 'serpentine' receptors. Most receptors are able to activate more than one G-protein subtype, which leads to the activation of various signalling cascades.

G-proteins

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Synonyms

- heterotrimeric GTP-binding proteins
- heterotrimeric guanine nucleotide-binding proteins

Definition

G-proteins are named for their ability to bind and hydrolyze the guanine nucleotide GTP. In the widest sense, the superfamily of guanine nucleotide binding proteins comprises two structurally distinct classes. The monomeric GTP-binding proteins (also called monomeric GTPases) are involved in a variety of cellular processes. The heterotrimeric GTP-binding proteins are primarily involved in transmembrane signal transduction, by coupling membrane receptors to various effector molecules. Traditionally, the term 'G-protein' is only applied to the latter group, the heterotrimeric GTP-binding proteins.

Family members of the trimeric guanine nucleotide-binding proteins reside at the inner side of the plasma membrane. When GDP that is bound to the α-subunit, is exchanged for GTP the α-monomer and the βγ-dimer dissociate and activate or inhibit target proteins. Sixteen distinct α-, eleven γ- and five β-subunits have been cloned to date.

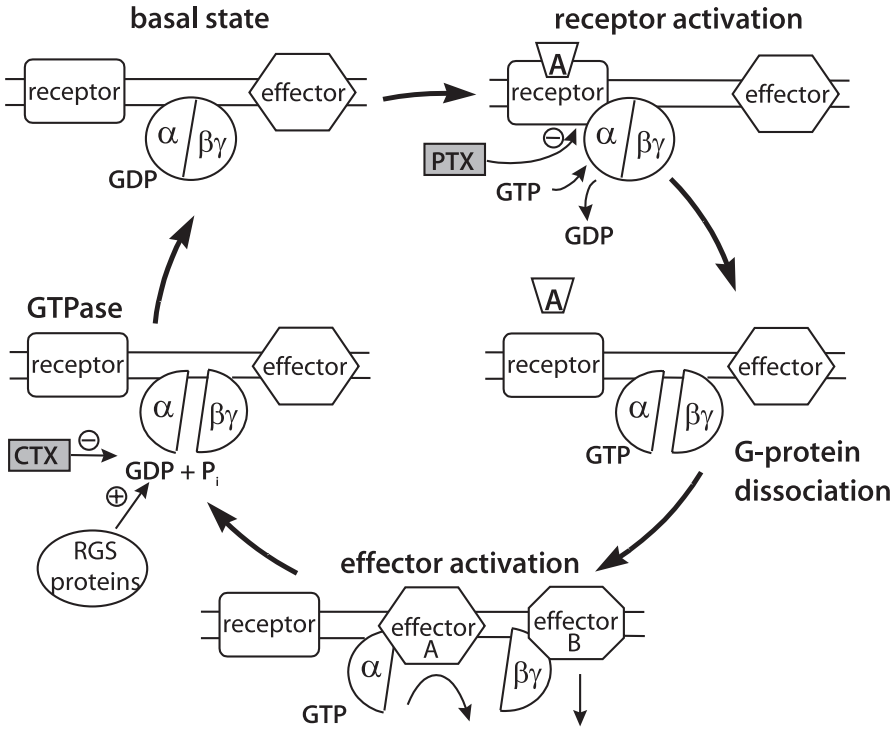
Characteristics

Cellular functions in a living organism are regulated and coordinated by a huge variety of extracellular signals, including hormones, growth factors, paracrine factors, neurotransmitters or sensory stimuli. Many of these signals are received by the cells via receptors on the plasma membrane. These transduce the incoming information by coupling to G-proteins that are attached to the inner side of the plasma membrane. More than 1000 genes that code for G-protein coupled receptors (GPCR) have been identified, making GPCRs one of the largest gene families of the mammalian genome. G-proteins that are activated by GPCRs, regulate various effectors such as enzymes and ion channels. The latter produce intracellular signals that result in specific cellular responses. G-protein functions are highly diverse. This is due to their composition of different α-, β- und γ-subunits, each of them products of different genes. The α-subunit of the heterotrimeric G-protein possesses structural and functional homologies to other members of the guanine nucleotide binding protein superfamily. The β- and γ-subunits of heterotrimeric G-proteins form an non-dissociable complex and represent a functional unit. Some G-proteins are very specialized such as those, expressed only in sensory cells. Others appear to have functions in a wide variety of cells and tissues. Many G-proteins seem to have overlapping distributions and functions, indicating complex functional relationships between different G-proteins.

Molecular and cellular regulation

In order to convey a signal from an activated receptor to an effector, the heterotrimeric G-protein undergoes an activation-inactivation-cycle which allows it to function as a regulatory molecular switch (Fig.). In the basal state, the βγ-complex as well as the GDP-bound α-subunit are associated. In this form, the G-protein is recognized by the appropriate activated receptor that interacts with the G-protein heterotrimer.

This interaction results in the GDP dissociation from the α-subunit of the heterotrimeric G-protein. GDP is then replaced by GTP. Binding of GTP to the α-subunit induces a conformational change, which in turn leads to the dissociation of the α-subunit from the βγ-complex. The GTP-bound α-subunit as well as the βγ-complex are now able to interact with effector proteins. The G-protein α-subunit has an intrinsic GTPase activity that terminates the G-protein activation. The resulting GDP remains bound to the α-subunit, which now reassociates with the βγ-complex. The reassociation of the heterotrimeric G-protein, induced by the hydrolysis of GTP to GDP, represents the inactivation mechanism of the βγ-complex. Two bacterial toxins interfere specially with the G-protein activation-inactivation cycle and have been useful tools in studying G-protein-mediated signalling. → Pertussis toxin blocks the interaction of activated receptors and various G-proteins whereas → cholera toxin leads to the constitutive activation of some G-proteins. A physiological regulation of the GTPase activity of the α-subunit occurs by several effector proteins. They interact with the GTP-bound



G-proteins. Fig. – The G-protein cycle. A, agonist; PTX, pertussis toxin; CTX, cholera toxin; RGS, regulator of G-protein signalling.

α-subunit and accelerate their GTPase activity, leading to G-protein inactivation. In addition, a recently discovered family of proteins, the so-called regulators of G-protein signalling (→ RGS-proteins), are also able to increase the GTPase activity rate of the G-protein α-subunit.

Mammalian G-Protein families

More than twenty G-protein α-subunits have been described in mammals. They can be devided into four subfamilies based on structural and functional homologies (Table). The main properties of individual G-proteins appear to be primarily determined by the identity of the α-subunit of the heterotrimeric G-protein. While some G-protein α-subunits show a very restricted expression pattern, others are expressed in a wide variety of tissues and some G-protein α-subunits like $G\alpha_S$, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{12}$ and $G\alpha_{13}$ appear to be expressed more or less ubiquitously. An individual cell expresses up to ten different G-protein α-subunits. The crystal structure of GDP- and GTP-bound transducin and $G\alpha_{i1}$ has revealed a clearer picture of the molecular processes underlying the guanine nucleotide binding and hydrolysis by G-protein α-subunits. The α-subunit consists of two domains. The GTPase domain is homologous to other GTP binding proteins and contains sites for the binding of guanine nucleotides, the βγ-complex, the receptor and the effector molecule. A second domain is highly helical and specific for G-protein α-subunits, its exact function is not clear.

Five G-protein β-subunits and eleven γ-subunits have been described in mammals. Crystal structure analysis of the βγ-complex showed that the β-subunit possesses a propeller-like structure, formed by seven β-sheets. The γ-subunit is located at one end of the β-propeller structure and is associated with the β-subunit by a tight interaction. With the exception of the β_5-subunit that is expressed mainly in the central nervous system, the currently known β-subunits exhibit a high level of sequence homology (79 – 90%). In contrast, G-protein γ-subunits are much more heterogeneous.

Functions

The βγ-complex has long been regarded as a more passive partner of the G-protein α-subunit. During the last years it has become clear that βγ-complexes also play a very important role in the regulation of various effectors. The best examples of βγ-regulated effectors are particular isoforms of → adenylyl cyclase and → phospholipase C. With few exceptions, no major differences appear to exist between different βγ-combinations with regard to their ability to regulate effector enzymes.

The stimulation-dependent regulation of adenylyl cyclases by G-protein-coupled receptors involves G-proteins of the G_s-family of which two main members are known, G_s and G_{olf}. The ubiquitously expressed $G\alpha_s$ gene gives rise to several splice variants. Four splice variants, two short forms ($G\alpha_{s\text{-}S}$) and two long forms ($G\alpha_{s\text{-}L}$), are closely related in their structure and appear to be functionally indistinguishable. All known adenylyl cyclase isoforms are activated by $G\alpha_s$. In addition, two more long splice forms of $G\alpha_s$, termed $XL\alpha_s$ and NESP55, have been described. The NESP55 protein is unrelated to $G\alpha_s$, whereas the $XL\alpha_s$ protein contains the essential parts of the $G\alpha_s$ protein. It is, however, not clear whether $XL\alpha_s$ is able to regulate adenylyl cyclases and whether it functions in transmembrane signal transduction.

The most widely expressed $G\alpha_{i/o}$-family members are the G_i-type G-proteins (G_{i1}, G_{i2} and G_{i3}) that have been shown to mediate receptor dependent inhibition of adenylyl cyclases type I, II, III, V, VI, VII and IX. Since the cellular levels of these G-proteins are usually relatively high they also represent an important source for βγ-complexes, which can regulate a variety of cellular effectors. The G-protein G_o is the most abundant G-protein in the mammalian nervous system. G_o is involved in the inhibitory regulation of voltage-dependent Ca^{2+} channels, a process which appears to be mediated by the βγ-complex of G_o.

Several G-protein α-subunits are primarily expressed in sensory cells and have been involved in the signal transduction of sensory stimuli. Rod-transducin ($G_{t\text{-}r}$) and cone-transducin ($G_{t\text{-}c}$) play well established roles in the phototransduction cascade in the outer segments of retinal rods and cones. They couple light receptors to downstream signalling components of the retinal phototransduction cascade. In contrast to the transducins, the function of gustducin ($G\alpha_{gust}$) in taste cells is less well understood. Between the four taste qualities sour, salty, bitter and sweet, the latter two tastes appear to be transduced through heterotrimeric G-proteins.

$G\alpha_q$ family members mediate the pertussis toxin insensitive regulation of phospholipase C β-isoforms. The G_q family consists of four members. Their α-subunits are encoded by individual genes with different expression patterns. $G\alpha_q$ and $G\alpha_{11}$ appear to be expressed more or less ubiquitously and are primarily responsible for the coupling of receptors in a pertussis toxin insensitive manner to phospholipase C β-isoforms. In contrast, the murine G-protein α-subunit $G\alpha_{15}$ and its human counterpart $G\alpha_{16}$ are only expressed in a subset of hematopoietic cells. The expression of $G\alpha_{14}$ is restricted to certain organs such as kidney, testis and lung. Receptors activating G_q family members in mammalian systems do not discriminate between G_q and G_{11}. There appears to be little difference between the abilities of both G-protein α-subunits to regulate phospholipase C β-isoforms. $G\alpha_q$ and $G\alpha_{11}$ indistinguishably activate the β1, β3 and β4 isoforms and both are equally poor regulators of phospholipase C β2. The β2 isoform is activated by G-protein βγ-subunits.

The G-proteins G_{12} and G_{13} constitute the G_{12} family and appear to be expressed ubiquitously. It has been difficult to selectively study the cellular processes regulated by G_{12} and G_{13} since G_{12}/G_{13}-coupled receptors also appear to activate G_q-family members. Most knowledge about the signalling processes, regulated by G_{12}/G_{13} is therefore based on the use of constitutively active mutants of $G\alpha_{12}$ and $G\alpha_{13}$. Mutants of both have been shown to cause rearrangements of the actin cytoskeleton by activating the monomeric GTPase Rho [→ RHO family proteins]. Effectors that are directly regulated by $G\alpha_{12}$ and $G\alpha_{13}$ are not known. However, the guanine nucleotide exchange factors (→ GEFs) for Rho, p115RhoGEF and PDZ-Rho-

GEF, have recently been shown to interact with $G\alpha_{12}$ and $G\alpha_{13}$ and represent putative effectors.

In recent years, genes of almost all G-protein α-subunits have been inactivated in mice. The resulting phenotypes of Gα deficient animals have provided some insight into the biological role of G-proteins demonstrating a crucial involvement of G protein mediated signalling cascades in multiple developmental processes as well as processes occuring in the adult organism.

Clinical Relevance

G-protein mediated signalling processes are operating in all human cells. They are involved in many physiological and pathological processes. Many clinically relevant drugs function either as agonists or as antagonists of → GPCRs and exert their effects through G-protein-mediated signalling pathways. Some diseases have been found to be caused by distinct defects in single G-protein α-subunits.

Gain-of-function mutations of the $G\alpha_s$ gene give rise to the *gsp* oncogene which has been found in almost 30% of thyroid toxic adenomas as well as in some thyroid carcinomas and growth hormone producing pituitary adenomas. The sporadic somatic mutation leads to the substitution of Arg201 -the same residue which is ADP-ribosylated by cholera toxin- and results in a constitutively active form of $G\alpha_s$ by blocking its GTPase activity. This leads to the activation of adenylyl cyclase that is independently of receptor agonists. The same sporadic mutation if occuring early in embryogenesis results in a mosaicim causing the McCune-Albright syndrome that is characterized by polyostotic fibrous dysplasia of the bone, precocious puberty and café-au-lait pigmentation of the skin. An analogous mutation of the $G\alpha_{i2}$ gene (the *gip2* oncogene) has been

G-proteins. Table – Mammalian G-protein α-subunits. [a]various splice variants; [b]species variants ($G\alpha_{15}$, mouse; $G\alpha_{16}$, human); [c]effector is regulated by βγ-subunits; PLC, phospholipase C.

class	subtype	expression	effectors
$G\alpha_s$	$G\alpha_s$[a]	ubiquitous	→ adenylyl cyclase (all types) ↑
	$G\alpha_{olf}$	brain, olfactory epithelium	→ adenylyl cyclase ↑
$G\alpha_{i/o}$	$G\alpha_{i1}$	widely distributed	→ adenylyl cyclase (types I,III,V,VI,-VII,IX) ↓
	$G\alpha_{i2}$	ubiquitous	(as above)
	$G\alpha_{i3}$	widely distributed	(as above)
	$G\alpha_o$[a]	neuronal, neuroendocrine cells	Ca^{2+} channel (L-/N-type) ↓[c]
	$G\alpha_{gust}$	taste cells, brush cells	?
	$G\alpha_{t\text{-}r}$	retinal rods, taste cells	cGMP → phosphodiesterase ↑
	$G\alpha_{t\text{-}c}$	retinal cones	(as above)
	$G\alpha_z$	neuronal, platelets	→ adenylyl cyclase ↓/?
$G\alpha_q$	$G\alpha_q$	ubiquitous	→ phospholipase C-β ↑
	$G\alpha_{11}$	almost ubiquitous	(as above)
	$G\alpha_{14}$	kidney, lung, spleen, testis	(as above)
	$G\alpha_{15/16}$[b]	hematopoietic cells	(as above)
$G\alpha_{12}$	$G\alpha_{12}$	ubiquitous	?
	$G\alpha_{13}$	ubiquitous	?

found in human ovarian sex cord stromal tumours and adrenal cortical tumours.

In some patients the loss of one intact $G\alpha_s$ allele, due to various inactivating mutations of the $G\alpha_s$ gene, has been diagnosed. The resulting phenotype depends on the parental origin of the mutated allele. Patients who inherit the inactive allele from their mothers have pseudohypoparathyroidism (PHP) type Ia and show resistance to the effects of various hormones such as parathyroid hormone (PTH) and thyroidea-stimulating hormone (TSH) that act via G_s-coupled receptors. They also show specific phenotypic features such as short stature, bone abnormalities and mild mental retardation, resulting in a syndrome termed Albright hereditary osteodystrophy (AHO). Patients who inherit the mutations from their fathers suffer from AHO only an do not exhibit hormone resistance, a condition called pseudopseudohypoparathyroidism (PPHP). These parent-of-origin specific phenotypes are most likely due to a combination of genomic and tissue-specific → imprinting and tissue-specific → haploinsufficiency of the $G\alpha_s$ gene, which are, even today, not completely understood.

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G-quadruplex DNA

Definition

G-quadruplex DNA is a guanine-rich, square-planar DNA structure held together by Hoogsteen base pairing.

Graft Acceptance and Rejection

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Definition

Graft acceptance and rejection refers to the fate of cells, tissues or organs transplanted between different individuals.

Characteristics

Graft acceptance and rejection depends upon the nature of the graft (cells, tissues or organs) and on the genetic differences between the donor and the recipient. Transplanted cells, such as bone marrow cells or hepatocytes, derive their vascular supply, nutrition and growth factors mainly from the graft recipient (Table).

Transplanted tissues, such as tumor fragments, pancreatic islets and skin, derive some of the vascular supply and growth factors from the donor and some from the recipient. Transplanted organs provide the entire vascu-

Graft Acceptance and Rejection. Table

graft type	source of vasculature	source of growth factors
cells	recipient	recipient
tissues	recipient and donor	recipient and donor
organ	donor	donor

lar supply and most growth factors, the recipient providing only hormones. The ability of cell and tissue grafts to undergo neovascularization and to be supported by growth factors of the recipient determines graft acceptance over a period of days to weeks. The acceptance of grafts also depends on the location and the biological compatibility between the donor and the recipient. For example, bone marrow cells may survive transplantation into some locations such as in bone marrow or in the blood stream, but not others such as into muscle. As another example, bone marrow cells may fail to engraft across species because the growth factors of the recipient are incompatible with the graft.

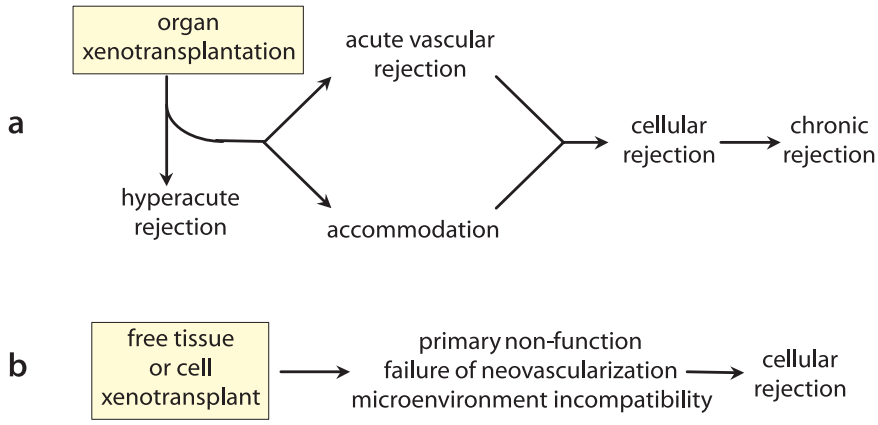
- The most important factor in determining graft acceptance and rejection is the genetic relationship between the donor and the recipient. The genetic relationship determines the homology of growth factors (discussed above) and the ability of the immune system of the recipient to recognize and destroy the graft.
- Grafts from one individual to themselves are referred to as → autografts. Autografts are always accepted if they are placed in a suitable location.
- Grafts between genetically identical individuals are called → isografts. Isografts are usually accepted unless the donor has acquired antigen through infection, chemical modification or mutation.
- Grafts between different individuals of the same species are referred to as → allografts. Allografts are almost always rejected unless the recipient is immunoincompetent or immunosuppressed or the donor and recipient are highly inbred and closely related.
- Grafts between individuals of different species are called → xenografts. Xenografts are always rejected unless the recipient of the graft is immunoincompetent or immunosuppressed.

The biological responses to transplantation are summarized in a Fig. Cell and tissue grafts are subject to primary non-function or failure of engraftment owing to the resistance mounted by natural killer cells, macrophages or other leukocytes. If this hurdle is bypassed, cell and tissue grafts are subject to cellular rejection mediated by T lymphocytes. Organ transplants may be subject to immediate or hyperacute rejection prior to transplantation if the recipient has antibodies specific for the transplant donor. Antibodies that form after transplantation of organ grafts may cause acute vascular rejection or chronic rejection. Organ grafts between different individuals are, like cell and tissue grafts, subject to acute cellular rejection.

Cellular and molecular parameters

The most important antigens determining the rejection or acceptance of grafts are the → major histocompatibility antigens. The major histocompatibility antigens are important because they are polymorphic; thus all individuals other than identical twins in an outbred population are likely to differ in major histocompatibility. Another reason major histocompatibility antigens are important is that they can be recognized directly by T lymphocytes, thus foreign cells can provide a strong stimulus. Recognition of major histocompatibility antigens by T lymphocytes provokes a severe and very rapid immune response. Major histocompatibility antigens may also stimulate the production of antibodies. Alloantibodies, as such, can cause vascular rejection of organ grafts and can be used in tissue typing (Fig. 1). Antigens that provoke less severe and rapid responses are referred to as minor histocompatibility antigens. Any protein or carbohydrate that distinguishes two individuals can potentially serve as a → minor histocompatibility antigen. Minor histocompatibility antigens are generally processed by antigen-presenting cells and presented to a very small fraction of T cells, thus accounting for the slower immune response.

A special type of immune response known as → graft-versus-host disease can occur after bone marrow transplantation. When the transplant consists of or includes mature T lymphocytes, those lymphocytes may attack the recipient of the transplant, thus giving rise to graft versus host disease. Graft versus host disease may be directed against major or minor histocompatibility antigens. Natural killer cells are par-



Graft Acceptance and Rejection. Fig. – The immunological response to xenotransplantation. The immune response to xenotransplantation can be classified according to whether the graft consists of isolated cells or free tissues, such as islets of Langerhans or of a primarily vascularized organ such as the kidney or heart. a) Vascularized organ grafts are subject to hyperacute and acute vascular rejection caused by the action of antidonor antibodies on donor endothelium. If hyperacute or acute vascular rejection are averted, the graft may undergo accommodation, a condition in which the graft appears to resist injury despite the return of anti-donor antibodies to the circulation and the presence of an intact complement system. A vascularized organ graft may also be subject to cellular rejection and chronic rejection more or less like the corresponding types of rejection observed in allografts. b) Free tissue grafts are subject to failure caused by primary non-function, failure of neovascularization or failure of the microenvironment to support the survival and function of the foreign tissue. If the free tissue or isolated cells engraft, they are then subject to cellular or humoral rejection.

ticularly important in resisting foreign bone marrow. Natural killer cells are lymphocytes that can act against a variety of foreign cells which lack self-MHC molecules or which are targeted by antibodies.

One basis for graft acceptance may be immunological → tolerance. Tolerance refers to the specific non-responsiveness of the immune system when an individual is confronted with a graft or an antigen that is generally considered capable of triggering immunity. Tolerance to self-antigens is acquired by developing T cells in the thymus and developing B cells in the bone marrow, and by mature B cells and T cells through various mechanisms and in various locations. Tolerance to foreign cells and antigens may be induced by various means; however, the induction of tolerance often requires severe treatments that lower the ability to respond broadly to foreign antigens.

Clinical aspects

Cell, tissue and organ grafts are often carried out to address the consequences of organ and tissue failure brought about by chemotherapy, cancer or the surgical removal of tumors. Bone marrow or hematopoietic stem cell transplantation is frequently undertaken to enable chemotherapy. Organ transplantation may be undertaken if the removal of the primary tumor or the complications of chemotherapy dictate. However, organ transplantation is avoided as much as possible because the immunosuppression needed retain an organ allograft may hasten the recurrence of a tumor.

Graft-versus-host Disease

Definition

Graft-versus-host disease refers to the clinical manifestations in different organ system (i.e. skin, gut, lungs, liver) secondary to recognition of differences in major histocompatibility complex antigens (MHC) or minor antigenic differences that are differentially expressed between the recipient and the donor. It is a type of re-

jection that develops from grafts, usually bone marrow transplants, containing immunocompetent T lymphocytes that directly recognize and attack recipient cells; → graft acceptance and rejection.

Graft-versus-leukemia

Definition

Graft-versus-leukemia (GVL) effect refers to the immune reaction by effector cells in the donor marrow against the malignant clone of cells after allogeneic bone marrow transplantation; → adoptive immunotherapy.

Granzyme B

Synonyms

- granzyme 2
- → CSP-B
- CGL-1
- CSP-B
- CTLA1
- CCPI

Definition

Granzyme B is a protease, necessary for targeted cell lysis in cell-mediated immune responses. It is linked to the activation cascade of → caspases, cleaving caspase 3, caspase 7 and caspase 10, activating their → apoptosis capacity. It is a protein of 247 aa and 27 kD that is localised in cytoplasmic granules of cytolytic T-lymphocytes and natural killer cells. The gene maps to 14q11.

GRB2

Definition

Growth factor receptor-bound protein 2 (Grb2); → Ras activation.

Growth Factor

Definition

A growth factor binds to and signals, through a plasma membrane receptor, to stimulate cellular growth and/or cell division. They bind to specific cell surface receptors and stimulate cellular proliferation, e.g. → PDGF or → TGF.

GSP

Synonyms

- GNAS1 (guanine nucleotide binding protein, alpha stimulating activity polypeptide 1)

Definition

Guanine nucleotide binding adenylate cyclase-stimulating protein; see → G-proteins.

GST

Definition

→ Glutathione S-transferase.

GTPase

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Synonyms

- GTP-binding proteins

Definition

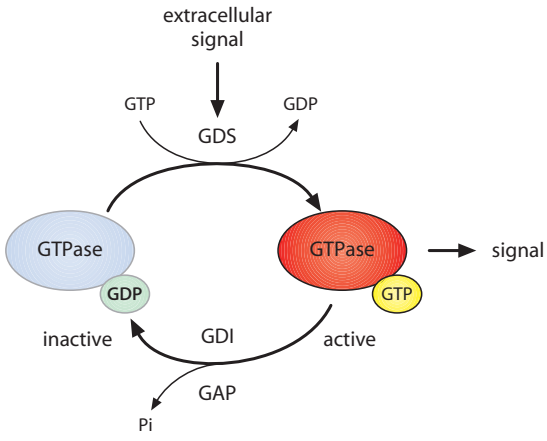
GTPases, also known as GTP-binding proteins, → G-proteins or small GTP-binding proteins are members of the Ras superfamily. They are en-

zymes that catalyzes the hydrolysis of GTP to GDP and inorganic phosphate. They are important intermediaries in signal transduction.

GTPases are proteins that work as molecular switches in the regulation of cell responses to extracellular signals. Their function is regulated by GDP/GTP-cycling, where GDP/GTP exchange promotes formation of the GTP-bound protein and GTP hydrolysis promotes formation of the GDP-bound protein. The intrinsic GDP/GTP exchange and GTP hydrolytic activities are typically low and are further accelerated by regulatory proteins. For some GTPases, the GTP-bound form represents the active form (e.g., → Ras and → Rho family proteins), whereas for others GDP/GTP cycling is required for activity.

Characteristics

The function of GTPases is regulated by an alternating cycle of on/off states depending on their binding to either GTP or GDP, respectively (Fig. 1). Thus, when bound to GTP, these molecular entities acquire a structural conformation that enables them to interact with other proteins called effectors. Effectors are then able to activate specific signalling pathways. When bound to GDP, the conformation of GTPases return back to an inactive state and the signal is interrupted. Frequently, a specific GTPase has the ability to regulate several effectors making their role plastic, versatile and complex (Fig. 2).



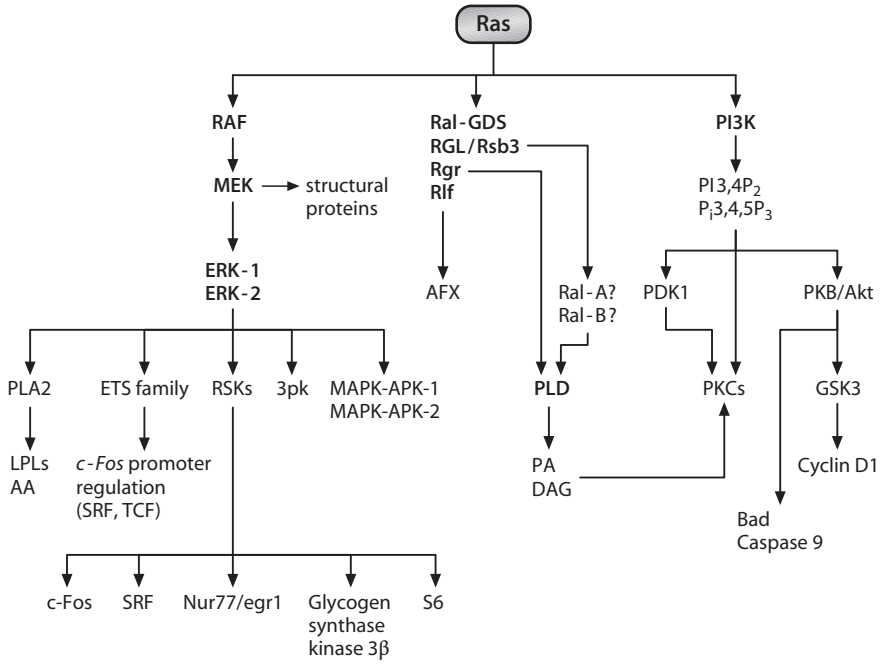
GTPase. Fig. 1 – Activation/inactivation cycle of GTPases.

More than a hundred different GTPases have been identified with some degree of similarity to the → Ras protein, the founder of this class of signalling molecules. All these molecules have been grouped on several branches according to their sequence homology. Three of these branches, the Ras branch itself, the Rho branch, and the Rab branch, include most of the best characterised members of the superfamily. Ras proteins are mostly involved in regulation of cell growth, senescence and differentiation, while the Rho branch is devoted to cell structure and polarity, motility and transcriptional regulation in proliferation and apoptosis. Finally, Rab proteins are involved in secretion and vesicular traffic.

Regulation of GTPases

Regulation of GTPases is achieved both by positive and negative mechanisms (Fig. 1). Specific exchange factors are responsible for the activating loop, catalysing the exchange of prebound GDP for GTP (more abundant in the cell cytoplasm). Exchange factors are regulated by extracellular signals such as receptor tyrosine kinases for growth factors, receptors coupled to heterotrimeric GTP-binding proteins or lipid metabolites-sensitive exchange factors (such as RasGRP). There are exchange factors with a highly specific activity towards members of the superfamily of GTPases, and other exchange factors with a broad spectrum, able to activate efficiently several members of the superfamily.

On the other hand, GTPase activating proteins (GAPs) catalyse, by a factor of at least 100-fold, the intrinsic ability of GTPases to hydrolyse GTP into GDP+Pi, rendering the protein inactive and ready for the next cycle of activation. In some cases, cycling of GTPases are modulated by other proteins in addition to exchange factors and GTPases, such as GDI (GDP dissociation inhibitor) that sequesters GTPases of the Rho branch in their inactive, GDP-bound state. In this case, exchange of GDP by GTP must be accompanied by removal of this inhibitor.



GTPase. Fig. 2 – Specific GTPases can interact with a number of different effector molecules. Here mammalian effectors of the Ras protein and their activated signalling pathways are depicted.

Clinical implications

The activity of several GTPases have been implicated in human diseases like cancer, immunodeficiency such as Wiscott-Aldrich syndrome, developmental disorders such as Aarskog-Scott syndrome or X-linked mental retardation. Alterations at both upstream regulatory elements (exchange factors) or defects on the inactivating process (GTPases) have been described as responsible for the dysfunction of the pathway.

At least two alternative mechanisms for activation of the oncogenic potential of GTPases have been found. Point mutations that reduce their GAP-stimulated GTPase activity would render proteins with a much lower efficiency to catalyse GTP hydrolysis. Such point mutations are found frequently in human tumours where members of the Ras branch are involved. Alternatively, cancer-promoting mutations can also affect components of the regulatory cycle, such as GAPs and exchange factors. This is found in proteins involved in the regulation of Ras such as in the case of neurofibromatosis type 1 (neurofibromin or NF1) and Rho activity (Dbl, Vav, Ost, Ect2, etc). In some cases, point mutations can promote Ras proteins to spontaneously exchange GDP for the more abundant GTP. In all cases, the endpoint result of these alterations is the same: a larger proportion of the GTPase, and for longer periods of time, will be bound to GTP, rendering the protein permanently in their activated state. Thus, it can interact and activate the corresponding signalling effectors.

Signalling pathways

GTPases control a large number of signalling pathways involved in regulation of cell growth, cytoskeleton structure, secretion, transcription and apoptosis. Specific effectors that are under the control of the specific GTPase mediate all these cellular functions. The GTPases most closely related to the carcinogenic process belong to the Ras and Rho branches.

In the case of Ras proteins, at least three major effectors have been identified in mammalian cells (Fig. 2). The Raf kinase, which is connected to the MEK/ERK kinases pathway, im-

pinges on transcriptional regulation of factors mediating cell proliferation such as ETS. Also, PI3K is involved in the regulation of survival signalling through activation of the PDK/PKB pathway. Finally, the Ral-GDS family of exchange factors, including Ral-GDS, Rlf, Rgr and Rgl/Rsb3 participate in important signalling processes such as transcriptional regulation (AFX transcription factors) and lipid-derived signalling through the Ral/PLD pathway, which have implications in cell growth control. Evidence has been generated that a combination of any of two from these three signalling pathways may be sufficient to acquire the transforming phenotype. In addition, cross talking among Ras-dependent and Rho-dependent signalling is an absolute requirement for full transformation.

Regarding the Rho branch, which is also implicated in the carcinogenic process, several effector molecules have been identified. These include the kinases PKN, ROKα/Rho kinase (Rock), PAKs, p70-S6K, ACKs and MLKs, lipid-related enzymes such as phospholipase D (PLD) and PI3K, and other molecules such as p140mDia, citron, rhophilin, rhotekin or WASP. Activation of these effector molecules results in changes in cell morphology and motility, as well as transcriptional activation of the → AP-1, SRF, → NFκB and STAT transcription factors [→ signal transducers and activators of transcription in oncogenesis]. Finally, Rho proteins play a role in the acquisition of the metastatic phenotype. The precise effector that is involved in each of these biological effects and in particular its relationship to cancer, has yet to be assigned.

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GTPase-activating Protein

Definition

GTPase activating protein is an effector protein of → Ras. This regulatory protein stimulates the intrinsic GTP hydrolysis activity of GTPases to promote formation of the GDP-bound protein; it interacts with GTP-bound Ras to transmit a given signal.

Guanine Nucleotide Dissociation Stimulator

Definition

Guanine nucleotide dissociation stimulator is an effector protein of → Ras, interacting with GTP-bound Ras to transmit signals.

Guanine Nucleotide Exchange Factor

Definition

Guanine nucleotide exchange factor (GEF) promotes the exchange of GTP for GDP on monomeric GTPases. Its function is comparable with that of G-protein-coupled receptors [→ G-protein-coupled receptor] (GPCRs) on heterotrimeric → G-proteins. These regulatory proteins accelerate the intrinsic GDP/GTP exchange activities of → GTPases to promote formation of the GTP-bound protein.

GVHD

Definition

→ Graft-versus-host disease.

GVL

Definition

→ Graft-versus-leukemia.

GZMB

Definition

→ Granzyme B.

H

H3

Definition

H3 is the *Rhesus* monkey homologue of → DMBT1, which is up-regulated in the endometrium during the progesterone-dominant phase.

H. pylori

Definition

→ *Helicobacter pylori*.

Hairy/E(Spl) Family

Definition

Hairy/E(spl) family is a group of → bHLH proteins comprising Hairy, E(Spl), deadpan, Hes-1, Hes-5, Her-1, Her-4, Hesr-1, Sharp-1 and Sharp-2. E(Spl) (Enhancer of split) refers to a gene complex in *Drosophila melanogaster* that contains a number of target genes of the Notch signaling pathway.

Hamartin

Definition

Hamartin is the protein encoded by the tumor suppressor gene, TSC1, which is responsible for hamartoma development in tuberous sclerosis complex.

Hamartoma

Definition

A hamartoma, or hamartomatous polyp, is a benign tumor characterized by goblet cell rich mucosa covering branching bundles of smooth muscle from the lamina muscularis mucosa.

Hamartomatous Polyp

Definition

A hamartomatous polyp, or hamartoma, is a benign tumor characterized by goblet cell rich mucosa covering branching bundles of smooth muscle from the lamina muscularis mucosa.

HAND Gene Family

Definition

Hand family is a group of bHLH proteins comprising Hand1 and Hand2, which are involved in heart and neural crest development.

HAND1

Definition

Heart and neural crest derivatives expressed 1 (Hand1), also known as Hxt, Ehand and Thing1, is a → bHLH protein member of the Hand family. Hand1 is expressed in cytotrophoblastic cells, prenatal and adult heart, and is required for trophoblast giant cell differentiation involved in cardiac looping, ventricular maturation and conotruncal development.

HAND2

Definition

Heart and neural crest derivatives expressed 2 (Hand2), also known as Hed, Dhand and Thing2 is a → bHLH protein member of the Hand family and is involved in heart and neural crest development.

Haploid

Definition

Haploid refers to a set of chromosomes containing one copy of each autosome and one sex chromosome; the haploid number n is characteristic of gametes of diploid organisms.

Haploinsufficiency

Definition

Haploinsufficiency describes a situation in which one intact allele of a gene is not able to carry out all functions observable in the presence of both intact alleles.

Haplotype

Definition

The haplotype is a linked set of alleles associated with one parental genome. It is the particular combination of alleles in a defined region of some chromosome, in effect the genotype in miniature. Originally used to described combinations of → MHC alleles, it is now used to describe particular combinations of → RFLPs or → microsatellite alleles.

Haptotaxis

Definition

Haptotaxis is the migration of cells in response to an insoluble gradient of chemoattractants.

HASH-2

Definition

Human achaete-scute complex (*Drosophila*) homolog-like 2 (Hash-2), also known as Ash2, Hash2 and Ascl2 is a human → bHLH protein, member of Achaete-scute family. The human ASCL-2 gene locus maps to 11p15.5.

HATH1

Definition

Human atonal (*Drosophila*) homolog 1 (Hath1), also known as atonal protein homolog 1 (atoh1) is a → bHLH protein member of Atonal family of 354 amino acids and 38 kD. The human ATOH1 or ATH1 gene locus maps at 4q22. Hath1 activates → E-box dependent transcription in collaboration with E12/E47, but the activity is completely antagonized by the negative regulator of neurogenesis Hes-1. It may play a role in the differentiation of subsets of neural

cells by activating E-box dependent transcription. Efficient DNA binding requires dimerization with an other bHLH protein.

Hayflick Limit

Synonyms

- Hayflick number
- irreversible replicative senescence
- mortality stage 1

Definition

The term Hayflick limit refers to the fact that the maximum number of cell divisions of a certain cell population is genetically fixed such that aging cells become non-dividing (senescent) at last. This replicative life span is probably defined by a 'critical' telomere length [telomerase [→ telomerase]]. Human fibroblasts for instance, divide *in vitro* 70 to 80 times, before multiplication reaches a plateau. Usually, the cells then increase in size, flatten in shape and enter an irreversible state of arrest, called senescence; → senescence and immortalization. An exception appear to be human mammary epithelial cells that can escape mortality stage 1, exhibit eroding telomere sequences and ultimately generate the types of chromosomal abnormalities that are seen during early → breast cancer development.

HB-EGF

Definition

Heparin-binding egf-like growth factor (Hb-egf) precursor, also known as Hbegf, Dtr (diphteria toxin receptor) (dt-r) and Egf-like membrane protein, may be involved in macrophage-mediated cellular proliferation. It is a protein of 208 amino acids and 23 kD, and is mitogenic for fibroblasts and smooth muscle but not endothelial cells. It is able to bind Egf receptors with higher affinity than Egf itself and is a far more potent mitogen for smooth muscle cells than Egf is. The human DTR or HEGF gene locus maps to 5q23.

HBV

Definition

→ Hepatitis B virus.

HECT Domain

Definition

Homologous to E6-AP C-terminus (HECT) domain is the defining feature of a large class of → ubiquitin-protein ligases, E3. The HECT domain forms a covalent thiolester linkage to → ubiquitin; → ubiquitination.

Hedgehog

Definition

In vertebrates there are three hedgehog (Hh) genes (*Sonic*, *Indian* and *Desert*) that encode secreted proteins involved in embryonic patterning of a wide range of tissues; → hedgehog signalling.

Hedgehog Signalling

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Definition

The hedgehog signalling pathway is a highly conserved pathway responsible for the correct embryonic patterning of a range of tissue and organ types including skin, hair, lung, limbs,

skeleton and neural system. Members of this signalling pathway have been implicated in the pathogenesis of a range of tumour types including

- → basal cell carcinoma (BCC)
- → medulloblastoma.

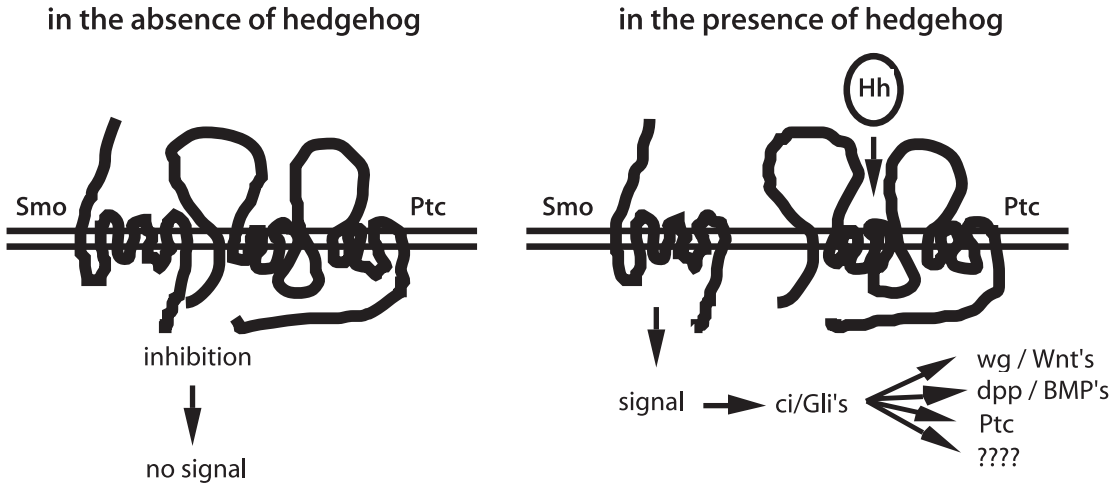
Characteristics

The → hedgehog proteins are secreted molecules that act on adjacent cells through a receptor complex involving two additional proteins, → patched and smoothened. The current model for hedgehog signalling suggests that in the absence of hedgehog, patched inhibits smoothened at the cell surface. However, when hedgehog binds to patched, the inhibition of smoothened signalling is released resulting in the activation of downstream target genes (Fig.).

The pivotal role of the hedgehog signalling pathway in tumour formation was first realised with the discovery that the *patched* gene is mutated in a rare inherited cancer predisposition syndrome known as → Gorlin syndrome or naevoid basal cell carcinoma syndrome (NBCCS). NBCCS is characterised by a range of embryonic developmental defects in addition to cancer predisposition. While the major tumours seen in NBCCS patients are BCCs, other neoplasms that occur at an increased incidence in this syndrome include medulloblastoma, ovarian fibroma, meningioma, fibrosarcoma, rhabdomyosarcoma and cardiac fibroma.

In addition to being responsible for NBCCS, abnormalities in the patched gene have also been shown to be involved in a large percentage of the common sporadic forms of BCC that occur in the general community as a result of factors such as sun exposure. It is now known that *patched* acts as a → tumour suppressor gene, with inactivation of both copies required for tumour progression. NBCCS individuals are born with one inactivated copy of the *patched* gene in every cell of their bodies, and tumour formation results when the other copy is inactivated in a given cell throughout life. This will occur more frequently and at a younger age than inactivation of both copies in the same cell during the life of a non-NBCCS individual. In addition to BCCs, mutations in *patched* have also been demonstrated in a subset of sporadic medulloblastomas, tricoepitheliomas (TEs), esophageal squamous cell carcinomas and transitional cell carcinomas of the bladder. In addition *smoothened* has been shown to act as an → oncogene, with activating mutations detected in both BCCs and medulloblastomas.

Because inactivation of *patched* and activation of *smoothened* have the same net effect of activating genes downstream of hedgehog signalling, it is likely that the nature of these downstream genes is fundamental to tumour development. The identity of a number of these



Hedgehog Signalling. Fig.

genes can be inferred from studies in the fruitfly *Drosophila melanogaster* and are likely to include members of the *Wnt* and *TGF*β gene families, both of which have been implicated in tumour formation. However, it is likely that other as yet unidentified targets of hedgehog signalling exist, and the identification of these molecules is likely to provide valuable insights into the molecular aetiology of tumorigenesis.

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HEIR-1

Definition

Id3 [→ Id proteins].

Helicase

Definition

Helicase is an enzyme that separates the complementary strands of a DNA duplex.

Helicobacter pylori

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Definition

Helicobacter pylori, identified in Australia in 1983, is a gram-negative spiral bacterium found in the mucus layer of the stomach.

Characteristics

H. pylori is found in the stomachs of about one third of adults in developed countries (where it has been decreasing in prevalence) and in about two thirds of adults in developing countries. It is strongly correlated with indicators of low socio-economic status, probably spread person to person, usually acquired in early life, and generally persists until old age unless eradicated with specific antibiotic treatment.

Treatment

A one week regimen of two broad spectrum antibiotics (eg, amoxicillin and clarithromycin) and an acid-suppressing agent (eg, omeprazole) can eradicate the infection in about 80% of individuals. Preventive and therapeutic vaccines are under development.

Associated neoplasms

Chronic *H. pylori* infection has been associated with two different gastric neoplasms:

1. *Gastric adenocarcinoma.* Overall, prospective epidemiological studies indicate that gastric cancer is 2 or 3 times as common in people chronically infected by *H. pylori*. Strong associations have also been reported between infection and putatively pre-cancerous lesions (such as atrophic gastritis and intestinal metaplasia). Long-term experimental *H. pylori* infection of Mongolian gerbils produces lesions that resemble human gastric cancer. Experts, however, remain divided about its causal relevance: in 1994 the International Agency on Research in Cancer declared *H. pylori* a 'carcinogen', but a consensus panel of the National Institutes of Health disagreed. Several randomised trials of *H. pylori* eradication in the prevention of gastric cancer are in progress, most of which should be reported by 2010. Even collectively, however, the present trials may well be too small and too brief to assess reliably a plausible reduction in risk.
2. *Mucosa associated lymphoid tissue (MALT) lymphoma.* Collectively, several small stu-

dies indicate complete histological remission in about three-quarters of patients with MALT lymphoma following *H. pylori* eradication treatment. More prolonged studies are needed to determine if these effects are sustained (since the mean duration of follow-up in the previous studies was only about a year). The relevance of *H. pylori* to this tumour is further suggested by the presence of lymphoid follicles in a high proportion of people with *H. pylori*-related gastritis (whereas such lesions are usually absent in normal stomachs) and by reports of MALT tumour stimulation *in vitro* by bacterial antigens.

Non-neoplastic diseases

In addition to gastric neoplasms, it has been suggested that chronic *H. pylori* infection is relevant to a variety of other conditions. Randomised trials have demonstrated that most recurrences of peptic ulceration can be prevented by *H. pylori* eradication. Preliminary associations of *H. pylori* infection with non-ulcer dyspepsia and other conditions (e.g, coronary heart disease) are being investigated.

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Helpervirus

Definition

A helpervirus provides functions absent from a defective virus, enabling the latter to complete the infective cycle during a mixed infection. It is required by some viruses, such as → AAV or incomplete → retrovirus, to undergo a productive infection.

Hematological Malignancies

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Definition

Hematological malignancies are a collective term for → leukemia and → lymphoma.

Characteristics

The hematopoietic system

The blood contains a number of different cell types, which can be divided into cells with and without a nucleus. The unnucleated cells are red blood cells (erythrocytes) and platelets (thrombocytes). The nucleated white blood cells consist of three major subclasses: granulocytes, lymphocytes and monocytes, which can be further categorized as eosinophil, basophil or neutrophil granulocytes, CD4- or CD8-positive T-lymphocytes, CD5-positive or negative B-lymphocytes and natural killer (NK)-cells. These cells are all derived from a common ancestor, the pluripotent progenitor cell in the bone marrow. In the bone marrow, different lineages are derived from this pluripotent progenitor. Precursor cells proceed through specific maturation steps before they, as mature circulating cells, leave the bone marrow in order to circulate in the bloodstream. Fig. 1 shows a simplified schematic representation of these processes. The pluripotent progenitor cell is shown in black in the middle, cells which are normally found only in the bone marrow (or thymus) are in blue, cells normally circulating in the blood are in red and cells found in tissues are in green.

Two major lineages can be defined: The myeloid lineage (shown in blue on the left

side) and the lymphoid lineage (shown in green on the right side). Platelets, erythrocytes, granulocytes, monocytes and dendritic cells are derived from the myeloid lineage. T- and B-lymphocytes, plasma cells and NK-cells are derived from the lymphoid lineage, although NK-cell precursors are not as exactly defined as B- or T-cell lymphoid precursors. The lineages, and the maturation steps involved, can be recognized using morphologic, cytochemical and immunophenotypic features. Similarly, hematologic malignancies are categorized according to such features.

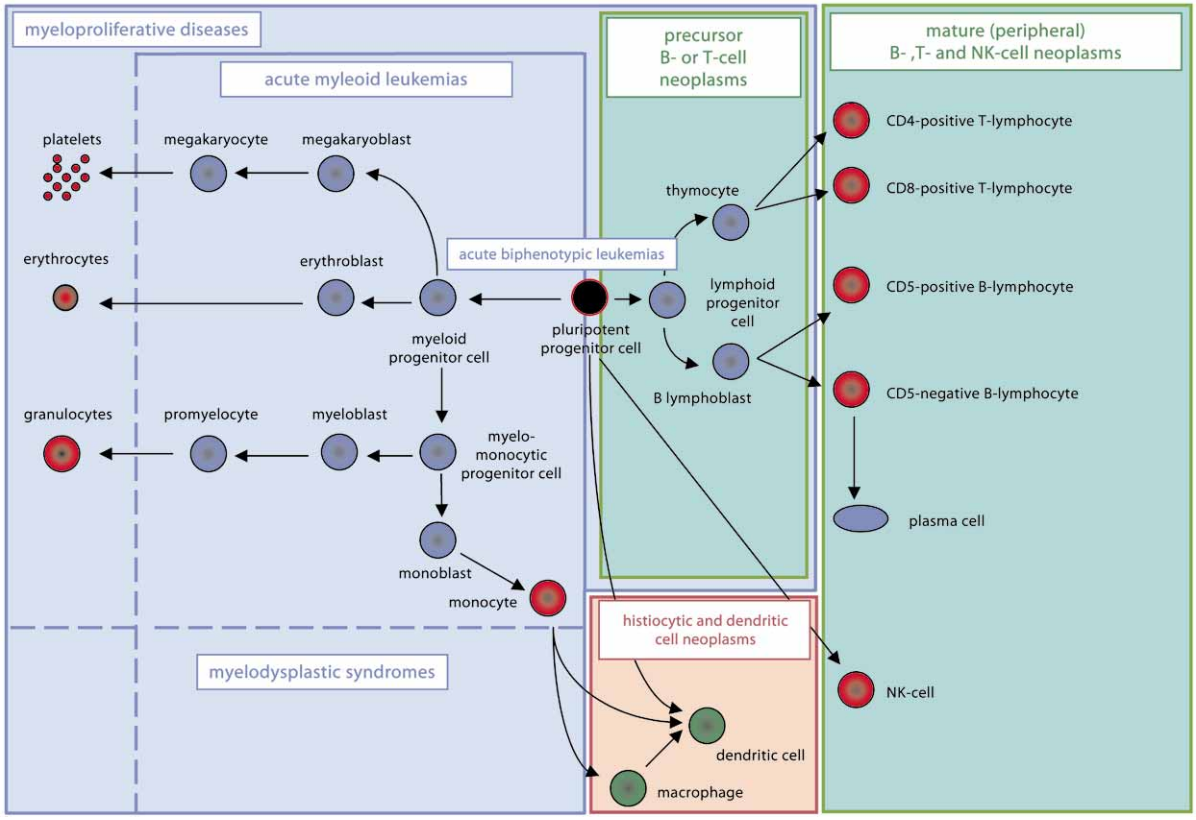
Classification

The World Health Organization (WHO) recently published an updated classification of neoplastic diseases of the hematopoietic and lymphoid tissues. In relation to the development of hematopoietic cells, the major WHO classes can be viewed as disease 'domains', based on the cell types primarily affected in each class and specific subclass. The principles of this classification are shown in Fig. 2.

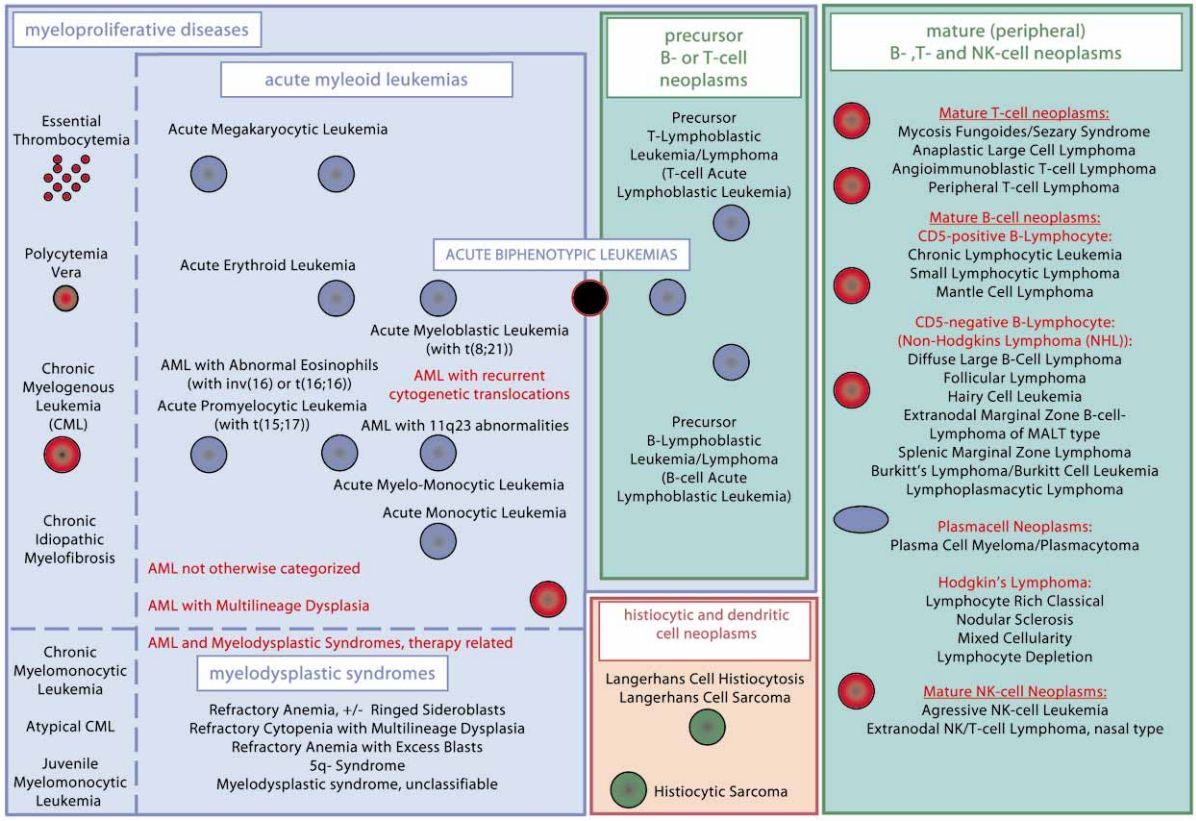
The domains of the myeloid neoplasms are on the left side, histiocytic and dendritic cell neoplasms are at the bottom and the domains of lymphoid neoplasms are on the right side. The WHO classification adopts the REAL classification of lymphoid neoplasms, whereas the classification of myeloid neoplasms has been revised in several categories compared to the FAB-classification. Each major class consists of subclasses, which subsequently categorize the specific disease entities, the most common of which are shown in Fig. 2.

Myeloid neoplasms

The class includes myeloproliferative diseases, myelodysplastic syndromes, myeloid diseases with both proliferative and dysplastic features and the acute myeloid leukemias.



Hematological Malignancies. Fig. 1



Hematological Malignancies. Fig. 2

- Myeloproliferative diseases are disorders of 'effective' hematopoiesis and primarily increase the level of one or more of the circulating myeloid cell lines resulting in essential thrombocytemia, polyctemia vera or chronic myelogenous leukemia (→ CML). Chronic idiopathic myelofibrosis is also recognized within this subclass, along with more rare disorders as chronic neutropilic leukemia, chronic eosinophilic leukemia and unclassifiable myeloproliferative diseases. The 'domain' of these diseases is however larger than the peripheral blood, since all of these diseases have the potential to transform into acute leukemia, including leukemias with a lymphoid phenotype. Furthermore, a small but distinct class of myeloproliferative diseases also show dysplastic features. Therefore the WHO classification has defined a class of myelodysplastic/myeloproliferative diseases, shown in the lower left corner of Fig. 2. This class includes chronic myelomonocytic leukemia, atypical chronic myelogenous leukemia and juvenile myelomonocytic leukemia.
- The myelodysplastic syndromes (MDS) are diseases of ineffective hematopoiesis, resulting in decreased levels of one or more of the circulating myeloid cell lines. As the myeloproliferative diseases, the myelodysplastic syndromes can also transform but only to acute myeloid leukemias, suggesting that the domains of these diseases may be somewhat smaller than that of myeloproliferative diseases. The class includes refractory anemia (with or without ringed sideroblasts), refractory cytopenia with multilineage dysplasia, refractory anemia with excess blasts, 5q-syndrome and unclassifiable myelodysplastic syndromes.
- The acute myeloid leukemias (AML) can be divided into sublasses (as shown in red in Fig. 2): AML's with recurrent cytogenetic translocations, AML with multilineage dys-

plasia (with or without preceeding myelodysplastic syndrome), therapy-related AML and myelodysplastic syndromes and AML not otherwise categorized. Conversion from myelodysplastic syndrome to ouvert AML is defined by a blast count exceeding 20% of the cells in the bone marrow.

- Finally acute biphenotypic leukemias are included among myeloid neoplasms in the WHO classification.

Lymphoid neoplasms

Includes two classes, precursor B- or T-cell neoplasms and mature (peripheral) B-, T/NK-cell neoplasms (Fig. 2, right side).

- The precursor neoplasms are the acute lymphoblastic leukemias (ALL) of either B- or T-lineage origin, but also include the presentation of these diseases as lymphoblastic lymphomas.
- Mature (or peripheral in the sense that these neoplasms arise in mature circulating lymphocytes) lymphoid neoplasms are divided into subclasses of T-, B- and NK-cell neoplasms. Shown in Fig. 2 are only the most common forms of lymphoma and not those associated with posttransplant states nor the numerous variant forms (1). Mature T-cell neoplasms include mycosis fungoides (and the leukemic variant of this lymphoma; Sezary syndrome), anaplastic large T-cell lymphoma, angioimmunoblastic T-cell lymphoma, peripheral T-cell lymphoma and a number of more rare diseases. The mature B-cell neoplasms can be divided into those arising in B-cells that express the membrane protein CD5 (B-cell chronic lymphocytic leukemia/small lymphocytic leukemia (CLL) and mantle cell lymphoma) and those arising in conventional CD5-negative B-lymphocytes or plasma cells: the non-Hodgkin lymphomas (NHL), → Hodgkin disease and plasma cell disorders, of which numerous specific lymphomas/leukemias exist. The CD5-negative mature B-cell neoplasms are the most common forms of malignant lymphoma and include diffuse large B-cell lymphoma, follicular lymphoma, extranodal marginal zone B-cell lymphoma of MALT (mucosa associated lymphoid tissue) type, splenic marginal zone lymphoma (with or without villous lymphocytes), Burkitt lymphoma/Burkit cell leukemia (formerly classified as ALL L3 in the FAB-classification) and lymphoplasmacytic lymphoma (with the clinical syndrome of Waldenströms macroglobulinemia) plus a large number of rarer conditions and variants (1). These B-lymphocyte lymphomas/leukemias have a very heterogenous clinical course and biology, from highly aggressive but potentially curable diseases such as diffuse large cell lymphoma and Burkitt lymphoma [→ Epstein-Barr virus, → malignant lymphoma], to indolent but incurable diseases as CLL and follicular lymphoma. Based on histology, Hodgkin disease is subclassified as either nodular lymphocyte-predominant Hodgkin lymphoma or classical Hodgkin lymphoma with the four specific subtypes: nodular sclerosis, lymphocyte-rich, mixed cellularity and lymphocyte depletion Hodgkin lymphoma. The plasma cell neoplasms are dominated by plasma cell myeloma (multiple myeloma or myelomatosis), but also include several variants from monoclonal gammopathy of unknown significance to localised plasmacytoma or disseminated plasma cell leukemia. NK-cell neoplasms are rare, but include both lymphoma and leukemia subtypes (Fig. 2, bottom right).

Other hematological neoplasms

Also shown in Fig. 2 are histiocytosis and the rare sarcomas arising from macrophages or dendritic cells. Not shown are the rare neoplasms arising in mast cells, including systemic mast cell disease or mastocytosis.

Diagnosis

The complexity of the hematopoietic system (Fig. 1) is reflected by the existence of a wide spectrum of distinct tumors, with numerous variants (Figs. 1 and 2). Optimal treatment of a given condition is dependent on exact classification of the disease. It is not sufficient to rely solely on morphology, cytochemistry and im-

munophenotype, since some specific conditions, which require specific treatments, can only be identified based on cytogenetics or even more sensitive molecular methods as fluorescent in-situ hybridisation (FISH), polymerase chain reaction (PCR) or DNA-sequencing of specific genes. The classification of hematological malignancies described here is based on these features, but also recognizes the clinical course of specific entities, such as the establishment of MDS- or therapy-related AML's as diseases with particularly poor prognoses compared to AML's with recurrent cytogenetic translocations. It is likely that the continued elucidation of molecular mechanisms underlying hematological malignancies and the application of new technologies, such as microarray technology, in this research will establish new entities and create a need for further revisions of the dynamic classification scheme in the near future.

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Hematopoiesis

Definition

Hematopoiesis is the process of blood cell formation from hematopoietic stem cells in the bone marrow.

Hemizygote

Definition

A hemizygote is a diploid individual that has lost its copy of a particular gene (for example, because of a → deletion) and which therefore has only a single copy.

HEN Family

Definition

Hen family is a → bHLH protein family comprising human Hen1 and Hen2, mouse Nhlh1 and Nhlh2, which are implicated in development of mammalian nervous system.

Hensin

Definition

Hensin is the rabbit homologue of → DMBT1, which induces a reversal of polarity and terminal differentiation of kidney epithelial cells.

Heparan Sulfate

Definition

Heparan sulfate (HS) is a linear, highly charged polysaccharide composed of repeating hexuronic (L-iduronic or D-glucuronic acid) and D-glucosamine disaccharide units that are substi-

tuted to a varying extent with N- and O-linked sulfate moieties and N-linked acetyl groups; → heparanase.

Heparan Sulfate Moieties

Definition

Heparan sulfate proteoglycans can be attached to surface molecules such as → CD44. Fibroblast growth factors (FGFs) have a high affinity for heparan sulfate; this binding is required for biological activity of the FGFs.

Heparan Sulfate Proteoglycans

Definition

The basic heparan sulfate proteoglycan (HSPG) structure consists of a protein core to which several linear heparan sulfate (HS) glycosaminoglycan (GAG) chains are covalently O-linked; → heparanase.

Heparanase

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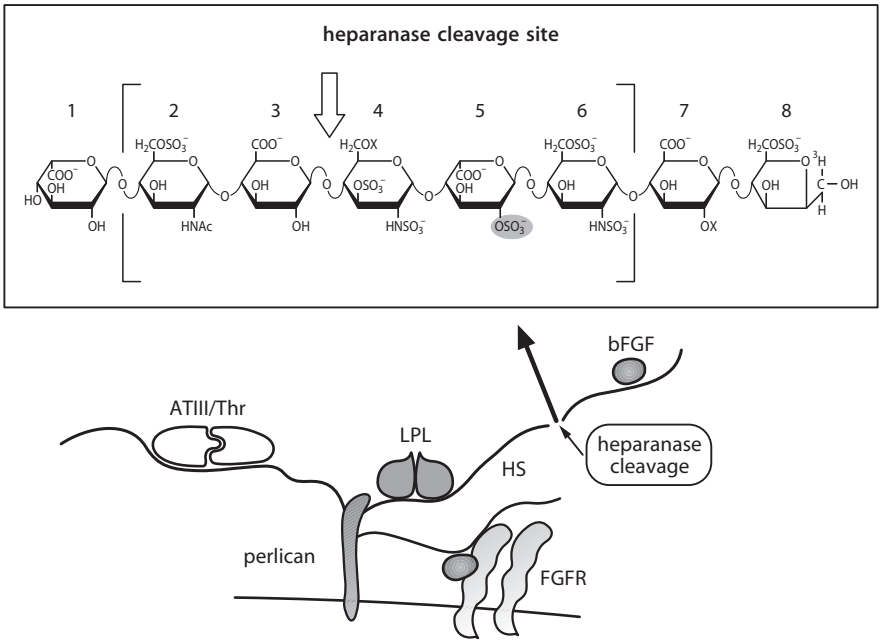
Definition

Heparanase is a mammalian enzyme (endo-β-glucuronidase) degrading heparan sulfate (HS), a ubiquitous strongly anionic linear polysaccharide associated with the cell surface and → extracellular matrix (ECM) of a wide range of cells of vertebrate and invertebrate tissues. The enzyme cleaves glycosidic bonds in HS with a hydrolase mechanism and is thus distinct from bacterial eliminases, called heparinases and heparitinase. The heparanase enzyme facilitates cell migration and egress from blood vessels and hence plays a role in tumor → metastasis, → angiogenesis, → inflammation and autoimmunity. Heparanase activity has been identified in a variety of human tumors (i.e., melanoma, carcinoma of the breast, liver, colon, prostate and pancreas; myeloid leukemia) and certain normal cells (i.e., cytotrophoblasts, platelets, neutrophils, activated T-lymphcytes). HS chains (Mr ~ 30,000) are cleaved by heparanase at only a few sites, resulting in HS fragments of still appreciable size (Mr ~ 5,000). Thus, the enzyme recognizes a particular and quite rare HS structure. A 2-O sulfate group on hexuronic acid residue located two monosaccharide units away from the cleavage site appears essential for substrate recognition by heparanase (Fig. 1). Heparanase activity (pH optimum in solution ~ 6.0) has been demonstrated in both lysosomal and endosomal cellular compartments and in the cell membrane. In human neutrophils the enzyme is localized in tertiary granules.

Characteristics

Heparan sulfate proteoglycans (HSPGs)

The basic HSPG structure consists of a protein core to which several linear heparan sulfate (HS) glycosaminoglycan (GAG) chains are covalently O-linked. The HS chains are typically composed of repeating hexuronic (L-iduronic or D-glucuronic acid) and D-glucosamine disaccharide units that are substituted to a varying extent with N- and O-linked sulfate moieties and N-linked acetyl groups (Fig. 1). The HS chains generally consist of clusters of sulfated disaccharide units separated by low or non-sulfated regions. Studies of the involvement of extracellular matrix (ECM) molecules in cell attachment, growth and differentiation revealed a central role of HSPGs in embryonic morphogenesis, angiogenesis, metastasis, neurite outgrowth and tissue repair. The HS chains, unique in their ability to bind a multitude of proteins, ensure that a wide variety of effector molecules (i.e., heparin-binding growth factors, chemokines, lipoproteins, enzymes) (Fig. 1) cling to the cell surface and ECM and thereby exert lo-



Heparanase. Fig. 1 – Scheme of heparan sulfate and the heparanase cleavage site (top), and of a basement membrane HSPG (perlecan) (bottom). Cleavage is associated with release of HS-bound growth factors and enzymes.

calized cellular effects. Moreover, transmembrane (syndecan) and membrane anchored (glypican) HSPGs have a co-receptor role in which the proteoglycan, in concert with other cell surface molecules, comprises a functional receptor complex that binds the ligand and mediates its action.

Molecular properties

Cloning and expression of a single human heparanase cDNA sequence was achieved using amino acid sequences derived from heparanase enzymes purified from human platelets, placenta, hepatoma cells and transformed embryonic fibroblasts (1, 2). The heparanase cDNA contains an open reading frame of 1629 bp, which encodes for a 61.2 kD latent polypeptide of 543 amino acids (Fig. 2). The mature active 50 kD enzyme, isolated from tissues, has its N-terminal 157 amino acids downstream from the initiation codon (Fig. 2), suggesting post-translational processing of the heparanase polypeptide (1, 2). Processing and activation occur during incubation of the full length recombinant enzyme with intact tumor cells. The nature of the cellular, possibly membrane bound enzyme(s), involved in activation of the latent heparanase has not been characterized. The sequence also contains a putative N-terminal signal peptide sequence (Met^{1} to Ala^{35}) and a candidate transmembrane region (Pro^{515} to Ile^{534}; Fig. 2). Alignment of the human, mouse and rat heparanase amino acid sequences, corresponding to the 50 kD human mature enzyme (Lys^{158} to Ile^{543}), demonstrated 80.0%, 79.7% and 92.7% identity between the human and mouse, human and rat, and mouse and rat heparanases, respectively (2). A 58–60% homology was found between these enzymes and the chicken heparanase. The fact that highly homologous cDNA sequences were derived from different species and types of normal and malignant cells is consistent with the notion that one dominant endoglucuronidase is expressed by all mammalian cells. Thus, unlike the large number of proteases that can solubilize polypeptides in the ECM, it appears that only one heparanase is used by cells to degrade the heparan sulfate side chains of HSPGs. The genomic locus that encodes heparanase spans ~40 kb. It is

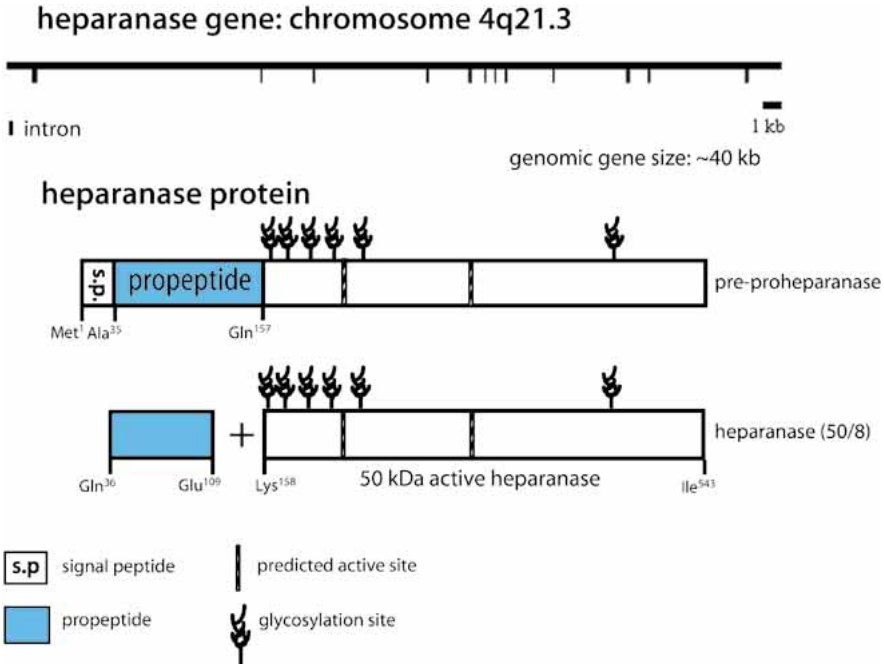
composed of 12 exons separated by 11 introns and is localized on human chromosome 4q21.3 (1, 2).

Preferential expression in human tumors

The heparanase mRNA and protein are preferentially expressed in metastatic cell lines, in specimens of human melanomas and carcinomas and in myeloid leukemia cells. In the colon, both the heparanase mRNA and protein are expressed at early stages of neoplasm, already at the stage of tubulovillous adenoma, but practically not detected in the adjacent 'normal looking' colon epithelium (3). Gradually increasing expression of heparanase is evident as cells progress from severe dysplasia through well differentiated to poorly differentiated colon carcinoma. Deeply invading colon carcinoma cells and adjacent desmoplastic stromal fibroblasts show the highest levels of the heparanase mRNA and protein (3). Both the heparanase gene and protein are also highly expressed in colon carcinoma metastasizing to the lung, liver and lymph nodes, as well as in the accompanying stromal fibroblasts (3). Preferential expression of the heparanase mRNA and protein is also noted in carcinomas of the prostate, breast, pancreas ovary and liver (1).

Involvement in tumor metastasis

A critical event in the process of cancer invasion and metastasis is degradation of various ECM constituents including collagen, laminin, fibronectin and HSPGs. The ability of HSPGs to interact with various ECM macromolecules and with different attachment sites on plasma membranes suggests a key role for this proteoglycan in the self-assembly and insolubility of ECM components, as well as in cell adhesion and locomotion. Cleavage of HS may therefore result in disassembly of the subendothelial ECM and hence play an important role in fundamental biological phenomena involving cell migration and ranging from pregnancy, morphogenesis and development to inflammation, angiogenesis and cancer metastasis. Expression of HS degrading heparanase correlates with the metastatic potential of tumor cells (1, 2, 4). Moreover, elevated levels of heparanase were detected in sera of metastatic tumor-bearing animals and cancer patients (4), in tumor speci-



Heparanase. Fig. 2 – Scheme of the human heparanase gene and protein.

mens and in the urine of patients with aggressive metastatic disease. Evidence for a direct causal role of heparanase in tumor metastasis was provided by the conversion of murine melanoma and lymphoma cells from low metastatic to highly metastatic phenotypes following transfection and over-expression of the heparanase gene (1).

Involvement in tumor angiogenesis

Angiogenesis represents a coordinated multicellular process that requires the functional activity of a wide variety of molecules including growth factors, ECM components, adhesion receptors and matrix-degrading enzymes. HSPGs and HSPG-degrading enzymes have been implicated in a number of angiogenesis-related cellular events including cell invasion, migration, adhesion, differentiation and proliferation. The heparin affinity of fibroblast growth factors (FGFs) and other heparin-binding growth factors appears to be the basis for their storage in → basement membrane (BM) and ECM, where they are bound to HS and can be released in an active form by HS degrading enzymes. The released angiogenic factors may then stimulate endothelial cell proliferation and migration associated with neovascularization.

An important early step in the angiogenic cascade is degradation of the subendothelial capillary BM by proliferating endothelial cells and formation of vascular sprouts. Heparanase, degrading the polysaccharide scaffold of BM, markedly contributes to the invasive ability of endothelial cells and their migration through the ECM toward the angiogenic stimulus. Apart from direct involvement in BM invasion by endothelial cells, the heparanase enzyme elicits an indirect angiogenic response by releasing HS-bound angiogenic growth factors from ECM and BM (Fig. 1) and by generating HS degradation fragments that stabilize and promote the mitogenic and angiogenic activity of heparin-binding growth factors (i.e., bFGF, → VEGF). In fact, recombinant mammalian heparanase, as well as heparanase secreted by platelets, tumor cells and inflammatory cells, release FGF as a complex with HS fragments. This yields a highly active form of FGF that readily interacts with high affinity receptors on the surface of endothelial cells and thus elicits an angiogenic response. The molecular size of the HS chain required for optimal stimulation of FGF receptor binding, dimerization and signaling is similar to that of HS fragments released by heparanase. A profound angiogenic response is elicited *in vivo* by lymphoma cells overexpressing the heparanase gene and embedded in a reconstituted BM (Matrigel). It appears that cooperative interaction between heparanases from tumor, inflammation and endothelial sources play an important role in the angiogenic cascade. The anti-cancerous potential of heparanase inhibitors is, therefore, not restricted solely to suppression of the invasive metastatic phenotype, but also to suppression of tumor angiogenesis.

Clinical relevance

Heparanase inhibiting molecules (e. g., non-anticoagulant species of heparin, polysulfated polysaccharides, polyanionic molecules) markedly reduce (>90%) the incidence of lung colonization induced by various tumor cells, in correlation with their anti-heparanase activity (4, 5). The occurance of a single heparanase species and its ability to promote both tumor angiogenesis and metastasis, the most critical steps in tumor progression, make it a promising target for cancer therapy. The heparanase inhibiting pentasaccharide, phosphomannopentaose sulfate (PI-88) is being tested in cancer patients as it reduces the vascularity, primary tumor growth and metastasis of mammary adenocarcinoma in rats. Anti-heparanase antibodies and molecular probes may also have a prognostic application in early detection of micro-metastases in tissues and body fluids (e.g., blood, urine, pleural effusions).

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Hepatolithiasis

Definition

Hepatolithiasis is a disease state characterized by the presence of multiple, small calculi forming in the small intrahepatic bile ducts, typically associated with chronic parasitic and bacterial infections. It is endemic in some areas of Asia, predisposing these patients to frequent attacks of cholangitis and an increased risk of → cholangiocarcinoma.

Hepatitis

Definition

Hepatitis is liver inflammation and necrosis of varying severity and etiologies, such as toxic (drugs, alcohol), metabolic, autoimmune or viral.

Hepatitis C Virus

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Synonyms

- HCV

Definition

Hepatitis C virus (HCV) belongs to the flaviviridae family (*Hepaciviruses* genus) and is a pathogenic human RNA virus, which causes chronic liver disease and hepatocellular carcinoma [→ liver cancer, molecular biology] (HCC).

Characteristics

The size of this enveloped virus is approximately 60 nm. Its nucleocapsid contains a single-stranded RNA genome of approximately 9.6 kilobases genome (Fig.) of plus(+) strand polarity that carries a single open reading frame. At least 6 major genotypes (1 to 6) with up to 3 subtypes (a to c) exist, which differ not only in their nucleic acid sequence but also in their pathophysiological properties. All structural and non-structural viral proteins are processed from a polyprotein precursor of 3,010 to 3,033 amino acids in the cytoplasm or endoplasmic reticulum of the infected cell.

Untranslated regions

The 5′- and 3′-untranslated regions (UTR) are highly conserved within the different genotypes. The 341 to 344 nucleotides 5′-UTR (Fig.) forms extensive stem-loop structures, which are important in translation initiation. It also harbours an internal ribosomal entry site. The 3′-UTR is composed of a poorly conserved variable region (28-42 nucleotides), a variable polypyrimidine stretch and conserved

aminoacids	protein	function
1-191	C	core
192-383	E1	envelope glycoprotein
384-746	E2	envelope glycoprotein
747-809	p7	?
810-1026	NS2	Zn auto-protease
1027-1657	NS3	Ser protease
		RNA helicase
1658-1711	NS4A	NS3 Ser protease cofactor
1712-1972	NS4B	?
1973-2420	NS5A	interferon-α-resistance
2421-3010	NS5B	RNA polymerase

Hepatitis C Virus. Fig. – Genomic organization and designation of HCV RNA, and amino acid positions and function of HCV proteins.

98 nucleotides at the 3′ end (x region). Both UTRs seem to be crucial for regulation of HCV translation and possibly also for controlling HCV replication, and are therefore candidate targets for experimental antiviral strategies.

Structural proteins

The core protein C (21 kD) polymerizes to an icosaedric capsid and binds RNA to form the nucleocapsid. The envelope proteins E1 (31 kD) and E2 (70 kD) form heterodimers whose formation is mediated by the chaperon calnexin. These dimers are embedded in a host-derived lipid bilayer. Within the E2 sequence there are two hot spots of mutations (hypervariable regions, HVR1 and HVR2). Mutations occuring during HCV infections are due to a selection driven by the host immune system and account for the regular existence of a variety of → quasispecies. The E2 protein is produced as two precursors, E2/NS2 and E2/p7 that differ in their C-terminus. The hydrophobic p7 is probably important for membrane anchoring of E2. The function of p7 or E2/p7 during the HCV life cycle is not known. All structural protein processing is performed by cellular signal peptidases.

Non-structural proteins

The non-structural protein NS2 (23 kD) is released from the polyprotein precursor both by host signal peptidases at the C-terminus of p7 and by a NS2/3 proteinase at the NS2-NS3 junction. NS3 (68 kD) has three different functions: It is part of the NS2/3 proteinase, its N-terminal third contains a serine proteinase and at the C-terminus a RNA-dependent NTPase/ helicase was discovered. Together with NS4A (6 kD) as a stable complexed cofactor, the N-terminal NS3 serine proteinase catalyses the cleavage between NS3-NS4A, followed by cleavage between NS5A-NS5B, NS4A-NS4B and NS4B-NS5A. The NS3 helicase can unwind double-stranded (ds)RNA, dsDNA and RNA/DNA heteroduplex molecules. For this purpose any NTP or dNTP is used as source of energy. NS4B is a 26 kD membrane associated protein of unknown function. NS5A (56-58 kD) represents two cytoplasmatic proteins, p56 and p58, which are both phosporylated at serine residues. This process may be essential for the viral replication cycle, but the biological functions of these proteins are not understood. There is some evidence that NS5A mediates interferon-alpha resistance of HCV. The NS5B (65 kD) protein is responsible for HCV RNA replication; it represents an RNA-dependent RNA-polymerase that is not found in humans and may therefore be another attractive target for antiviral strategies. The RNA minus(-) strand is transcribed in the host cytoplasm into a plus(+) strand RNA. This serves as a template to produce new minus(-) strands for packaging into the envelope. Both steps are accomplished by the NS5B protein.

Cellular and molecular regulation

Although the HCV genome does not harbour acutely transforming oncogenes, the HCV core as well as the NS3 gene are candidate genes whose products may mediate malignant hepatocyte transformation.

Oncogene complementation assays using HCV *core* and H-→ *ras* or c-→ *myc* oncogenes showed that HCV *core* cooperates with these oncogenes and transforms primary rat embryo

fibroblasts to the tumorgenic phenotype. Focus formation, soft agar growth and tumor development was also shown in nude mice using Rat-1 fibroblasts. Also in this model a loss of contact inhibition, morphological changes and anchorage- and serum-independent growth occurred when HCV *core* and v-H-*ras* were cooperatively expressed. The most striking evidence of an oncogenic potential of HCV comes from an HCV *core*-transgenic mouse model, in which hepatic tumors arose in up to 30% of animals of two different strains. Interestingly, all tumors occurred in male animals. However, other mice transgenic for HCV core or C-terminally truncated (amino acid 384-715) HCV *core* failed to develop histological or biochemical signs of liver disease.

The postulated underlying molecular mechanisms of HCV core-induced hepatocyte transformation are manifold. They comprise sequestration of LZIP in the cytoplasm leading to a loss of CRE-dependent transcription and regulation of cell proliferation and subsequently to morphological transformation of NIH 3T3 cells. Other mechanisms may be interference with → apoptosis or → transactivation of cellular *c-fos*, *c-myc*, *p53* or β-*interferon* promoters.

Stable expression of the 5′-portion of the NS3 gene was able to induce focus formation and soft agar growth in NIH 3T3 cells. Only recently it was demonstrated that internal cleavage of the NS3 protein occurs at cleavage sites FCH(1395)//S(1396)KK and IPT(1428)//S(1429)GD within the HCV RNA helicase domain in presence of NS4A. These findings were confirmed in two different isolates of HCV of genotype 1b. The 5′-portion of NS3 was more oncogenic than the full-length NS3 protein.

Clinical relevance

An estimated 2% of the world's population is chronically infected with HCV. It accounts for 20% of acute and 70% of chronic → hepatitis cases. HCV is mainly transmitted by blood. Since screening and treatment of blood products for HCV has been routinely performed, post-transfusion hepatitis has become extremely rare. Intravenous drug addiction is today a major way of HCV transmission. Acute hepatitis is often clinically inapparent, and in chronic hepatitis symptoms occur mainly in later stages of disease. A high chronicity rate of up to 75% of acute infection and its silent course account for the pathogenic potential of this virus, which includes extrahepatic manifestations. 20% of chronically infected patients develop → liver cirrhosis and 2 to 5% per year will progress to hepatocellular carcinoma (HCC).

Therapy for HCV infection comprises the combined use of 3 to 6 MU of α-interferon administered subcutaneously three times a week and 800-1200 mg of the nuleoside analog → ribavirin p.o daily for 6 to 12 months dependent on the genotype and viral load. Roughly 40 to 45% of previously untreated or relapse patients benefit from the treatment, i.e. eliminate HCV and normalize liver enzymes. In addition, this treatment is capable of improving histological signs of liver damage. Its effect on HCC prevention can not clearly be judged at present.

Recent therapeutic innovations comprise → pegylated interferons that allow single weekly dosing due to slow effector release from subcutaneous depots and a decrease of systemic side effects. Sustained response rates up to 70 % can be reached. A synthetic consensus interferon-alpha showed promising effects in monotherapeutic use in a preliminary trial and is presently being evaluated in combination with ribavirin. Major future achievements are expected from the development of inhibitors of HCV protease, helicase or RNA-dependent RNA polymerase, which may improve effectivity of antiviral treatment similarly to the situation in HIV infection, but are not yet available for clinical use. Therapeutic nucleic acids such as antisense oligodeoxynucleotides, RNAs or ribozymes may be another experimental concept for the treatment of HCV infection in the future.

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Hepatitis Viruses

Definition

Hepatitis viruses are an inhomogeneous group of DNA (e.g. hepatitis B virus) or RNA (e.g. hepatitis C, δ, A, E) viruses that cause liver disease. Hepatitis viruses B, C and δ can induce chronic liver disease and hepatocellular carcinoma [→ linkage group].

Hepatoblastoma

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Definition

Heptoblastoma is a childhood malignant embryonal liver tumor consisting of immature epithelial cells with or without additional mesenchymal component.

Characteristics

Hepatoblastomas are the most frequent malignant liver tumors of childhood. Their annual incidence is 0.5-1 cases per million children under 15 years of age in Western countries. The affected children are most frequently between 6 months and 3 years old. Hepatoblastoma has also been detected in utero by prenatal ultrasound examination. Since liver tumors lack early clinical symptoms, hepatoblastoma patients often present with locally extended tumors at diagnosis. However, distant metatases usually occur very late in the disease progression. The patients frequently have highly elevated → α-fetoprotein levels. In these cases this oncofetal antigen can be useful as a senstive diagnostic marker and also as a marker for the monitoring of treatment response.

Pathological classification

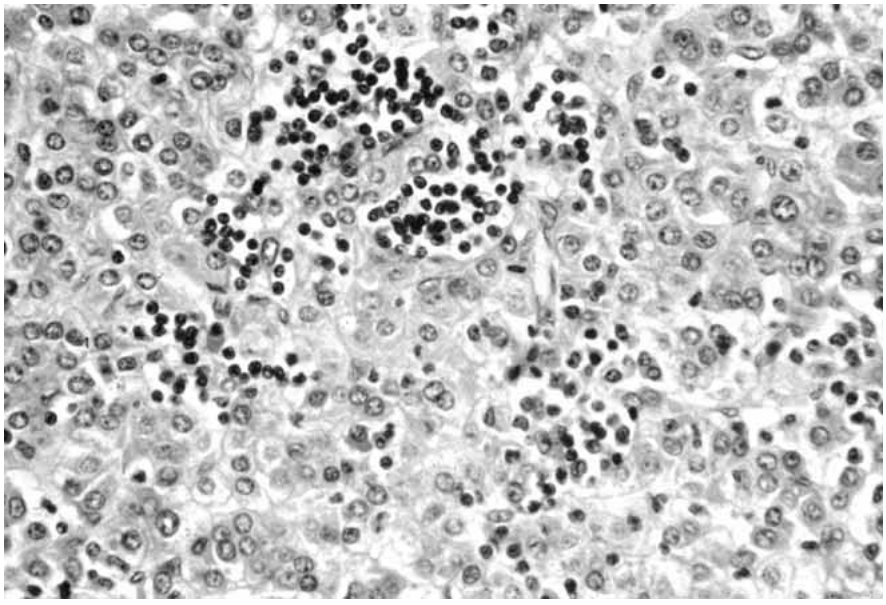
Hepatoblastomas always consist of immature epithelial liver cells resembling fetal or embryonal liver cells. Approximately one third of the cases contain additional mesenchymal components and are then termed 'mixed' hepatoblastomas. According to Weinberg and Finegold (1983) the epithelial component is further classified into well differentiated 'fetal', less differentiated 'embryonal' and undifferentiated 'small cell anaplastic' categories. The latter is rare, as are 'macrotrabecular' or 'teratoid' variants. Small cell anaplastic as well as macrotrabecular variants indicate a bad prognosis. A histopathological hallmark of hepatoblastomas, in particular of the fetal differentiated cases is the occurence of hematopoietic foci mainly consisting of erythropoietic or thrombopoietic progenitor cells mimicking fetal hematopoiesis in the liver (Fig.).

Staging

In the last 30 years the treatment modalities and outcome of hepatoblastoma patients has significantly improved. It turned out that hepatoblastomas are responsive to chemotherapy. Today, most hepatoblastoma patients are enrolled in multicenter studies and receive a stage and risk adapted multimodal therapy. Different staging systems are used including the American and German four-graded postoperative staging system, the Japanese TNM classification and the European SIOP PreTreatment Extent of Disease (PRETEXT) grouping system.The patients are stratified into standard and high risk patients, the latter often presenting with extended, multifocal or metastatic disease.

Therapy and outcome

In current protocols most patients receive a neo-adjuvant chemotherapy with cisplatin



Hepatoblastoma. Fig. – Histopathology of an epithelial hepatoblastoma showing foci of hematopoietic cells.

and doxorubicin, in some studies combined with ifosphamid, 5-fluorouracil or carboplatin. The combination carboplatin and etoposide has also been effective. The overall survival after chemotherapy and resection is 70 – 80%. Unfortunately, approximately one quarter of the patients still die from the disease, so that predictive factors are important to early recognize these high risk patients and to offer them an intensified therapy. Recently, high risk patients have been treated with prolonged preoperative chemotherapy (SIOP, USA) or with mega-therapy (Germany).

Predictive factors

Several histological, clinical as well as serological factors have been evaluated for their predictive value. In particular, the extension of the tumor in the liver, multifocality, vasculare invasion and the presence of metastases have been of predictive importance in most studies. The decline of alpha-fetoprotein levels under chemotherapy predicts the clinical response. In contrast, the impact of distinct histological features such as mixed versus epithelial, or fetal versus embryonal differentiation and DNA ploidy of the tumors is still under discussion and the data is controversial.

The aetiology of hepatoblastomas is unknown. Environmental factors do not seem to play a major role in the pathogenesis of hepatoblastomas. Preterm infants may have an elevated risk for the development of hepatoblastomas. Although most hepatoblastomas occur sporadically, familial cases have been described. The incidence is highly elevated in families with adenomatous polyposis coli (FAP [→ APC Gene in Familial Adenomatous Polyposis]) and → Beckwith-Wiedemann Syndrome.

Genetic and molecular characteristics

Cytogenetic analyses have revealed several recurrent numerical aberrations as well as structural alterations. Most frequently trisomies of chromosomes 1, 2, 8 and 20 have been described as well as a recurrent translocation t(1;4)(q12;q34) in approximately 15% of hepatoblastomas. Approximately 50% of cases show gain of material of chromosome 2q. A few cases have an → amplification of material from chromosomal band 2q24, indicating the location of a hepatoblastoma related oncogene. Microsatellite analyses have uncovered frequent allelic losses of chromosomal regions of chromosome arms 1p and 11p. The losses of chromosomal region 11p15.5 are always of maternal

origin indicating the existence of one or more imprinted hepatoblastoma associated gene(s) expressed from the maternal allele.

Studies on the mutational status of the → *TP53* gene resulted in conflicting data. Whereas *TP53* mutations were described in a small Japanese tumor collection, these were absent in other studies. Similarly, somatic missense mutations were described in one study but not confirmed by others. In contrast, recent studies indicated that the activation of the → WNT/wingless signalling pathway by activating mutations in the β-*catenin* gene is a frequent event in hepatoblastomas. Approximately 50% of the hepatoblastomas show point mutations or deletions of exon 3 encoding the protein degradation targeting site of the β-catenin protein, resulting in a destruction of N-terminal phosphorylation sites that are necessary for protein degradation. This leads to the accumulation of a mutated protein which transfers oncogenetic signals to the nucleus and increases transcription of specific target genes of the TCF/LEF family of transcriptions factors such as cyclin D1 and c-myc. Similar mutations have been described in other tumor entities including colorectal cancer. However, hepatoblastoma represents the malignant tumor with the highest incidence for β-catenin mutations.

Cellular characteristics

Hepatoblastoma cells resemble liver progenitor cells during embryonic and fetal development suggesting that hepatoblastomas are derived from such progenitors. Hepatoblastoma cells are still dependent on growth factors and use specific growth factor signalling systems. The important fetal mitogen, insulin-like growth factor-II, has been demonstrated to be highly over-expressed in hepatoblastomas. The encoding → *IGF2* gene maps to chromosome 11p15.5, a region frequently altered in hepatoblastomas and related embryonal tumors.They also produce several hematopoietic cytokines such as interleukin-1, stem cell factor, erythropoietin and thrombopoietin that induce hematopoietic foci in hepatoblastomas. Hepatoblastoma cells over-express the receptor → Met for hepatocyte growth factor (HGF; → scatter factor) and proliferate in response to HGF *in vitro*. Concentrations needed for this effect are uaually found in the serum of patients after liver surgery. This may explain why hepatoblastomas often show rapid regrowth after partial resection. Therefore it is now common sense that the tumors should undergo primary resection, only when they can be removed without residual tumor cells.

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Hepatocyte Growth Factor

Definition

Hepatocyte growth factor (→ HGF).

Hepatocyte Growth Factor Receptor

Definition

→ Met.

Hepatocyte Growth Factor-like Protein

Definition

→ Macrophage-stimulating protein.

Hepatocyte Stimulating Factor

Definition

→ Interleukin-6.

Hepatoma

Definition

Hepatoma is a primary malignant tumor of the liver.

HER-1

Definition

→ Epidermal growth factor receptor.

HER-2/neu

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Synonyms

- *ERBB2*
- *c-erb-B2*
- *p185neu*
- *v-erb-B2*
- avian erythroblastic leukemia viral oncogene homolog 2

Definition

The proto-oncogene HER-2/neu (ERBB2) is located on chromosome band 17q21.1 and encodes a transmembrane → receptor tyrosine kinase. The name for the HER-2 protein is derived from 'Human Epidermal growth factor Receptor' as it features substantial homology with the → epidermal growth factor receptor (EGFR).

Characteristics

The HER-2/neu (p185neu) protein belongs to a family of closely related growth factor receptors including.

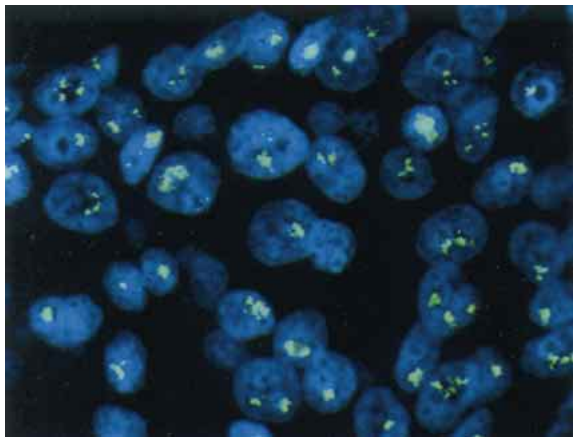
- HER-1 (ERBB1), EGFR
- HER-2 (ERBB2)
- HER-3 (ERBB3)
- HER-4 (ERBB4)

HER-2/neu gene amplification and protein overexpression have been identified in 10 to 34% of breast cancers, and patients whose breast cancers contain HER-2/neu aberrations have a poor prognosis. The prognostic impact of HER-2/neu → amplification was initially most convincing in patients with axillary lymph node metastases. By contrast, a prognostic role was less compelling in patients with localized (lymph node negative) breast cancer. Various studies came to different conclusions regarding

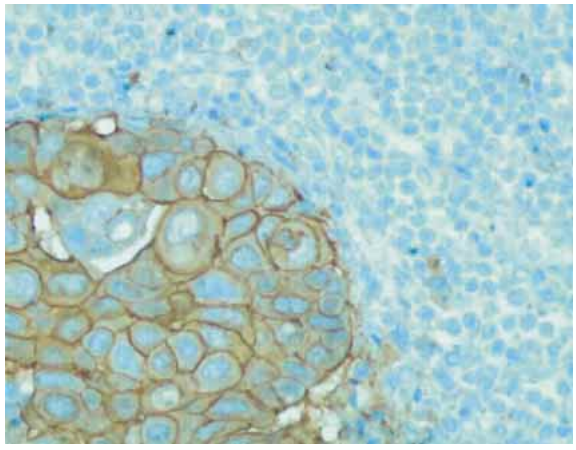
a link between HER-2/neu gene or protein abnormalities and survival in lymph node negative patients. Reviews of these non-correlating studies have focused on the immunohistochemical methods used to identify over-expression of the HER-2/neu protein. Technical inconsistencies in the detection of HER-2/neu protein by immunohistochemistry have included both the differing sensitivities and specificities of the commercially manufactured anti-HER-2/neu antibodies and the several antigen retrieval techniques used in formalin-fixed paraffin embedded tissues. Also, the lack of a standardized interpretation protocol results in significant interobserver variability. Notably, fluorescence *in situ* hybridization technique (FISH) evaluations have consistently demonstrated a relationship between HER-2/neu gene amplification and breast cancer recurrence and disease-related death in both lymph node-negative and -positive cases.

ELISA-based measurements of HER-2/neu protein in breast cancer cytosols also correlate with disease outcome. Serum-based HER-2/neu evaluation can also be accomplished using the ELISA method, although consensus as to the clinical utility of this technique has not been achieved. Southern- and slot-blotting methods are less effective since the DNA of tumor cells extracted from the primary carcinoma sample are diluted by DNAs from benign breast tissue and inflammatory cells. More recent studies support an association between HER-2/neu gene amplification or protein over-expression with poor clinical outcome in gastrointestinal, pulmonary and genitourinary neoplasms.

Clinical efficacy of the drug, HerceptinTM, has stimulated significant expansion of HER-2/neu testing in the management of breast cancer. Herceptin is a humanized monoclonal antibody administered intravenously that is particularly effective when combined with cytotoxic agents (either taxotere or Adriamycin plus Cytoxan). A 27% response rate with these treatment approaches has been described for patients with advanced metastatic breast cancer refractory to conventional treatment. Recently, clinical trials of Herceptin plus cytotoxic therapy have begun for patients with prostate, lung, ovarian and pancreatic cancers. The various products available for HER-2/neu testing are shown in the Table.



HER-2/neu. Fig. 1 – HER-2/neu gene amplification detected by FISH. Note clusters of signals in each nucleus.



HER-2/neu. Fig. 2 – HER-2/neu protein overexpression detected by immunohistochemistry. Note intense continuous membrane staining pattern.

In addition to its role in predicting prognosis and response to Herceptin therapy, HER-2/neu testing in breast cancer has also been used to select other treatment options. Although the ability of HER-2/neu status to predict response to anti-estrogen therapy has not achieved consensus, the enhanced response of HER-2/neu positive tumors to chemotherapy regimens containing Adriamycin is generally well-accepted in clinical oncology practice.

HER-2/neu. Table – Reagents for HER-neu analysis.

product name	source	method	indication
inform	VentanaMedical	FISH	prognosis
HercepTest	Dako	IHC	herceptin response
PathVysion	Vysis	FISH	prognosis, taxotere response
CB11	VentanaMedical	IHC	herceptin response
serum test	Oncogene Science	ELISA	prognosis, therapy response

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HER-4

Definition

→ ERBB4.

Hereditary Hamartosis Peutz-Jeghers

Definition

→ Peutz-Jeghers-syndrome.

Hereditary Mutation

Definition

A hereditary mutation is carried in the DNA of a reproductive cell. When reproductive cells carrying a mutation combine to produce offspring, the mutation will be present in all of the offspring's body cells. This fact makes it possible to test any of the cells, including blood cells, for the presence of a particular disease gene.

Hereditary Papillary Renal Carcinoma

Definition

Hereditary papillary renal carcinoma (HPRC) is a form of inherited cancer characterized by a predisposition to develop multiple renal tumors.

Herpesviruses

Definition

Herpesviruses are enveloped DNA viruses. Humans are the natural host for 8 herpesviruses; → human herpesvirus 6.

HES-1

Definition

Hes-1, also known as hairy (*Drosophila*) homolog 1 is a → bHLH protein member of Hairy/E(Spl) family. The human HES-1 gene locus maps at 3q28-q29 and the mouse hes-1 gene locus at chromosome 16 (27.00 cM).

Heterochromatin

Definition

Heterochromatin (in contrast to euchromatin) is a condensed form of chromatin in higher eukaryotic cells that was originally identified by light microscopy. It remains condensed during interphase and is inactive in DNA transcription. Heterochromatin is thought to contain genes that are permanently turned off in a cell and in all of its progeny. Two forms of heterochromatin can be distinguished:

1. Constitutive heterochromatin has chromosomal regions that are condensed in all cells. It is thought to contain DNA that is never transcribed in any cell. In human chromosomes it is localized around the centromere of each mitotic chromosome. Constitutive heterochromatin contains simple repetitive sequences, often referred to as satellite DNA.
2. Facultative heterochromation has chromosomal regions that are condensed only in certain, not all cells. The total amount of facultative heterochromatin can vary greatly in different cells, e.g. embryonic cells have less facultative heterochromatin than differentiated cells. Facultative heterochromation is not known to have great amounts of repetitive DNA. The best example is the X-chromosome in females, where one of the two copies is inactiveted during very early development. Inactivation of the two parentals occurs randomly in different cells. Since, during cell division, the inactivated state is stably transmitted to the daughter cells, a clonal inheritance occurs. This has the consequence that in some areas of the female body the paternal chromosome may be active and in others the maternal chromosome. This effect can manifest itself through a phenotype. For instance, if the two paternal chromosomes carry genes for different fur or skin colours, patched patched fur or skin may be phenotypically displayed.

Heteroduplex (hybrid) DNA

Definition

A heteroduplex (hybrid) DNA is generated by base pairing between complementary single strands derivated from the different parental duplex molecules and occurs during genetic recombination.

Heterogeneity, genetic

Definition

Genetic heterogeneity is the situation where a single phenotype or disease is connected with different genotypes. Different genotypes may represent different mutations in one particular disease gene (allelic heterogeneity) or mutations in different genes (locus heterogeneity).

Heterophilic Adhesion

Definition

Heterophilic adhesion occurs between two undefined cell types that can be mediated by either identical (homophilic) or different (heterophilic) adhesion molecules.

Heterotrimeric GTP-binding Proteins

Definition

Heterotrimeric GTP-binding proteins are GTP-binding proteins with three subunits; the α subunit has the ability to bind GTP, while the β and γ subunits do not bind to GTP. Both the α and $\beta\gamma$ subunits are able to specifically interact and activate a variety of effector molecules.

Heterotypic Adhesion

Definition

Heterotypic adhesion is mediated by undefined adhesion molecules between identical cell types (homotypic) or two different cell types (heterotypic).

HGF

Definition

Hepatocyte growth factor (HGF) is a protein that stimulates growth of several cell types including hepatocytes; → scatter factor (SF).

HGF Activator

Definition

HGF activator is a 34 kD serine protease that is produced as an inactive pro-enzyme and proteolytically cleaved to its active form. Unlike plasminogen activators, which cleave and activate scatter factor (SF; HGF) in stoichiometric ratios, HGF activator functions to convert proSF to mature active SF in enzymatic quantities.

HGFL

Definition

→ Macrophage-stimulating protein.

Hh

Definition

→ Hedgehog.

HHV-4

Definition

Human herpesvirus 4

HHV-6

Definition

→ Human herpesvirus 6 is a member of the β-herpesvirinae subfamily, an etiologic agent of exanthem subitum and a benign pediatric disease. It is also possibly associated to lymphoproliferative diseases and Hodgkin Disease.

HIF

Definition

Hypoxia-inducible factor (HIF) is a heterodimeric transcription factor that regulates oxygen homeostasis and physiologic responses to oxygen deprivation.

High Mobility Group Protein

Definition

High mobility group (HMG) proteins are architectural factors that bind to and induce conformational changes in DNA. These changes allow other proteins, such as transcription factors, to bind and thereby regulate transcription as well as other DNA-dependent activities. HMGI proteins, which are highly conserved across species, bind the minor groove of DNA through three AT-hook binding domains and are thought to play a role in cell differentiation and proliferation.

High Resolution Mass Spectrometry

Definition

High resolution mass spectrometry is the separation and analysis of chemical compounds based on their fragmentation, ionization and migration in response to a charge field. Recent advances in sensitivity, resolution and data analysis allow for femtomole quantities of proteins to be identified and for post-translational modifications to be detected and mapped in the sequence.

High Throughput Screens

Definition

High throughput screens are rapid screens involving parallel assays used in pharmaceutical research to identify potential targets or drugs from large combinatorial libraries of reagents.

Highly Repetitive DNA

Definition

Highly repetitive DNA is the first component to reassociate after denaturation and is equated with satellite DNA.

HILP

Synonyms

- → BIRC4

Definition

Human → IAP-like protein. HILP is an evolutionary conserved cell death suppressor that exerts major anti-apoptotic efffects by inhibiting caspases.

Hippocampal

Definition

Hippocampal, as related to the hippocampus, the latter being a specific structure within the brain.

Histidine Protein Kinase

Definition

Histidine protein kinase is an enzyme that contains a histidine autophosphorylation site and transfers phosphate to another protein, typically on a histidine or aspartate amino acid.

Histogram

Definition

Histogram is the frequency distribution of a measured parameter (e.g., pO_2 histogram).

Histologic Diagnosis

Definition

Histologic diagnosis means a diagnosis that is made by taking a piece of a patient's tumor (a biopsy) and examining it under the microscope. It is essential to have this performed before treating a patient for cancer. This is the only way one can be certain that one is actually dealing with a true cancer.

Histone Deacetylases

Definition

Histone deacetylases are enzymes that remove the acetyl group of acetyl lysine moieties in the

N-terminal tail of histones. The modification of histones in chromatin silences gene expression.

Histone H4

Definition

Histone genes encoding five classes of histone protein are clustered on chromosomes 1 and 6. The *H4* gene consists of a single exon flanked by histone gene-specific motifs. The *H4* promoter sequence contains consensus binding sites for histone nuclear factor D (HiNF-D) and interferon regulatory factor 2. HiNF-D is a multicomponent protein containing cyclin A, CDC2 and an → RB-related protein, which links *H4* gene regulation directly to cell cycle control.

Histones

Definition

Histones are conserved DNA binding proteins of eukaryotes that form the nucleosome, the basic subunit of chromatin.

HLA

Definition

HLA is the acronym for human leukocyte antigen, a term for MHC in human; → HLA class I.

HLA Class I

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Definition

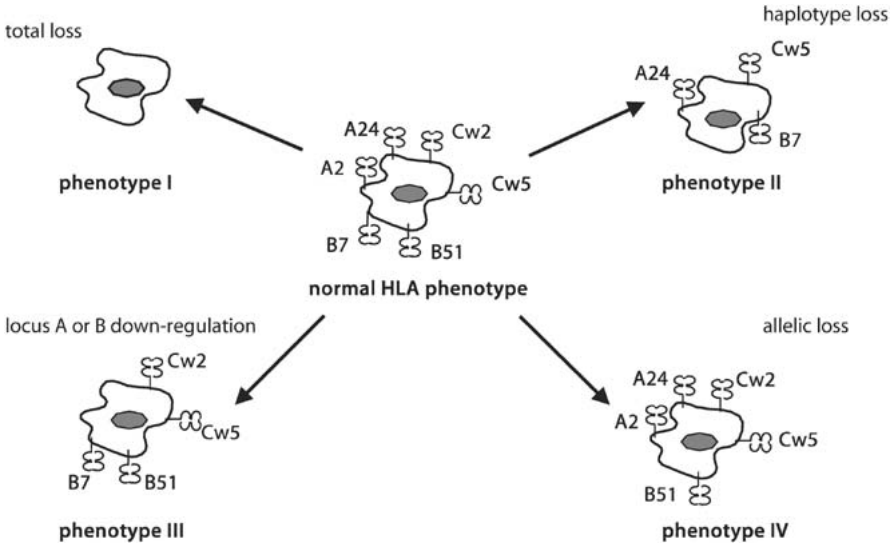
Major histocompatibility complex (MHC) is a complex of genes that encodes MHC molecules that are required for antigen presentation. The major histocompatibility system, → HLA in human, was first discovered in the search for polymorphic antigens to match for transplantation. Today, much information has been discovered regarding the physiological function of HLA to distinguish normal from infected or transformed cells. This system is a collection of genes arrayed over 2-3 centimorgans of DNA on chromosome 6 in humans. The region of MHC is subdivided into three classes based on functional aspects of the genes within each class.

Characteristics

The MHC class I and II molecules (HLA-ABC and HLA-DR,DP,DQ respectively) are cell-surface glycoproteins closely related in structure and function. HLA class I molecules form heterodimers and consist of two polypeptide chains, a heavy chain of 340 amino acids encoded in the MHC and a light non-polymorphic chain, β_2m, encoded on chromosome 15. The structure of HLA class II molecules is similar to that of class I and is composed of a heterodimer transmembrane glycoprotein chain, ab. The two molecules have a distinct distribution among cells that have been shown to have a key functional role in immune response.

- MHC class I molecules are expressed on the surface of nearly all nucleated cells.
- MHC class II molecules are expressed on → antigen-presenting cells (APC).

MHC molecules are receptors for peptides, derived from self and foreign antigens which are captured within the cleft of the HLA molecules. Processed foreign or self antigen complexed to HLA class I or II molecules is recognized by a specific membrane-bound T cell receptor on the surface of → T cells. The major function of HLA class I molecules is to alert T cells to the presence of intracellular extraneous peptides derived from intracellular pathogens (viruses, some bacteria) and tumor cells. HLA class I molecules can also interact with → natural killer cells (NK cells) to prevent NK-mediated cell lysis.



HLA Class I. Fig. – Altered HLA class I phenotypes in human cancers. A tumor cell with a normal phenotype can evolve to several altered phenotypes: a) phenotype I corresponds with HLA class I total losses; b) phenotype II with HLA haplotype losses; c) phenotype III with HLA-A or -B-locus-specific losses and d) phenotype IV with HLA allelic losses (A, B, and C).

Bioactivity

Amongst different reasons for the poor immunogenicity of tumor cells, the most frequent is the down-regulation of the expression of major histocompatibility complex molecules, of importance because it prevents the presentation of tumor antigen peptides to T cells (Table 1). Human HLA class I downregulation is a widespread phenomenon in tumor biology and probably reflects a mechanism by which tumor cells can escape immune response because of the role of HLA molecules in presenting immunogenic peptides to T cells.

There have been many studies of processes that lead to a low cell surface expression of HLA molecules. It has been demonstrated that all the biosynthesis stages of these molecules are suitable targets for this strategy during tumorigenesis. The result is the attenuation of expression of one, various, or all of the alleles, which gives a selective advantage because the cells are then invisible to CTLs. This strategy, acquired in a relatively short period of time during tumor progression, is also used by viruses for immune evasion but in their case as a consequence of evolution. Several viral gene products have been identified that target specific components of the MHC class I biosynthetic pathway.

Alterations in the expression of HLA antigens are a far from sporadic phenomenon, as could be deduced from the first immunohisto-

HLA Class. Table 1 – Mechanisms of immune escape.

mechanism	effect
structural and regulatory HLA gene defects	loss of CTL recognition
defects in antigen-processing machinery	loss of CTL recognition
loss or decrease of tumor antigen expression	loss of CTL recognition
loss of Fas	absence of T-cell induced apoptosis
expression of FasL	induction of apoptosis in Fas+ CTLs

logic studies, done with monoclonal antibodies, and can affect from 39% to 88% of tumors derived from HLA+ epithelia (Table 2). These studies provide the basis for defining various phenotypes in tumor (Fig.):

- *Phenotype I:* total HLA loss.Tumor cells frequently exhibit a complete loss of expression of HLA antigens. This is a relatively common phenotype (9 – 52%), readily detected in most human tumors with mAbs. It can be associated with defects in β2-microglobulin, synthesis, transporter associated processing and hypermethylation of cis-acting regulatory elements of the HLA promoter. Mutations in the β2-microglobulin gene have been identified in melanoma and colon carcinomas and range from single base mutations to partial gene deletion.
- *Phenotype II:* HLA haplotype loss. Loss of an HLA haplotype has been shown in melanoma, pancreas and colon tumor cell lines. Loss of a full chromosome 6 or deletion of a large genomic region has been found in the majority of cases. Chromosomal non disjunction or mitotic recombination has been proposed to underlie this phenotype.
- *Phenotype III:* HLA locus loss. Loss of class I locus expression of HLA-A (range 3 – 19%) or HLA-B (5 – 19%) has been documented in several tumors. The mechanisms of locus downregulation may be transcriptional since there are differences in HLA class I locus promoter sequences and in mRNA levels for some alleles in tumor versus normal cells. Furthermore, in some tumors, such as melanomas, the selective down-regulation of HLA-B locus has been related to oncogenic products.
- *Phenotype IV:* HLA allelic loss.It is difficult to define the precise frequency of tumors which show the loss of only one HLA allele because of defects in the repertoire of allele-specific antibodies. The molecular mechanisms underlying the selective allele loss might result from point mutations, partial deletions of HLA class I genes, or splicing defects. Complex phenotypes have also been shown representing combinations of these phenotypes.

HLA Class. Table 2 – Frequency of HLA Class I altered phenotypes in invasive tumors.
The results are expressed as percentage of alterations of HLA, including total or partial allele losses.

tumor type	total
breast	88
cervix	63
colon	81
larynx	79
melanoma	51
pancreas	39
prostate	85

Functional significance of HLA class I loss: Implications for immune evasion

The ability of virus-infected and tumor cells to put themselves into an immune privileged situation depends on many shared factors. The level of expression of HLA class I molecules can also influence the presentation and immunogenicity of CTL (cytotoxic T lymphocytes) epitopes and the modulation of NK-cell responses, so that tumor phenotypes which fail to upregulate their HLA expression in response to cytokines may affect clinical progression. The tumor HLA-loss phenotypes may result from the need to avoid recognition both by specific CTLs and NKs. This tumor evolution may involve multiple steps, thereby generating heterogeneity of HLA expression even within an individual cancer. For instance, the metastatic capacity of different tumor clones measured in spontaneous metastasis assays has been correlated with high MHC class I expression and low NK sensitivity in some tumor models.

It is clear that the HLA class I expression of a tumor is central to the efficacy of any cell mediated immunotherapy. This is particularly important when considering peptide vaccines designed on the basis of their presentation by a particular HLA allele, since the tumor phenotype will be critical to the efficacy of such an approach and to its proper evaluation in clinical trials. Evidence for immunoselection of tumors that have lost the expression of several

HLA molecules under selective pressure to escape T-cell cytotoxicity was recently demonstrated in patients who had undergone → immunotherapy. In patients with structural HLA loss, the defects are irreversible and peptide-based vaccines will be of limited value. Furthermore, some patients have tumors that display constitutive loss of HLA class I. It is therefore important to develop strategies to classify altered HLA phenotypes and identify the molecular mechanisms that give rise to these HLA alterations. The contribution of the HLA immunophenotype of the tumor tissue will be an important factor to considerer in order to design the most effective immunotherapeutic protocol and to fully understand the tumor development.

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HMG

Definition

→ High mobility group protein.

HMG-CoA Reductase

Definition

3-Hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase (ec 1.1.1.34), belongs to the HMG-CoA reductase family. It is a transmembrane glycoprotein of 888 amino acids and 97 kD involved in the control of cholesterol biosynthesis, being the rate-limiting enzyme of sterol biosynthesis.

HMGI-C

Definition

HMGI-C is a member of the → HMG family of proteins.

HMLH1-3

Definition

The human mut L homologue 1 to 3 (hMLH1-3) genes encode proteins that are components of the human mismatch repair (MMR) system; defective MMR may cause → microsatellite instability in tumors.

HMSH2-6

Definition

The human mut S homologues 2 to 6 (hMSH2-6) genes encode proteins that are components of the human mismatch repair (MMR) system; defective MMR may cause → microsatellite instability in tumors.

HNPCC

Definition

Hereditary nonpolyposis colorectal cancer (HNPCC); → Lynch syndrome.

Hoarseness

Definition

Hoarseness is one form of dysphonia that is defined as a rough or noisy quality of voice. However, hoarseness is often used interchangeably with → dysphonia. It is a symptom of both local laryngeal pathology and systemic disease. It is not only a distressing symptom for patients, but is also often the early presenting symptom of serious disease such as cancer of the larynx.

Hodgkin Disease

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Definition

Hodgkin disease (HD) is unique among the neoplasms derived from lymphoid tissues, i.e. malignant lymphomas. In fact, many characteristics of HD are controversial, in particular the epidemiological, virological, genetic, histopathological, immunological, and biological findings. It is now clear that HD is not a single disease with variants, but rather a group of at least two diseases, namely nodular lymphocyte predominant Hodgkin disease (nLPHD), and classic Hodgkin disease (cHD).

Characteristics

While nLPHD is a B-cell neoplasm, it is not clear whether the B-cell derived displastic cells, i.e. the L-H or popcorn cells are monoclonal or polyclonal. Moreover, it is not clear whether nLPHD should be treated in that it shows slow or no progression at all. In contrast cHD contains four histotypes, i.e. NSHD (nodular sclerosis HD), MCHD (mixed cellularity HD), LDHD (lymphocyte depletion HD), and cLRHD (lymphocyte rich HD) that require treatment because otherwise they are fatal.

Immunological and molecular biological studies of cHD at the single cell level suggest that HRS (Hodgkin and Reed Sternberg) cells in most cases are monoclonal derivatives of late germinal center B-cells, but in few cases are derivatives of cytotoxic T-cells and although less likely of NK-cells. The major event in the pathogenesis of B-cell related cHD is the blockage of apoptotic pathway. Epstein-Barr Virus (→ EBV) might be involved in the postulated dysfunction of the apoptotic pathway, leading to the genesis of classic HRS cells.

The essential elements for diagnosis of HD are the following:

- lymphomegaly, single or multiple, not painful, often monostational and with variable dimensions over time;
- frequent presence of one or two systemic symptoms (fever, night sweat, loss of weight);
- presence of the Reed-Sternberg cell in the lymphonodal biopsy.

The most characteristical clinical presentation of HD is a young adult, showing an asymptomatical lymphnode swelling. The enlarged lymphgland, which is usually not tender, occurs most widely in the neck, often in the supraclavicolar fossa but it may also be discovered in the mid- or right neck or in the axilla. Another common presentation of the illness is the discovery of an anterior mediastinal mass on the routine chest radiographic examination.

Treatment of patients with HD has been one of the most significant successes in twentieth century clinical medicine. This was once uniformally a fatal disease, now is curable in approximately 75% of patients at many major medical centers worldwide. The management of these patients, however, is often difficult and requires particular attention to details of the staging and treatment program. This is a necessary procedure in order to obtain good results by keeping the potential serious toxicities and morbidities of the therapy to a minimum. Most of the serious side effects of the therapy of HD are not evident for at least five-twenty years or more after treatment is completed. These might be described as problem of success, since

they require many years of survival, free of HD recurrence to be recognized. As they have become evident, treatment programs have been modified in an effort to reduce their incidence and severity to a minimum. Therefore, the management of patients with HD continues to evolve. Treatment recommendations however, may differ somewhat among physicians and investigators with great experience in the treatment of HD.

A therapeutic program for a patient with HD should not be initiated without definitive diagnosis by an experienced hematopathologist. Appropriate diagnostic studies and stage determination should be made before start of a therapy. Almost all patients benefit from consultation with both an experienced medical oncologist or hemathologist and a radiation oncologist to jointly plan a treatment program, although not all patients require both modalities, chemotherapy and radiation therapy, in their initial management. Generally, the standard recommended treatment for patients with HD depends on the stage of the disease. There are special clinical situations and settings in which the standard approach must be modified, for example in patients with HIV infection. Briefly, patients with stage IA and IIA supradiaphrammatic may be managed with full dose extend field radiation without chemotherapy. Those patients with bulky disease request management with combined chemo- and radiation therapy modality. Patients with stage IA and IIA infradiaphrammatic may be cured with radiation therapy alone. Patients with stages I and IIB are usually treated with chemotherapy alone or with a combined modality approach, i.e. chemotherapy and radiation therapy. Patients with stage IIIA may be treated with a combined modality approach, while those in stage IIIB are generally treated using chemotherapy alone as in a those with stage IV. ABVD (adriamycin, bleomycin, vinblastine and dacarbazine) is the most effective single chemotherapy regimen in HD.

There are a lot of clinical problems that require the intervention of experienced medical oncologists. In particular, pulmonary infiltrates, epidural cord compression, herpes zooster and postsplenectomy sepsis. Moreover, there are clinical problems after therapy including complications and late effects of the therapy, either from radiation therapy or from chemotherapy, e.g. sterility, second-tumors, coronary heart, lung and artery diseases from radiation therapy, etc. Recently, in order to avoid these serious long-term side effects, treatment centers try to use a combined chemo- and radiation therapy approach with a short but intensive chemotherapy regimen in combination with limited field, low-dose radiation therapy. This approach could be successful, in order to maintain the high rate of cures decreasing the potential serious side-effects in the follow-up. Finally those patients who relapsed or become resistant may be salvaged by high dose chemotherapy with stem cell support. This approach has been shown to cure some of the patients who otherwise would have died from HD.

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Hodgkin Disease, clinical oncology

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Synonyms

- Hodgkin's disease
- Hodgkin lymphoma

Definition

Hodgkin disease is a form of cancer - a type of malignant lymphoma. It is a malignancy of Reed-Sternberg cells and variants (Hodgkin cells, collectively). It occurs in about 3 to 4

per 100,000 population and represents approximately 25% of all cases of → lymphoma.

Characteristics

Pathology

Hodgkin disease is identified by the recognition of Reed-Sternberg cells and variants in the appropriate milieu, which usually consists of varying numbers of small lymphocytes, histiocytes, eosinophils and plasma cells. Typically, the cellular milieu consists of greater than 99% of the reactive non-neoplastic elements, with the Hodgkin cells representing a rare dispersed population within involved tissues (and therefore difficult to study, except by single cell dissection). Hodgkin disease can be separated histologically into classical type and nodular lymphocyte predominance type. The classical type is generally subdivided into nodular sclerosis, lymphocyte-rich subtypes, mixed cellularity and lymphocyte depletion subtypes, based on their histologic appearance. Cases of nodular sclerosis are characterized by the presence of thick fibrous bands that traverse the affected lymph node and a variant of Hodgkin cell, the lacunar cell, which shows artifactual retraction of the cytoplasm in formalin-fixed sections. Cases of the lymphocyte-rich subtype have a marked predominance of small lymphocytes and have few identifiable Reed-Sternberg cells, while cases of lymphocyte depletion have a relative paucity of small lymphocytes and often show diffuse fibrosis. The mixed cellularity subtype lacks thick fibrous bands and has an intermediate number of Hodgkin cells. The Reed-Sternberg cells of classical Hodgkin disease are unique polyploid or multinucleated cells with large inclusion-like nucleoli, while variants consist of similar mononucleated cells with large nucleoli. In nodular lymphocyte predominance, a nodular pattern is often seen in the absence of broad fibrous bands and there is a predominance of small lymphocytes. The Hodgkin cells in the nodular lymphocyte predominance type are known as L&H cells and have multilobated nuclei with less prominent nucleoli than the cells in classical Hodgkin disease. Classical Hodgkin cells have a characteristic immunohistochemical profile, being → CD30$^+$, → CD15 (X carbohydrate hapten)$^+$, → CD20 (B lineage marker)$^{+/-}$ and → CD45 (leukocyte common antigen)$^-$, while the L&H cells in the nodular lymphocyte predominance subtype are generally CD30$^-$, CD15$^-$, CD20$^+$ and CD45$^+$. CD30 is a member of the tumor necrosis factor receptor superfamily and is a late activation marker of lymphoid cells. The reactive lymphocytes are usually helper T cells in classical Hodgkin disease. In nodular lymphocyte predominance, B cells make up the predominant cell type in the nodules, however, the L&H (lymphocytic and/or histiocytic) cells are usually surrounded by a collar of CD57$^+$ helper T cells.

Basic aspects

Recent studies of the variable-region genes of the immunoglobulin receptors have demonstrated that the vast majority of cases of Hodgkin cells are derived from germinal center B cells. In nodular lymphocyte predominance Hodgkin disease, the precursor cells are usually mutating germinal-center B cells, and the mutation pattern suggests that the cells have been selected for expression of functional immunoglobulin receptors. In classical Hodgkin's disease, ongoing mutations are not identified. In fact, some of the mutations identified result in stop codons that would ordinarily lead to elimination of the cell in the germinal center through apoptosis. This suggests that the Reed-Sternberg cells of classical Hodgkin disease are preapoptotic, crippled, germinal center B cells. There is some data to suggest that escape from apoptosis may arise through deregulation of NFκB, possibly due to mutations in the IκBα molecule that is the natural inhibitor of NFκB, or overexpression of the tumor necrosis factor receptor-associated factor (TRAF) 1 molecule. In rare cases, Reed-Sternberg cells may be derived from T cells. Hodgkin cells produce a variety of cytokines, including:

- tumor necrosis factor
- interleukins 1-10 and 13
- lymphotoxin-α
- tumor necrosis factor

- transforming growth factor-β
- interferon-γ
- granulocyte-macrophage colony-stimulating factor
- macrophage colony-stimulating factor.

The secretion of these cytokines probably accounts for the abundant cellular milieu as well as some of the unusual clinical findings, such as systemic symptoms. Although high levels of → p53 protein have been identified in Hodgkin disease, only a minority of case have detectable mutations of the *TPp53* gene. About 40% of cases of the classical form of Hodgkin disease are associated with the → Epstein-Barr virus. In these cases, monotypic episomes of the Epstein-Barr virus can be identified in the Hodgkin cells; these cells have a latency pattern II. Epstein-Barr virus-associated cases are particularly found in younger patients and with mixed cellularity and lymphocyte depletion subtypes, particularly in economically disadvantaged populations. There are few cytogenetics studies of Hodgkin disease since Hodgkin cells are rare in involved tissues, they are difficult to grow in culture and few cell lines exist. Most reported karyotypes are extremely complex with frequent polyploidy; no characteristic abnormalities have been consistently identified. Single cell studies have demonstrated the absence of the t(14;18) chromosomal translocation that is the hallmark of follicular B-cell lymphoma.

Clinical

Patients with Hodgkin disease usually present with an enlarged lymph node. Patients with the nodular sclerosis subtype have a predilection for disease above the diaphragm, particularly mediastinal node involvement. Patients with lymphocyte depletion often present with abdominal nodal involvement, while patients with nodular lymphocyte predominance often present with isolated disease in the upper neck. Some patients may also have systemic, or 'B', symptoms-weight loss $>$ 10% during the previous six months, documented fever and night sweats. There is a slight male predominance and a bimodal age distribution in Western populations, with peaks of incidence in young adulthood and old age. Among economically disadvantaged populations, the first peak of incidence occurs earlier in childhood, particularly for males. There is a relatively low incidence of Hodgkin disease among Asian populations. Hodgkin disease usually starts at a single site and progresses in an orderly manner through the lymphatic system to local lymph nodes before disseminating hematogenously to distant sites, particularly the bone marrow, spleen or liver. Hodgkin disease is typically staged from I to IV.

- Stage I represents involvement of a single lymph node structure
- Stage II is involvement of two or more lymph node regions on the same side of the diaphragm
- Stage III is involvement of lymph node regions or structures on both sides of the diaphragm
- Stage IV is involvement of extranodal sites

Prognosis is directly related to stage. Hodgkin disease is typically treated by radiotherapy and/or multidrug chemotherapy, with an overall 5-year survival of greater than 80%. Patients who relapse usually do so within the first three years after treatment and have a significantly worse prognosis. High-dose chemotherapy with autologous bone marrow transplantation or peripheral blood stem-cell transplantation has become a standard therapy for patients who fail conventional chemotherapy regimens. Novel immunotherapies have been proposed, including treatment with interleukin-2, bi-specific antibodies, immunotoxins and radioimmunoconjugates. Patients with the nodular lymphocyte predominance subtype may behave differently than the classical forms of Hodgkin disease, with a better overall survival but a greater number of recurrences that are independent of time after treatment.

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Homeobox Genes

Definition

Homeobox genes are a class of developmental regulatory genes that encode homeoproteins, which function as transcription factors to regulate downstream targets, turning on (activating) or turning off (repressing) other genes that in turn regulate developmental processes. Aberrant expression of homeobox genes has been implicated as causal factors in leukemia and solid tumors; → homeobox genes and cancer.

Homeobox Genes and Cancer

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Definition

Homeobox genes are a class of developmental regulatory genes that encode homeoproteins. Homeoproteins function as transcription factors to regulate downstream targets, turning on (activating) or turning off (repressing) other genes that in turn regulate developmental processes. Aberrant expression of homeobox genes has been implicated as causal factors in leukemia and solid tumors.

Characteristics

Homeobox genes are often referred to as master control genes. They are active during embryonic development and regulate important processes such as → morphogenesis and cellular → differentiation. They were first identified in Drosophila as the homeotic cluster (*HOM-C*) genes and were later found to have homologs in many species. They are now known to be highly evolutionarily conserved and are present in animals, plants and fungi.

Homeobox genes share a common sequence motif (the homeobox), which is 180 nucleotides in length and encodes a 60 amino acid region (the homeodomain). The homeodomain mediates DNA binding to sites containing a → TAAT sequence that are found in the transcriptional regulatory elements of target genes. Upon DNA binding, homeoproteins are thought to act as transcription factors to regulate downstream targets.

There are two major subclasses of vertebrate homeobox genes:

- the clustered genes, or *HOX* genes, and
- the non-clustered genes.

The non-clustered genes are sub-divided into many subfamilies, which are classified on the basis of sequence similarities within their homeobox regions. The *HOX* family of homeobox genes, as well as most other families of homeobox genes, is thought to control cellular proliferation and differentiation during development. The *HOX* genes are expressed beginning in → gastrulation and are involved in patterning the body axis from the branchial arches to the tail.

What is most notable about *HOX* genes is their positioning on four chromosome clusters. Not only are the sequences of the individual genes highly conserved across species, the order of the genes on the chromosomes is conserved as well. Moreover, the physical order of the genes on the chromosome correlates with their spatial and temporal expression patterns along the anteroposterior axis of the embryo.

Many studies have reported a link between deregulated homeobox gene expression and abnormal cellular proliferation, which implicates homeobox genes not only as development regulators, but also as potential protooncogenes and tumor suppressor genes. The downstream

targets of homeobox genes are postulated to include extracellular matrix proteins, adhesion molecules and growth factors. Because these target genes are likely to be important for tumorigenesis as well as development, their misexpression may upset the delicate balance of cell proliferation, differentiation and apoptosis, thereby contributing to carcinogenesis.

Hox genes and leukemia

In addition to their functions during development, homeobox genes also play important roles in adult tissues. For instance, the *HOX* genes are expressed in specific patterns during lineage determination in → hematopoiesis. *HOX* genes have been demonstrated to be important for normal blood cell formation; in addition, abnormal expression of *HOX* and other homeobox genes contributes to the development of leukemia and lymphoma.

A common mechanism by which abnormal gene expression contributes to leukemia is through translocation of two chromosomal regions, which may produce fusion proteins with novel properties or impaired activities. For example, in human acute myeloid leukemia (AML) a translocation between chromosomes 7 and 11 fuses the nucleophorine gene *NUP98* in frame with *HOXA9*. It is thought that the resulting chimeric protein is no longer able to interact with *HOXA9* target genes. Another example of a homeobox translocation in leukemia is the fusion of *PBX1* homeobox gene and the *E2A* gene. Normally, PBX1 forms transcriptionally active protein complexes with HOX proteins; its fusion with E2A alters such interactions, which is thought to direct homeoproteins to different targets, thereby inducing leukemogenesis.

Like PBX, other divergent homeoproteins interact with specific HOX proteins to potentiate or modulate their effects, and their genes are potential targets for deregulation in carcinoma. For example, it now appears that the *MEIS1* homeobox gene is co-activated with *HOXA9* in human myeloid leukemia. In addition, translocation of the *MLL* gene is a common event having been found to be fused to more than 25 other genes in leukemia. While *MLL* is not a homeobox gene, it is homologous to a Drosophila protein that is an important regulator of *HOM-C* genes. The leukemogenic MLL fusion proteins are thought to disrupt *HOX* gene expression in hematopoietic progenitor cells, thereby contributing to myeloid or lymphoid acute leukemias.

Homeobox genes and solid tumors

A common feature of *HOX* gene expression in certain adult organs, such as kidney, lungs and colon, are the significant differences in expression pattern between normal and cancer tissue. For example, in the kidney *HOXC11* is present in tumors but not in normal tissue. Conversely, *HOXB5* and *HOXB9* are normally expressed in the kidney but expression is often lost in tumors. Other *HOX* genes, such as *HOXD4*, are expressed in both normal kidney and in kidney tumors, although they express a different-sized transcript in the tumors versus the normal tissue. In each of these cases the significance of these differential gene expression patterns remains undetermined.

Similar observations have been made in the colon. Misexpression of some *HOX* genes (*HOXA9*, *HOXB7* and *HOXD11*) is found in primary colon cancer and metastatic lesions originating from colorectal tumors. In addition, *HOXB6*, *HOXB8*, *HOXC8* and *HOXC9* are misexpressed at specific stages of colon cancer progression. And, while *HOXB7* and *HOXD4* are both expressed in normal and neoplastic colon, the size of the transcripts differ. Again, the significance of these observations for disease initiation or progression is unclear.

CDX1 and *CDX2* homeobox genes are expressed in normal colon, while their expression is reduced in colon tumors. Interestingly, there appears to be an inverse correlation between the levels of protein expression and the severity of the dysplasia. This correlation, in conjunction with other data, suggests that expression of *CDX1* and *CDX2* is important for maintaining normal colon differentiation and that their loss of expression may promote a cancer phenotype. These observations have been supported by mutant mouse models in which loss of *CDX* genes results in colon tumors.

Misexpression of certain homeobox genes has also been implicated in prostate cancer. For example, the *GBX2* homeobox gene is overexpressed in several metastatic prostate cell lines, suggesting a possible role in cancer progression. Conversely, loss of function of the *NKX3.1* homeobox genes has been implicated in prostate cancer initiation. Notably, *NKX3.1* maps to a hotspot that is frequently deleted in many prostate cancer samples, and mutant mice lacking *NKX3.1* display prostatic epithelial dysplasia. Like, the *CDX* genes, these mutant mouse models provide excellent support for a functional role of these homeobox genes in cancer.

At this point much of the data is rather circumstantial and based on altered expression patterns. Nonetheless, the available evidence suggests that homeobox genes may provide an important link in understanding the delicate balance of development, cell-cycle control and cancer. The specific molecular events that occur between the misexpression of homeobox genes and the progression to cancer remains a topic of active investigation.

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Homing Peptides

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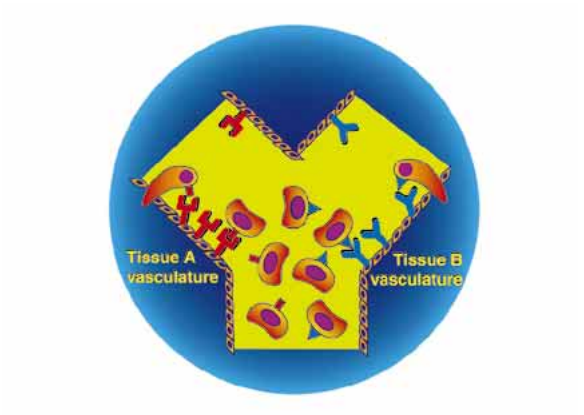
Definition

Homing peptides are peptides that home to specific sites in the vasculature *in vivo*.

Characteristics

Peptides capable of homing to selected target tissues through the circulation have been identified by *in vivo* screening of peptide libraries displayed on phage. The peptides bind to endothelial (and possibly pericyte) surface proteins that are selectively expressed in the vasculature of individual tissues. Homing peptides have been obtained for the vasculature in a large number of individual normal tissues that reveal a previously unsuspected degree of endothelial specialization. Screening for tumor homing has yielded a collection of peptides that home to tumor vasculature.

The normal organs for which vascular homing peptides have been described in the literature include brain, kidney, lung, skin, pancreas, retina, intestine, uterus, prostate and the adrenal gland. Success with so many tissues indicates that many, perhaps all, organs modify



Homing Peptides. Fig. – Homing peptides direct phage binding to specific sites in the vasculature.

the endothelium of the vasculature. The vasculature of tumors is also specialized. Tumors depend on → angiogenesis to grow and undergo → metastasis. The actively growing tumor vessels are biochemically and structurally different from normal resting blood vessels. For example, the integrins αvβ3 and αvβ5, as well as receptors for various vascular endothelial growth factors, are expressed at elevated levels in tumor blood vessels; each of these receptors plays a critical role in angiogenesis. Other promising marker candidates of tumor vasculature include an alternatively spliced form of fibronectin, CD34, collagen type VIII, endosialin, endoglin, aminopeptidases N and A and melanoma-associated proteoglycan.

Several peptide motifs that selectively direct phage into tumors have been identified by phage screening *in vivo* and *in vitro*. One of these motifs contains the sequence RGD (arginine-glycine-aspartic acid) embedded in a peptide motif previously shown to bind selectively to αv integrins. The αvβ3 integrin, together with αvβ5, is one of the known markers of angiogenic vessels. The phage carrying these sequences home into tumors in a highly selective manner and this homing is inhibited by cognate peptides. Tumor homing is independent of the origin of the tumor. It depends on the angiogenic characteristics of tumor vasculature, with neovasculature in the retina and in arthritic synovium targeted.

Determining the nature of the receptors for the homing peptides is in progress. So far, a membrane dipeptidase has been identified as the receptor for a lung-homing peptide, and another enzyme, aminopeptidase N, binds the tumor-homing NGR (asparagine-glycine-arginine) peptides in tumor vasculature. Each of these enzymes is expressed in certain normal epithelia, but their vascular expression is restricted to the target tissue (lung or tumors).

Biological significance

Various lymphoid tissues possess specific receptors that mediate tissue-specific homing of leukocytes. The homing peptide receptors may serve in similar cell trafficking roles. Furthermore, one possible explanation for tissue-specific tumor metastasis is that tumor cells may recognize specific homing peptide receptors in blood vessels at the preferred site of metastasis.

Clinical significance

Coupling of doxorubicin or an → apoptosis-stimulating peptide to tumor-homing peptides greatly enhances the anti-cancer activity of these drugs in mice and lowers their toxicity. The homing peptides may also be useful for the targeting of cells, liposomes and microdevices to tumors. Drug and gene delivery strategies similar to the tumor-targeting may be applicable to targeting of pathological conditions other than tumors.

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Homogeneously Staining Region

Definition

Homogeneously staining region (HSR) is a region within a chromosome lacking the typical banding pattern after staining with Giemsa, indicative of DNA → amplification. In tumor cells indicating → oncogene amplification, in some cases also amplification of genes encoding proteins for drug metabolism.

Homologous Chromosomes

Definition

Homologous chromosomes carry the same genetic loci. A diploid cell has two copies of each homologue, one derived from each parent.

Homologous Recombination Repair

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Definition

Homologous recombination repair is a DNA repair process that includes the invasion of an undamaged DNA molecule by a damaged molecule of identical or very similar sequence. Resynthesis of the damaged region is accomplished using the undamaged molecule as a template.

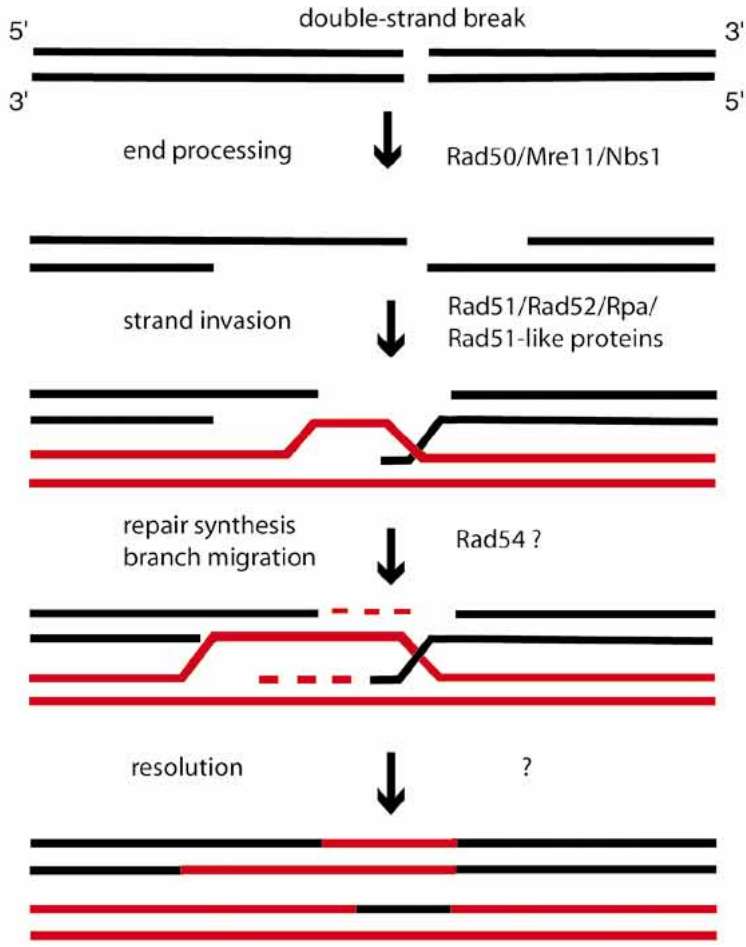
Characteristics

Homologous recombination repair has been found in all organisms examined from bacteria to man. It has an important role in repairing DNA damage with high fidelity by correcting damage with the use of information copied from an homologous undamaged molecule. Sister chromatids (duplicated chromosomes following DNA replication) or the paternal and maternal copies of chromosomes provide the required homology (sequence identity or near-identity over a few hundred DNA base pairs). In somatic cells, if the homologues have some sequence differences the copying process may alter the sequence of the damaged chromosome to the same as that of the undamaged chromosome, potentially revealing mutations (→ loss of heterozygosity). Additionally, recombination between repeat sequences may lead to high frequencies of sequence variation as seen in certain → minisatellite sequences. In germ cells, homologous recombination is vital for the reassortment of chromosomes during meiosis to create genetic diversity in organisms.

Cellular & Molecular Regulation

The proteins that mediate homologous recombination repair have functions and often structures that have been conserved in evolution. These proteins have to seek out homologous regions of chromosomes, exchange DNA strands, copy sequence from the undamaged strand and finally resolve the DNA structures arising from exchange. This complex series of events is best understood in bacteria but recently many of the components and functions of homologous recombination repair have been identified in mammalian cells. Evidence suggests that DNA double-strand breaks commonly trigger repair by homologous recombination; these breaks may be caused by the interaction of DNA with chemical radicals, produced as a consequence of cellular metabolism, or by external damaging agents such as ionizing radiations. Meiotic recombination is also driven by DNA double-strand breaks, but these are formed enzymatically at specific sites in DNA.

In bacteria, the → RecA protein is central to homologous recombination through its ability to search for homologous regions of DNA and promote strand exchange. RecA forms a polymer on DNA to give a nucleoprotein filament, acting as a DNA-dependent ATPase. Filament formation occurs very rapidly on single-stranded DNA; in purified solutions the rate can approach 1000 RecA monomers assembled per minute. RecA will also polymerise on double-stranded DNA but needs a short single-stranded gap to start the process, potentially targeting the protein to sites needing repair. On binding RecA and in the presence of homologous double-stranded DNA, strand exchange occurs to form junctions between the two DNA molecules. A number of other proteins are involved in the early stages of homologous recombination repair in bacteria; to generate the single-stranded DNA required for RecA-



Homologous Recombination Repair. Fig. – A model for the repair of a DNA double-strand break by homologous recombination in human cells. The main steps are noted to the left, and the proteins involved (where known) to the right. The Rad51-like proteins include Xrcc2 and Xrcc3. The broken DNA molecule (black) is processed to give long 3′ single stranded regions; these invade an undamaged homologous molecule (red). The branched-out undamaged strand acts as a template to repair the break. Repair synthesis is accompanied by branch migration. Resolution involves cutting the junctions between the two molecules; here it is shown without crossing-over, potentially leading to gene conversion where the homologous molecules differ in sequence.

mediated strand exchange different proteins (RecBCD, RecJ, RecQ, or RecE) may be used depending on the initial state of the DNA. For example, during bacterial conjugation the RecBCD protein will unwind DNA from a break and cut the unwound DNA at a specific sequence-recognition site, forming a long 3′ single-stranded region suitable for invading homologous double-stranded DNA. Other proteins assist in the loading of RecA onto single-stranded DNA and/or strand-invasion activities (RecF, RecO, RecR and single-stranded DNA binding protein). During recombination the junctions formed at sites of exchange migrate along the DNA molecules to complete gap repair, and finally the junctions must be cut to free the participating DNA molecules. Again some of these functions are supplied by different proteins, in this case either RuvABC or RecG, indicating the presence of more than one pathway for homologous recombination.

While much of the mechanistic detail of homologous recombination repair has been described in bacteria, it is clear that the principles

of this mechanism have been retained by all organisms. RecA-like proteins have been found in eukaryotes; in particular the → Rad51 protein from the yeast *Saccharomyces cerevisiae* (Rad51p) has both structural and functional similarities to RecA. Rad51p leads to the formation of nucleoprotein filaments on DNA and the promotion of homologous pairing and strand exchange reactions *in vitro*. However, in addition to Rad51p there are three other RecA/Rad51-like proteins in yeast; Rad55p, Rad57p and Dmc1p. Biochemical studies suggest that these RecA/Rad51-like proteins do not have redundant functions but rather have distinct roles to play in the early stages of repair. The Dmc1 protein functions exclusively in meiosis, with loss of its function leading to sterility, while the other RecA-like proteins operate in both mitosis and meiosis. Rad55p and Rad57p exist as a dimer and appear to act as a cofactor for the assembly of Rad51p onto single-stranded DNA. A three-protein complex (Rad50p/Mre11p/Xrs2p) has been found that promotes the early stages of homologous recombination but is also involved in a number of other repair and DNA maintenance functions; the Mre11 protein in particular has nuclease activities that may process double-strand breaks to form single-stranded regions associated with strand invasion. Other yeast recombination proteins, such as Rad52p and Rad54p, interact with Rad51p and promote Rad51-mediated strand exchange. Rad52p interacts with single-stranded DNA binding protein (Rpa) and facilitates the loading of Rad51p onto single-stranded DNA lacking secondary structure. Rad54p is structurally related to a family of putative DNA helicases but its precise function is unknown.

Rad51 also occurs in human cells. Despite millions of years of evolutionary divergence, the human protein is remarkably similar in structure and function to the yeast Rad51 protein. Additionally, six further → RecA/Rad51-like proteins have recently been identified in humans. Two of these, Xrcc2 and Xrcc3, were found through their ability to complement the sensitivity of certain mammalian cell mutants to DNA-damaging agents. The remainder, including a protein closely similar to the yeast Dmc1, were found by searching for RecA/Rad51-like proteins in the human protein sequence databases. These proteins are presently the subject of intensive study but there is already evidence that some may function like the yeast Rad55 and Rad57 proteins in forming dimers that help Rad51 to function efficiently. The increase in the numbers of RecA/Rad51-like proteins in higher organisms also suggests that certain specialized functions may have evolved to deal with specific types of DNA damage or with tissue-specific recombination events. The yeast → Rad50 and → Mre11 proteins are also conserved in mammalian cells, but a structural homologue of the yeast Xrs2 protein has not been found in humans. Instead the Nbs1 [→ Nijmegen breakage syndrome] protein (also known as p95 or nibrin), defective in the radiosensitive and cancer-prone human disorder → Nijmegen breakage syndrome, appears to be a functional analogue of Xrs2. The features of Nbs1-deficient cells are very similar to those of ataxia-telangiectasia cells, and recently the human Mre11 protein has been shown to be mutated in individuals with an ataxia-telangiectasia-like disorder. Proteins similar to the yeast → Rad52 and → Rad54 have also been found in humans, and in the case of Rad54 it has been shown that the human protein is able to function in place of the yeast protein. The Rad52 protein is not so well conserved in structure between yeast and humans, although it is possible that another Rad52-like protein remains to be discovered in humans. In biochemical experiments, however, the human Rad52 protein has been shown to stimulate Rad51-mediated DNA-strand transfer reactions, probably at an early stage of Rad51 filament formation. Proteins involved in the later stages of recombination repair have yet to be identified in yeast or mammalian cells. The interactions of these proteins in the early stages of repair of a DNA double-strand break by homologous recombination are illustrated in the Figure.

To study the effects of loss of recombination genes in mammals, several of the genes have been disrupted in mice ('knockout mice'). Surprisingly, disruption of *Rad51* in mice is lethal for embryonic development and cells could not

be cultured from tissue derived from the knockout animal. This finding suggests that the mammalian *Rad51* gene has an important role in essential aspects of DNA metabolism or in development. It also suggests that the other *Rad51-like* genes cannot replace the function of *Rad51* itself. Disruption of some of the *Rad51-like* genes in mice also leads to embryonic lethality, showing that these similarly have important functions in the organism. In contrast, knocking-out other recombination genes in mice is not necessarily lethal. Disruption of the meiosis-specific *Dmc1* gene, as well as similar knockout experiments with the mouse *Rad52* and *Rad54* genes, yielded viable progeny. With the possible exception of the *Rad52* knockout mouse, each has severe defects in aspects of mitotic and/or meiotic processes, consistent with roles in homologous recombination repair.

The potential importance of homologous recombination repair genes in genetic stability and in development has been highlighted by several recent discoveries. Firstly, it has been found that the human Rad51 protein interacts physically with the tumour-suppressor protein p53, which has a central role in the control of the cell cycle and apoptosis. Additionally, Rad51 knockout embryos survived longer in a p53-deficient background, suggesting at least part of the growth problems experienced by Rad51 knockouts arises from unrepaired damage triggering → cell-cycle checkpoints leading to growth arrest. Equally important, Rad51 interacts with the breast cancer-susceptibility gene products Brca1 and Brca2. Disruption of the → *Brca1* or → *Brca2* genes in mice gives embryonic lethality and shows a similar elevation of chromosomal aberrations as that found in *Rad51*-disrupted cells. The link between the Brca proteins and homologous recombination repair has been extended by showing that Brca1 and Brca2-deficient cells have a large reduction in ability to repair double-strand breaks in homologous substrates integrated into the genome. Brca1 is phosphorylated in response to DNA damage and this event is dependent on the protein kinase mutated in the cancer-prone disorder ataxia-telangiectasia (Atm). Atm may act as a sensor of DNA damage, especially double-strand breaks, and there is evidence supporting a model in which Brca1 phosphorylation triggers the activity of Rad51 and Brca2 to initiate homologous recombination repair. However, Brca1 also associates with the human Rad50 protein and has a role in the → transcription-coupled repair pathway, so its action is likely to be broader than this model suggests.

The role of recombination proteins in influencing development in multicellular organisms has been illustrated by recent findings in the fruit fly, *Drosophila melanogaster*. Mutations in the *spindle* class of genes lead to patterning defects in the oocyte and embryo, apparently due to mis-localization and/or failure to accumulate normal levels of oocyte signalling molecules. The cloning and sequencing of these genes showed that they are homologues of *RAD51* or *RAD54*. Mutation in these genes leads to the accumulation of DNA damage, which triggers a meiotic recombination checkpoint that down-regulates some of the genes essential for embryonic development. Interestingly, oocytes that are mutant for both *spindle*-class genes and the *Drosophila* homologue of *ATM* (*mei-41*) can bypass this meiotic arrest and show normal developmental patterning, suggesting that loss of ability to signal damage fails to trigger this checkpoint. The downside of this checkpoint loss is that DNA damage may be carried through into later cell divisions, resulting in genomic instability.

The mechanisms of homologous recombination repair are still being investigated, but it is clear that this repair pathway is of considerable significance in all organisms. Other pathways can repair certain types of DNA damage; for example, in mammalian cells, → non-homologous end joining is an alternative way to repair DNA double-strand breaks. However, simple end joining of broken DNA is prone to loss of sequence (DNA deletion) at the damage site, while homologous recombination repair can rejoin breaks with high fidelity. In bacteria it is clear that a major role of homologous recombination repair is to sort out problems arising during DNA replication, in particular where a replication fork stalls due to damage in its path. In yeast and mammals there is also evidence that homologous recombination pro-

teins are particularly active during DNA replication. Additionally, certain types of DNA damage such as →DNA interstrand cross-links formed by agents such as mitomycin-C may be resolved only by the homologous recombination repair pathway. In support of this idea, cell lines lacking homologous recombination repair genes are very sensitive to mitomycin-C and other DNA cross-linking agents.

Clinical Relevance

Loss of homologous recombination repair leads to unrepaired damage in the genome, which in turn can give rise to genomic instability. Both Mre11 and Nbs1 have been found to be associated with rare human disorders with complex phenotypes, including radiation sensitivity and cancer-proneness (ataxia-telangiectasia and Nijmegen breakage syndrome, respectively). The association of homologous recombination repair proteins with p53 and the Brca proteins also suggests that this repair pathway has potentially important connections with predisposition to cancer. The links are strongest with breast cancer, through the Brca proteins and possibly the Atm protein. Additionally, loss of one of the human Rad51-like proteins (Rad51L1) has been associated with uterine leiomyomas.

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Homophilic Adhesion

Definition

Homophilic adhesion is →adhesion between two undefined cell types can be mediated by either identical (homophilic) or different (heterophilic) adhesion molecules; →cell adhesion molecules.

Homotypic Adhesion

Definition

Homotypic adhesion is mediated by undefined adhesion molecules between identical cell types (homotypic) or two different cell types (heterotypic).

Homozygote

Definition

A homozygote is an individual with the same allele at corresponding loci on the homologous chromosome.

Homozygous Deletion

Definition

Homozygous deletion is the physical loss of both copies of the same allele or the same chromosomal segment of a pair of homologous chromosomes.

Hormone

Definition

A hormone is a protein that binds with high affinity to a receptor to activate a signal transduc-

tion pathway leading to growth, differentiation, embryogenesis or other responses; some hormones act tissue specifically.

Hormone Replacement

Definition

Hormone replacement is the administration of a hormonally active compound to compensate for the absence of a natural hormone, for example estrogen after menopause.

Hotspot

Definition

A hotspot is a site at which the frequency of mutation (or recombination) is increased.

Household Genes

Definition

→ Constitutive genes.

Housekeeping Genes

Definition

Housekeeping genes are expressed in all tissues and during all developmental stages and encode proteins essential to all cells.

HPLC

Definition

High performance liquid chromatography (HPLC) linked to an electrochemical detector is capable of measuring → adducts to DNA and other products resulting from → oxidative DNA damage.

HPMS1-2

Definition

hPMS1-2 are human homologues of mut L that if inactivated in yeast cause a high frequency of post-meiotic segregation. These genes encode proteins that are components of the human mismatch repair (MMR) system; defective MMR may cause → microsatellite instability in tumors.

HPRC

Definition

Hereditary papillary → renal carcinoma.

HSF

Definition

Hepatocyte stimulating factor (HSF); → interleukin-6.

HSP

Definition

Acronym for heat shock protein. HSP89α, HSP90β; the *HSP89α* and *HSP90β* genes are composed of 11 and 12 exons, respectively. The regulation of *HSP* gene expression in eukaryotes is mediated by the conserved heat shock transcription factor (HSF). HSF acts through heat shock elements (HSEs) composed of three contiguous inverted repeats of a 5 bp sequence, nGAAnnTTCn; upon heat stress, HSF binds to HSEs as a trimer.

HSR

Definition

→ Homogeneously staining region.

HSV-TK

Definition

Herpes simplex virus thymidine kinase; → HSV-TK/Ganciclovir mediated toxicity.

HSV-TK/Ganciclovir Mediated Toxicity

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Definition

HSV-TK/Ganciclovir mediated toxicity is a form of → suicide gene therapy in which the cDNA for a viral enzyme, the herpes simplex virus thymidine kinase (HSV-TK), is transferred to tumor cells that are then treated with the antiviral drug ganciclovir (GCV). Expression of HSV-TK enables cells to phosphorylate GCV to a monophosphate derivative. Cellular enzymes convert the monophosphate to GCV triphosphate, which elicits toxicity through incorporation into DNA.

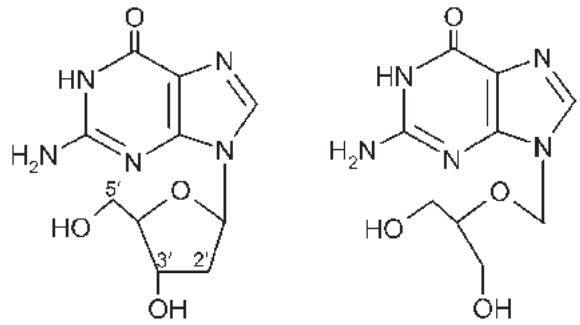
Characteristics

Toxicity in HSV-TK-expressing cells

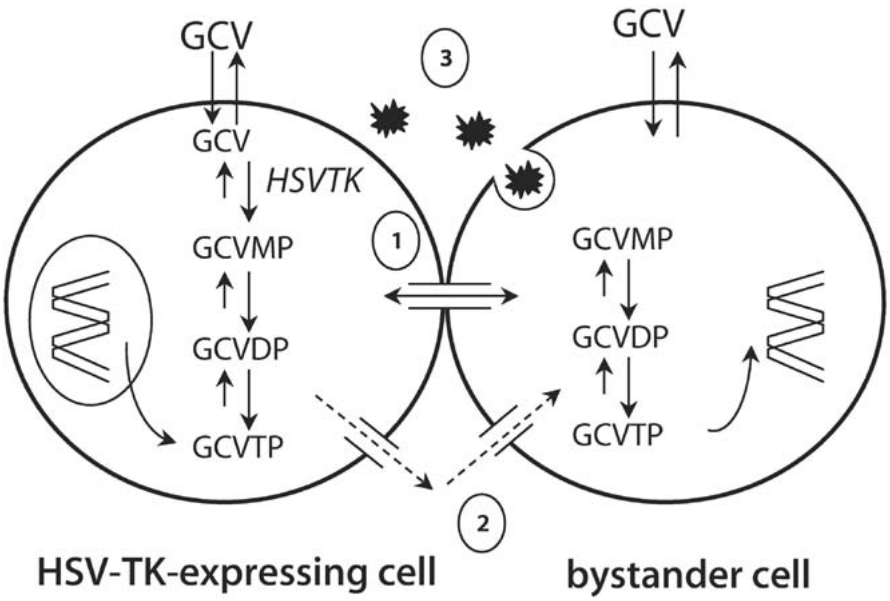
GCV is an acyclic analog of 2′-deoxyguanosine (Fig. 1). It was originally discovered as an antiherpesvirus agent, and it is used clinically in the treatment of cytomegalovirus infection. GCV is normally not toxic to mammalian cells because they lack an endogenous kinase that would phosphorylate the drug to the monophosphate form. The ability of mammalian cells genetically engineered to express HSV-TK to be killed by GCV was first shown in murine sarcoma cells. Since then numerous reports have demonstrated similar results in many different cell types.

After uptake into the cells, GCV is phosphorylated by HSV-TK to its monophosphate form (Fig. 2). GCV has a relatively high Km value for HSV-TK of approximately 50 μM, and in this concentration range it does not function as a substrate for any of the mammalian kinases, which accounts for its selective toxicity to HSV-TK-expressing cells. GCV monophosphate is then acted upon by the successive actions of cellular kinases to achieve the diphosphate and triphosphate derivatives. The phosphorylated derivatives of GCV are the major metabolites found in cells that express HSV-TK, with the triphosphate present in highest amounts. GCV does not appear to be a substrate for degradative enzymes.

At the triphosphate level, GCV can compete with the endogenous dGTP for incorporation into DNA by mammalian DNA polymerases. Since GCV has the equivalent of both the 5′ and 3′ hydroxyls of deoxyguanosine, it can theoretically be incorporated into DNA and allow further extension of the DNA strand (internucleotide addition). Studies using cell-free DNA polymerizing system, as well as studies in intact cells, have demonstrated that GCV monophosphate is incorporated into internucleotide linkages in DNA. GCV and its metabolites do not interfere with RNA or protein



HSV-TK/Ganciclovir Mediated Toxicity. Fig. 1 – Structure of ganciclovir (GCV).



HSV-TK/Ganciclovir Mediated Toxicity. Fig. 2 – Activation of GCV in HSV-TK-Expressing cells and putative bystander mechanisms. GCV is selectively phosphorylated to the monophosphate in HSV-TK expressing cells. Further phosphorylation can be accomplished by cellular enzymes. There are three proposed mechanisms for the transfer of phosphorylated metabolites to induce killing of bystander cells: **1** direct transfer through GJIC channels, **2** release from an HSV-TK-expressing cell and uptake from the media, **3** engulfment of apoptotic vesicles from dying, HSV-TK-expressing cells.

synthesis. The incorporation of GCV monophosphate into DNA is the primary lesion that results in cell death. For many cell types GCV induces cell death through → apoptosis.

Compared to other substrates for HSV-TK, GCV is significantly more toxic and more mutagenic to cells (2, 3). Comparisons between the cytotoxicity of GCV and the structurally related acyclovir in HSV-TK-expressing cells have consistently shown that GCV is far superior in its ability to kill cells (2). The action of acyclovir is primarily cytostatic, whereas GCV induces cell killing at low clinically achievable concentrations. Although GCV triphosphate accumulates in cells to a relatively low level of 10-20 μM, this is sufficient to produce several logs of cell death. This high toxicity may be attributable to the avid incorporation and lengthy retention of GCV into DNA. GCV triphosphate and its incorporation into the nascent DNA strand do not produce strong inhibition of DNA synthesis, so cells incorporate high levels of this drug, complete DNA replication and go on to divide. Studies have demonstrated that daughter cells become irreversibly blocked when they enter S-phase, suggesting that GCV monophosphate cannot serve as a template for DNA replication. A strong G2/M block has also been observed in some cell types after GCV treatment. The cell cycle position in which cells become blocked may depend on the concentration of GCV.

Toxicity to non-HSV-TK-expressing cells (bystander effect)

With current gene transfer technologies, only a small percentage of tumor cells will express the foreign gene. For this approach to be successful in cancer treatment there must be a mechanism by which cells that do not express the transgene (bystander cells) can be killed. It was noted early on that when only a fraction of the cell population expressed HSV-TK treatment with GCV resulted in killing of both the HSV-TK-expressing and HSV-TK-nonexpressing bystander cells. The strong bystander cell killing effect of HSV-TK/GCV has resulted in complete re-

gressions of experimental tumors in animals, which has spurred clinical interest in this approach (4).

In suicide → gene therapy, bystander cell killing generally occurs through the transfer of a toxic metabolite produced in a cell expressing the transgene to bystander cells. The bystander killing with HSV-TK/GCV was an unexpected finding since the toxic metabolite, GCV triphosphate, is negatively charged and therefore would not readily pass through cell membranes to kill bystander cells. However, studies have demonstrated that GCV mono-, di- and triphosphate accumulate in bystander cells when co-cultured with HSV-TK-expressing cells and GCV (5). It has been demonstrated that there are several mechanisms by which phosphorylated GCV can pass from an HSV-TK-expressing cell to a bystander cell.

Several possibilities exist to explain bystander effects:

- The best studied of these mechanisms is → gap junctional intercellular communication (GJIC). GJIC allows the direct exchange of small molecules (<1000 daltons) between neighboring cells. With a molecular weight ranging from approximately 350 to 510 daltons, GCV mono-, di- or tri-phosphate could potentially be transferred between cells through gap junctions. Tumor cell lines show varying levels of GJIC activity, in which some are totally devoid of GJIC whereas others show nearly 100% communication between cells. Many investigators have shown that increasing the GJIC activity of tumor cells enhances the bystander cell killing, consistent with the notion that phosphorylated GCV is transferred from HSV-TK-expressing to bystander cells through gap junctions (6).
- Another possible mechanism to explain the bystander killing with HSV-TK/GCV is through the formation of apoptotic vesicles (7). It has been shown that as HSV-TK-expressing cells die after GCV exposure apoptotic vesicles form and are engulfed by neighboring cells. Through this mechanism, either HSV-TK or phosphorylated GCV could be transferred to bystander cells. This mechanism would only work in cells that die through apoptosis following GCV treatment.
- There appears to be at least one other mechanism to account for bystander killing with HSV-TK/GCV. Bystander cell killing has been demonstrated in tumor cells with low or no GJIC when cultured with HSV-TK-expressing cells and GCV. In addition, in tumor cells with GJIC in fewer than 3% of the population, phosphorylated GCV has been detected in bystander cells after co-culture with HSV-TK-expressing cells and GCV (5). Phosphorylated GCV was detected in bystander cells as early as 4 hours after GCV addition to the co-culture and prior to the appearance of apoptosis. While this mechanism for transfer has not been fully characterized, it appears to result from the release of phosphorylated GCV from an HSV-TK-expressing cell to the surrounding medium with subsequent uptake by a bystander cell. Regardless of the mechanism for transfer of phosphorylated GCV, it is not known whether GCV mono-, di- or tri-phosphate or possibly all three metabolites are transferred.

Experimental tumors in animals

To test the efficacy of HSV-TK/GCV, investigators have implanted cell lines that stably expressed HSV-TK into animals and then administered GCV systemically. Although this approach does not accurately mimic methods for inducing HSV-TK expression in existing tumors, it allowed researchers to determine under ideal conditions whether HSV-TK/GCV would have antitumor activity. In addition, it allowed for testing of the bystander effect by implanting a known percentage of HSV-TK-expressing tumor cells in the presence of vector control cells. This approach showed good efficacy, producing complete regressions in animals even when only 10% of the tumor cells expressed HSV-TK. These studies showed promise for the clinical utility for HSV-TK/GCV with evidence of a strong bystander effect *in vivo*.

Further studies have utilized a number of different viral vectors (retrovirus, adenovirus,

herpes simplex virus or adeno-associated virus) containing the cDNA for HSV-TK to transduce tumors implanted in animals. While the percentage of cells that expressed HSV-TK did not exceed 10% for *in vivo* gene transfer, the results of treatment with GCV were still impressive with some animals showing complete tumor regressions. Thus, under conditions that more accurately represented the approach that would be used clinically, efficacy was demonstrated for HSV-TK/GCV. These successful animal studies fueled the clinical interest in HSV-TK/GCV and to date it is one of the more commonly used gene therapy approaches in cancer treatment.

Mechanism of the bystander effect *in vivo*

With demonstration of transfer of phosphorylated GCV between HSV-TK-expressing and non-expressing cells *in vitro* as an important component of the bystander effect, it was expected that a similar mechanism would explain the bystander effect noted *in vivo*. While this may explain a portion of the bystander cell killing in experimental tumors, it was quickly noted that there appeared to be an immune component as well (4). Tumors in immune-competent animals showed more regressions than the same tumor in immune-deficient animals, implying that the immune system mediated some of the antitumor activity. The most compelling evidence of the involvement of the immune system is the demonstration of a distant bystander effect. When two tumors were injected in opposite flanks of the animal, only one of which expressed HSV-TK, both tumors could regress following treatment with GCV. It has been postulated that the dying tumor cells that express HSV-TK become immunogenic and assist in the regression of the non-HSV-TK-expressing tumor cells. These studies have shown that, *in vivo*, the assistance of the immune system in promoting tumor regression is a powerful addition to the HSV-TK/GCV approach.

Clinical relevance

Since the initial reports of tumor regression in animals treated with HSV-TK/GCV, many clinical trials utilizing this approach have been initiated. Based on reports of brain tumor regressions in experimental animals, glioblastomas were targeted for HSV-TK/GCV treatment in clinical trials. In addition, several trials have focused on treatment of ovarian cancer with HSV-TK/GCV. These trials have primarily employed retroviral or adenoviral vectors to transduce tumor cells to express HSV-TK. Initial results have shown that these vectors are safe to use in humans, although some toxicity was detected with high doses of virus particles.

Although most of these initial trials were Phase I studies designed to test the safety of the vector administration, clinical outcomes were reported. Results from these and from Phase I/II trials have not demonstrated strong antitumor activity with HSV-TK/GCV. There are several possible reasons that the results have not been more dramatic:

- First, most patients in these trials had been previously treated with surgery, chemotherapy and/or radiation therapy, so these trials were treating tumors that were already resistant to initial therapy and therefore less likely to respond to the gene therapy.
- Second, despite the administration of high numbers of virus particles and multiple administrations of the vector in some trials, it is likely that fewer than 1% of the cells in the tumor expressed HSV-TK. Thus, while strong bystander activity has been demonstrated for HSV-TK/GCV in experimental tumors, at least 10 times more cells expressed HSV-TK in the animal models.

Future directions for cancer therapy with HSV-TK/GCV

Considering the low efficiency of gene transfer for current methodologies, it is likely that the major limitation to the therapeutic efficacy of HSV-TK/GCV is the inability to transduce a larger percentage of the tumor. Improved vector delivery systems that will allow transgene ex-

pression in more tumor cells could make an important impact. Structural modifications to HSV-TK that improve affinity for GCV have been shown to be efficacious in preclinical models (8). However, the low extent of gene transfer may still limit these improved HSV-TKs.

One aspect of this treatment that has not received much attention is that of dose-scheduling for GCV. The current dosing regimen is based on that used for treating patients with herpes virus infections. Given the limited period of time that cells express HSV-TK after gene transfer, increasing dose intensity to match the duration of expression of the transgene may improve therapy. Pharmacokinetic and pharmacodynamic studies are needed to determine the optimal dose and schedule of drug administration for HSV-TK/GCV tumor therapy.

With the current low efficiency of gene transfer regardless of the vector used, methods to improve bystander cell killing could make a major impact. Preclinical models have shown that transfer of vectors for connexin expression can improve bystander killing. However, to increase bystander killing by this mechanism requires connexin expression in a large percentage of both the bystander and HSV-TK-expressing cells, and thus with the low level of gene transfer by current methodologies this may not impact tumor therapy significantly. Recent reports demonstrate that the addition of hydroxyurea, a ribonucleotide reductase inhibitor, to HSV-TK/GCV regimens can synergistically increase bystander cell killing both *in vitro* and *in vivo* in animal models. This appears to work through increasing the GCV triphosphate: dGTP value, resulting in greater GCV incorporation into DNA (9). Pharmacologic manipulation to increase bystander cell killing would be expected to make a more dramatic improvement in efficacy than methods requiring gene transfer.

Suicide gene therapy in cancer treatment is still in its infancy. Although the promising results in preclinical models have not been realized in clinical studies, there is a great deal that can be tested to enhance this therapeutic approach. Improving gene transfer, optimizing drug administration regimens, increasing bystander cell killing through immunologic or pharmacologic means, and combining HSV-TK/GCV with other therapies may contribute to greater efficacy with this novel approach to cancer treatment.

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hTERT

Definition

Human telomerase reverse transcriptase (hTERT) is a catalytic subunit of → telomerase.

HTF islands

Definition

*Hpa*II tiny fragments (HTF); → CpG islands.

HTLV

Definition

→ Human T-cell leukemia virus.

HTLV-1

Definition

Human T-cell lymphotropic virus type-I (HTLV-1) is a human → retrovirus associated with a clinically aggressive form of adult T-cell leukemia (ATL) and the degenerative neuromuscular diseases, tropical spastic paraparesis and HTLV-I associated myelopathy (TSP/HAM).

HTLV-1 TAX

Definition

HTLV-1 Tax is a transactivating protein of region X, a transforming protein encoded by the X-region of the → HTLV-I genome. Tax trans-activates a variety of cellular and viral promoters through interaction with cellular transcription factors.

HUGO

Definition

Human Genome Organisation. An international organisation that, among other tasks, defines the nomenclature for genes and proteins; http://ash.gene.ucl.ac.uk/nomenclature/.

Human Herpesvirus 4

Definition

→ Epstein-Barr virus.

Human Herpesvirus 6

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Definition

The family *Herpesviridae* is comprised of viruses characterized by a distinct morphology. Virion particles are enveloped and have a diameter of approximately 200 nm. The icosaedral capsid contains a double stranded linear DNA molecule with a molecular weight of 80 to 150 million.

Characteristics

According to their biologic and molecular properties, → herpesviruses are classified in three subfamilies (*alphaherpesvirinae*, *betaherpesvirinae*, *gammaherpesvirinae*). Humans are the natural host for eight herpesviruses: herpes simplex 1 (HSV-1), herpes simplex 2 (HSV-2), varicella zoster virus (VZV), human cytomegalovirus (HCMV), Epstein Barr virus (EBV), human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7) and human herpesvirus 8 (HHV-8), also known as Kaposi sarcoma associated herpesvirus.

All herpesviruses have the important biologic characteristic of establishing life-long latent infections within specific cells of the host after primary infection. Latent virus may occasionally reactivate and produce recurrent infections. Both animal and human herpesviruses have been implicated in the etiology of animal or human neoplasms. The human herpesviruses with oncogenic or transforming potential include EBV, HSV-2, HCMV, HHV-8 and HHV-6.

HHV-6 is a *betaherpesvirinae* encoding approximately 100 genes and has a selective tropism for T-lymphocytes and macrophages. Viral strains can differ in biological properties such as pathogenic potential, *in vitro* cell tropism, reactivity with monoclonal antibodies, and are consequently divided in two variants: HHV-6A and HHV-6B. Infection with HHV-6, usually acquired in early infancy, is highly prevalent in humans. The virus causes Exanthem Subitum, a benign childhood disease, but infection can remain asymptomatic or result in febrile episodes without relevant symptoms. As a result of primary infection the virus establishes latent infection and resides silently in T-lymphocytes and in macrophages. In fact, small amounts of viral DNA are commonly detected in the blood of healthy adults. Occasionally, it may reactivate, especially in immunocompromised individuals (HIV-infected, transplant recipients). HHV-6 reactivation has been linked to disease especially in bone marrow transplant recipients. HHV-6 is present also in salivary glands and may be shed with saliva.

Clinical aspects

A possible role of HHV-6 in human oncogenesis has been suggested since its original isolation from patients with B- and T- cell lymphoproliferative diseases. Subsequently, HHV-6 DNA sequences have been detected in a variable number of non-Hodgkin lymphomas, in sporadic cases of leukemia, angioimmunoblastic lymphoadenopathy, cerebral lymphoma and also in neuroglial tumors, oral carcinoma, cervical carcinoma. However, an etiological role of the virus in these cases is not likely, whereby only a small number of cells are infected by HHV-6, even in the case of clonal lymphoproliferations. Nonetheless, the potential association with Hodgkins disease has more strength. Viral sequences are present in about 30% of cases and viral antigens can be detected in Reed-Sternberg cells, which appear to be the neoplastic element in this non-clonal lymphoproliferative disease. Furthermore, HHV-6 can reactivate EBV and enhance its gene expression. This synergistic interaction could be significant in the development of EBV-associated oncogenesis. Additionally, HHV-6 can integrate in host cell chromosomes and enhance the tumorigenic potential of human papillomavirus.

The tumorigenic role of HHV-6 is supported by *in vitro* studies that show a transforming potential on murine and human cells. Three different fragments of viral DNA immortalize primary cells or morphologically transform continuous cell lines. These cells injected in nude mice develop tumors. The protein encoded by the viral gene DR7, encoded by one of the transforming fragments, has the ability of transactivating heterologous promoters and binds to the antioncogene p53, altering its ability to regulate genes important in growth control.

In conclusion, the etiologic role of HHV-6 in human neoplasias is still undefined. The observation that only a small proportion of cells shows footprints of viral infection, even in neoplasias with a clonal origin, suggests the possibility that the virus might simply be a passenger in the tumor tissues. On the other hand, the detection of viral sequences in lymphoproliferative diseases and the *in vitro* transforming potential of selected fragments of viral DNA support the hypothesis that the virus might be involved in the oncogenetic process, at least in a fraction of cases.

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Human Papillomaviruses

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Synonyms

- human papillomavirus (HPV)
- human wart virus

Definition

Human papillomaviruses (HPV) are members of a heterogenous group of viruses; to date, there are almost 100 different viruses (HPV 1, 2, etc.) identified by DNA sequence analysis (genotypes). Papillomaviruses of a number of animal species have been found (e.g. bovine papillomavirus, canine oral papillomavirus). With very few exceptions papillomaviruses infect only their natural hosts. According to structural similarities they are grouped together with the polyomaviruses (e.g. simian virus 40 of monkeys) as the family of *papovaviridae*.

Characteristics

Virus particle

Human papillomavirus consists of an isosaedral virus capsid (without a lipid-containing envelope) of 55 nanometer in diameter that is built up of 360 copies of the major structural protein (L1) and 12 copies of the minor capsid protein (L2). Enclosed in the protein shell is one molecule of a circular double stranded DNA of about 8000 basepairs, which constitutes approximately 20% of the virus particle by weight. The total molecular weight of a papillomavirus particle is approximately $25x10^6$ daltons. In addition to the genes *L1* and *L2* coding for the structural proteins, the genome contains also 6-8 'early' genes. These code for 'non-structural' proteins required for the regulation of viral gene expression (E2), for DNA replication (E1, E2, E6, E7) and virus maturation (E4). Transcription of the entire genome proceeds in one direction, i.e. only one strand contains the coding information. The major promoters are located in a 1 kb region ('upstream regulatory region'; URR), which contains the origin of replication.

Virus replication

Papillomaviruses infect basal cells of stratified epithelia. Binding to and uptake into target cells appears to be mediated by a specific receptor(s), although the precise mechanism is not known. Low levels of DNA replication occur within basal cells, which appears to be necessary for persistence of papillomavirus infection. In suprabasal cells, vegetative DNA replication is initiated. By the aid of the viral proteins E6 and E7 the cells of the stratum spinosum that have entered the program of epithelial differentiation reinitiate the S-phase of the cell cycle. This switch provides the virus with the cellular machinery that is required for its own DNA replication. The E7 protein was found to bind to the hypophosphorylated form of the retinoblastoma [→ retinoblastoma protein, cellular biochemistry] protein (pRB), a critical molecule in the control of the G1/S boundary during the cell cycle. As a consequence, transcription factors of the E2F family are released from a complex with pRB and trigger expression of genes that are necessary for DNA replication and cell division, such as Myb and cyclin A and cyclin E. There is evidence that E7 can also override cell cycle control at the G2/M checkpoint via interaction with and functional inactivation of inhibitors of cyclin-dependent kinases ($p21^{Waf1/Cip1}$ and → p27). Additional effects of the E7 have been described that may lead to cell proliferation, such as binding to members of the → AP-1 transcription factor family.

The papillomavirus protein E6 binds to the product of the tumor suppressor gene *p53*, leading to its → ubiquitination and rapid degradation. This gene, which by deletion or mutation is involved in more than 50% of human cancers, is crucial for cell-cycle control, like

the members of the RB-family. Activated in response to stress (e.g. by DNA damage), the p53 protein leads to cell cycle arrest and eventually to → apoptosis. It is assumed that one of the functions of the E6 protein is to counteract the side effect of E7-dependent hyperproliferation, i.e. p53-induced apoptosis. Additional cellular proteins were found to be targeted by E6, e.g. → telomerase, ERC-55 (a putative calcium binding protein), paxillin (a cytoskeleton protein) and the interferon regulatory factor-3 (IRF-3). The consequences of such interactions are still unknown, but in the latter instance it is tempting to speculate that the E6 protein interferes with activation of interferon (as an antiviral principle) as the transcriptional activity of IRF-3 is inhibited after binding. The interaction between the E6 and E7 and cellular proteins was most intensively studied in case of the HPV type 16, which is the prototype of a cancer-associated papillomavirus. It is unclear, however, whether the role of the E6 and E7 proteins in virus replication are comparable between different HPV types. The interaction of these proteins with cellular proteins could not be demonstrated for certain HPV types.

Although it was observed that papillomavirus particles bind to a variety of different cells in culture, there is no evidence for persitence or replication of papillomaviruses other than in skin or mucosa. The conditions of differentiating epithelia are very difficult to reconstitute in cell culture, thus the propagation of papillomaviruses in experimental systems was impossible until recently. By the aid of → organotypic cultures established from human keratinozytes, the functions of papilloma virus genes can be studied. This system, however, is still insufficient to generate papillomavirus progeny on a preparative scale. Thus the molecular analysis of papillomavirus mostly depends on the molecular cloning of the DNA of the viral genome. Recent progress has been made by characterization of virus-like particles (VLP) that are generated by expression of the major structural protein L1 (with or without L2) in recombinant vectors (e.g. baculovirus or yeast). VLPs were also used to study early steps of the virus life cycle.

Pathogenesis of papillomavirus infection

Papillomaviruses are mostly transmitted by direct skin contact (e.g. during sexual intercourse), but also infection via contaminated objects has been reported. Replication of human papillomaviruses induces benign proliferations (skin warts, genital warts, laryngeal papillomas) within the affected skin or mucosa. Depending upon the site of infection and the condition of the patient (e.g. age), such lesions often regress spontaneously. Malignant progression of HPV-induced lesions is uncommon and depends upon the site of infection and on the HPV type. Despite the plethora of HPV types, there is a striking association of certain types to particular disease (e.g. HPV 1-4 in skin warts, HPV 6 and HPV 11 in genital warts and laryngeal papillomas).

Papillomavirus infection of the genital tract is very common. In women it is detected at high frequency (in 5 – 50% of women depending on age and sexual behavior). Those infections remain frequently subclinical and are diagnosed only by detection of viral DNA in cervical swabs. In males, although presumably equally common, HPV infection is detected less frequently. Clinical manifestations of genital papillomavirus infections are genital warts (condylomata acuminata) or intraepithelial neoplasias of the uterine cervix (CIN), vulva (VIN), penis (PIN) or the perianal region (AIN). CIN is considered a precursor to cervical cancer. However, the risk for malignant progression varies greatly (1 – 30%) depending upon the degree of severity defined as CIN I-III, which have been recently reclassified as low grade (CIN I) and high grade (CIN II+III). The second important parameter is the presence of particular HPV types. Thus papillomaviruses infecting the genital tract are classified as low risk types (e.g. HPV 6, 11) and high risk types (e.g. HPV16, 18, 31, 45).

Bioactivity

The role of certain (high-risk) HPV types in the development of anogenital cancers, most notably tumors of the uterine cervix, has been de-

monstrated by a large number of epidemiological studies and experimental observations.

- Viral DNA of 14 different HPV types (most frequently HPV 16, followed by HPV 18, 45, 31) was found in more 99% of large numbers of cervical cancer biopsies analyzed in worldwide studies. Also, cells established from cervical cancer and kept in culture for many years as cell lines, such as the HeLa line, contain HPV genomes; virus-specific mRNA and some proteins can be detected in cell lines and in biopsies, although fewer samples have been analyzed for gene expression.
- An infectious etiology of cervical cancer has been suspected for many decades; recently the risk factors for cervical cancer and infection by high risk HPV types (e.g. number of sexual partners) were shown to be identical.
- Follow-up studies demonstrated that there is no development of high-grade CIN (the endpoint of the follow-up) without the presence of high-risk HPV.
- CIN-like changes can be induced experimentally in organotypic cultures of human keratinocytes and in the epithelia of → transgenic mice expressing the E6 and E7 proteins.
- Cells of rodent and human origin can be transformed in culture by the expression of the E6 and E7 genes. Following inoculation, such cells grow as malignant tumors in small laboratory animals. The E6 and E7 proteins are required not only during the initial steps of transformation but also for the maintenance of the transformed state of the cell. Suppression of gene expression (by as yet undefined host factors or by introduction of an E6/E7-specific → antisense nucleic acid) leads to reduction of proliferation or even to loss of the malignant phenotype. The constant expression of the E6 and E7 proteins within the infected epithelium favors the accumulation of mutations in the cellular genome leading to the activation of oncogenes or inactivation of negatively regulating factors and ultimately to the development of cancer.

Despite the causative role of papillomaviruses, it is obvious that only a minority of infected women (1-5%, depending upon geographic region) develop cervical cancer, which with few exceptions occurs at least 2-3 decades after primary infection. Cervical cancer is several-fold more prevalent in developing countries than in affluent societies, whereas the prevalence of HPV infection is comparable across societies. Hence it is likely that other influences such as personal hygiene and sexual infections (e.g. chlamydia) may act as cofactors. Clearly, the regular attendence in cervical cancer screening programs (not available in poor resource countries) that are based on the detection of abnormal cells obtained from cervical swabs (→ Papanicolaou test) is the most effective protection against the disease. Other widely used cofactors such as smoking or use of oral contraceptives seem to be of minor influence.

Human papillomaviruses may play a role in the development of other cancer types as well (e.g. some cancers of the respiratory tract) but the evidence is less complete. Non-melanoma skin cancer is another candidate for being associated with papillomavisue infection, but the types that may be involved (e.g. HPV 20) are as yet only scarcely characterized.

Clinical Relevance

Papillomavirus infections, at least in their initial stages, are usually unrecognized by the immune system. In terms of expression of cytokines and the presence of immune cells there is no difference between a wart and normal skin. However, based on animal experiments and by studying the natural history of papillomavirus infections it was concluded that the immune system plays an important role both in preventing papillomavirus infections and in eliminating the clinical manifestations of infections (regression of warts). For instance, patients suffering from immunosuppression (e.g. by receiving immunosuppressive drugs after renal transplantation) have an increased prevalence of HPV-associated disease. It is not clear which event triggers HPV-specific immune responses during the natural courses of infection and why eventually such immune responses are

ineffective (cervical cancer occurs in immunological normal women). Nevertheless it is generally accepted that vaccination against papillomavirus infection (both as prophylactic means and as immune therapeutic treatment) that will become available within a few years will ultimately lead to the reduction of HPV-associated cancers.

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Human T-cell Leukemia Virus

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Synonyms

- human T cell leukemia virus (HTLV)

Definition

The human T cell leukemia virus (HTLV), a human retrovirus, was first identified in the early 1980s and is associated with adult T cell leukemia (ATL) and topical spastic paraparesis (TSP), a neurologic condition. Two related but distinct types of HTLV exist; HTLV-I and HTLV-II. HTLV-I is more common worldwide and is the virus most consistently found associated with disease.

Characteristics

→ Retroviruses are 100 nm, lipid-enveloped RNA viruses. Their genome consists of two identical RNA molecules located within the virus capsid. The viral genes include the gag (core region), pol (polymerase), env (the envelope) and certain other genetic regions that produce proteins associated with transformation or pathogenesis (e.g., X region). The X region encodes the viral proteins Tax and Rex that are involved in upregulating virus expression. Both HTLV-I and II infect $CD4^+$ lymphocytes; however HTLV-II appears to preferentially infect $CD8^+$ T cells. Although lymphocyte subsets are not altered to the same extent as in HIV infection, recent studies have demonstrated that HTLV-II, and to a lesser degree HTLV-I, are associated within an increased incidence of bacterial infections including pneumonia, bronchitis and urinary tract infections.

These viruses may be present in many people without causing disease. The viruses are spread by blood, genital secretions and by mother to child transmission often via maternal milk. Transmission of HTLV-I and HTLV-II appears to be primarily by cell to cell contact since there is low progeny production and very little virus release from the infected cell. Instead, HTLV-I provirus is propagated by lymphocyte division, resulting in pauci-clonal infection with the occasional emergence of monoclonal proliferation. This latter event can result in frank leukemia.

HTLV-I is most prevalent in southwestern Japan, the Caribbean, parts of South America and in central Africa. HTLV-II is endemic among Indian tribes in North, Central and South America and in central African pygmies. Within the last 30 to 50 years a low level epidemic of HTLV-II has affected injection drug users and their sexual partners in the United States and Europe.

Leukemia

Clinical Presentation. ATL occurs in 2 to 4 percent of HTLV-I-infected persons and takes up to 30 years to develop. Infection in childhood is thought to predispose to the development of

adult T cell leukemia (ATL), since the mothers of ATL cases are more likely to be HTLV-I seropositive than the mothers of healthy HTLV-I carriers. ATL can be distinguished from other human T cell leukemias by its occurrence predominantly in adulthood with an acute course, the morphologic properties of the leukemic cells (indented and lobulated nuclei), the generalized lymphadenopathy with an absence of mediastinal lymph node involvement, frequent lytic changes in the bone associated with hypercalcemia and in some cases infiltration of the skin. At the present time no specific therapy is available for ATL. Chemotherapy can reduce the tumor mass but long-term survival has not been achieved. Hypercalcemia can be managed by standard protocols. There have been case reports of hairy cell leukemia, large granular cell leukemia and $CD8^+$ cutaneous lymphomas occurring in HTLV-II infected persons, with the latter found particularly in whose with concomitant HIV infection. However in contrast to HTLV-I, an etiological role for HTLV-II in hematologic malignancy has not been proven in epidemiologic studies.

Pathology. HTLV, by infecting $CD4^+$ helper T cells, establishes a chronic infection that over time develops into leukemia. The mechanism for cell transformation is not yet fully explained but appears to be related to expression of the X region viral proteins or it may be the induction of cellular genes that cause continuous replication and subsequent chromosmal changes within cells. The result is autonomous cell growth and development of malignancy. Some investigators believe that the production of the lymphocyte growth factor interleukin-2 (IL-2) drives the $CD4^+$ lymphocytes to proliferate, particularly since there is an up-regulation of IL-2 production and expression of the IL-2 receptor on the infected cell surface. The leukemic cells, once proliferating, spread through the body and can induce a variety of syndromes including bone lesions due to osteoclastic activity. ATL differs from cutaneous T cell leukemia by the absence of leukemic cell infiltration of the epidermis despite presence of tumor cells in the dermis and subcutaneous tissue. Moreover, the bone marrow and lungs are usually not involved in ATL.

Neurologic disease

Clinical Presentation. Cases of tropical spastic paraparesis (TSP) have occurred in the Caribbean (e.g., Jamaica, Dominican Republic, Martinique, Trinidad) in Latin America, Africa and India. In Japan, a similar disease, called HTLV-associated myelopathy (HAM), is frequently diagnosed in HTLV-I endemic areas. This neurologic disease occurs more frequently in females than males, reflecting either sexually acquired infection or a female predilection for the immunologic basis for its pathology. Recent studies have demonstrated that a neurologic syndrome identical to TSP/HAM is found in HTLV-II infected persons, although the occurrence of neurologic disease may be less common than with HTLV-I. When first observed, TSP may suggest transverse myelopathy or multiple sclerosis. TSP can occur rapidly (within 3 to 4 months after blood-borne HTLV-I infection) but generally also takes several years to develop in infected people. Symptoms include bilateral weakness of the legs, back pain, leg numbness and loss of sensation in the feet. Sometimes bladder and bowel dysfunction is common. Mental capacity is usually normal and examination of the cerebral spinal fluid does not usually reveal abnormal findings, although tests for HTLV antibody and provirus are often positive.

Pathology. Because of the very high titer of antibody to HTLV-I and the presence of lymphocytes within the central nervous system, TSP has been considered to result from an autoimmune-like response of the host. Inflammation of nerve roots is caused by the infiltrating HTLV-1 infected cells in the spinal cord. Cellular and cytokine-mediated inflammatory responses directed at HTLV-I initiate the damage to neural tissue, followed by gliosis in advanced cases. Treatment with corticosteroids has shown some improvement but generally TSP has a slow unremitting course.

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Hutchinson-Weber-Peutz-Syndrome

Definition

→ Peutz-Jeghers-syndrome.

Hyaluronic Acid

Definition

Hyaluronic acid (HA) is a high molecular weight sugar consisting of multimers of 3 repeats of a basic disaccharide repeat (glucuronic acid and N-acetyl glucosamine joined in β1→3). The hexamers are joined in β1→4 formation between N-acetyl glucosamine and glucuronic acid. HA is present in high concentration in extracellular spaces and promotes cell migration in embryogenesis and neoplastic transformation. The aminoterminal region of → CD44 can bind HA.

Hybridization of Nucleic Acids

Definition

Hybridization is the pairing of complementary RNA and DNA strands to give a DNA-DNA, RNA-RNA or RNA-DNA hybrid. Hybridization can be done in 'wet' conditions, for instance to determine the genetic complexity of DNA (→ Cot); filter-bound DNA is used to identify or isolate homologous sequences by hybridization; DNA contained in chromosome spreads is used to determine the chromosomal localization of genes (fluorescence *in situ* hybridization, → FISH).

Hybridoma

Definition

A hybridoma is a cell line produced by fusing a myeloma cell with a lymphocyte, which continues indefinitely to express the immunoglobulins of the parent cells.

Hydroxamic Acid

Definition

Many synthetic inhibitors of → matrix metalloproteinases are based on the principle of coupling a zinc-chelating hydroxamic acid functional group with a short peptide or peptidomimetic sequence designed to target the inhibitor to the enzyme active site. More recent studies have utilized alternative chelators and nonpeptidic compounds in attempts to further engineer both selectivity and specificity.

Hyperacute Rejection

Definition

Hyperacute rejection is the destruction of engrafted organs that usually occurs within 24 hours of transplantation. It is caused by preformed antibodies and activation of complement on donor cells; → graft acceptance and rejection.

Hyperchlorhydria

Definition

Hyperchlorhydria is the increased gastric acid secretion; → gastrinoma.

Hyperdiploid

Definition

Hyperdiploid cells have one or more chromosomes or chromosome segments added to their normal number; in contrast to: → hypodiploid.

Hypergastrinemia

Definition

Hypergastrinemia is increased serum gastrin; → gastrinoma.

Hypermethylation

Definition

Hypermethylation is the overall increase in DNA → methylation at a certain → CpG island. It is the transcriptional silencing of a gene via methylation of 5′ CpG islands in the promoter region of a gene. Promoter hypermethylation represents an epigenetic mechanism that can act as an alternative to mutations to disrupt tumor-suppressor gene function and can predispose to genetic alterations through the inactivation of DNA-repair genes. The methylation of G:C rich regions within the regulatory domains of genes by DNA methyl transferases is a common mechanism for blocking expression of tumor suppressor genes during tumor progression.

Hyperplasia

Definition

Hyperplasia is an increase in the number of cells in a tissue or organ. It is the stimulation of cell division in a tissue giving rise to increased tissue mass or thickness. Hyperplasia is measured as increased rate of DNA synthesis (e.g. labeling index) or number of cellular layers.

Hypodiploid

Definition

Hypodiploid cells are deficient in one or more chromosomes or chromosome segments; in contrast to → hyperdiploid.

Hypoxia

Definition

Hypoxia is a state of oxygen deprivation (→ oxygenation of tumors); critically decreased O_2 partial pressures at a defined location in a tissue that lead to a compromised functional status or a progressive change in a biological parameter (e.g., radiation sensitivity). Hypoxia thresholds or critical O_2 levels that characterize the upper limit of hypoxia, below which activities progressively change, can encompass pO_2 values from 25 mmHg (start of reduced cell kill in standard radiotherapy) down to 0.02 mmHg (below this level cytochromes aa3 and c are no longer fully oxidized) with all other critical O_2 levels for specific cellular functions or activities distributed in between. Causative (pathogenetic) factors for the emergence of tumor hypoxia include:

- transient cessations in blood flow through a vascular bed (ischemic hypoxia, perfusion-limited hypoxia, ‘acute hypoxia’);

- a reduced capability to transport O_2 in the blood, e.g., during tumor-associated anemia or upon perfusion of tumor blood vessels with plasma only (anemic hypoxia);
- an increase in diffusion distances for O_2 (diffusional hypoxia, 'chronic hypoxia').

HYRC1

Definition

Acronym for 'hyper-radiosensitivity of murine severe combined immunodeficiency 1 (SCID) mutation, complementing 1′. HYRC1 is a DNA dependent serine/threonine protein kinase ((DNA-PK) catalytic subunit, involved in DNA double-strand break repair by end joining (non-homologous recombination) and V(D)J (where V is variable, D is diversity, and J is joining) immunoglobulin rearrangements. The gene maps to 8q11.

Hysterectomy

Definition

Hysterectomy is the surgical removal of the uterus; → uterine leiomyoma.

- total hysterectomy: resection of the uterus without removal of any uterine ligaments;
- modified hysterectomy: resection intermediate between total and radical hysterectomies;
- radical hysterectomy: standard surgery for invasive carcinoma of the cervix; resection of the uterus with anterior, lateral and posterior ligaments of the uterus in addition to regional lymphadenectomy;
- extended radical hysterectomy: radical hysterectomy done after cutting the internal iliac, inferior gluteal and internal pudendalic arteries.

IAP

Definition

→ Inhibitor of apoptosis protein, → baculovirus IAP repeat.

IC_{50}

Definition

IC_{50} is the molar concentration of a receptor ligand in a competition experiment between two ligands at which half of the agonist is bound to the receptor.

ICAM

Definition

Intercellular adhesion molecule (ICAM) is a specific cell surface protein involved in cell adhesion.

ICE

Definition

Interleukin-1β-converting enzyme (ICE) is a → caspase that can process pro-interleukin-1β to mature interleukin-1β. It can also process interferon-γ-inducing factor efficiently *in vitro* and *in vivo*. It was identified as the first member of the mammalian → caspase gene family, now referred to as caspase 11. It is the mammalian homologue of *Caenorhabditis elegans* cell death protein Ced-3 and consists of two subunits of 22 kD (p20) and 10 kD (p10).

ICE-LAP3

Definition

→ Caspase 7.

ICE-LAP6

Definition

→ Caspase 9.

ICErel-II

Definition

→ Caspase 4.

ICF Syndrome

Definition

ICF stands for: immunodeficiency, centromeric instability and facial abnormalities. The ICF syndrome is an extremely rare autosomal recessive disorder. It is characterized by the presence of variable immunodeficiency, instability of the pericentromeric heterochromatin and decon-

densation in chromosomes 1, 9 and 19, resulting in multibranched chromosomes and mild facial abnormalities; → methylation.

ICH

Definition

Interleukin-1 β converting enzyme family of cysteine proteases; belongs to apoptosis-related proteases.

ICH-1, also ICH1; synonymes: NEDD2; CASP2, → caspase 2.

ICH-2, synonyms: CASP4, → caspase 4.

Id Proteins

Definition

Inhibitor of DNA (Id) binding proteins are a family of related nuclear helix-loop-helix-proteins implicated in the control of differentiation and cell cycle progression. Id nuclear proteins interact with transcription factors and prevent them from binding to DNA. The primary targets of Id proteins are the basic helix-loop-helix (→ bHLH) transcription factors, which regulate cell-type-specific gene expression and expression of cell cycle regulatory genes. Generally, they act as positive regulators of cell growth and as negative regulators of differentiation. Id proteins lack a basic DNA-binding domain, therefore heterodimers between Id and bHLH proteins cannot bind to DNA. This mode of regulation is referred to as dominant-negative. Id proteins act as dominant-negative antagonists of other helix-loop-helix transcription factors; Id1, Id2, Id3, Id4, → E-box [Norton JD, Deed RW, Craggs G, Sablitzky F (1998) Id helix-loop-helix proteins in cell growth and differentiation. Trends Cell Biol. 8: 58-65].

- Id1, inhibitor of DNA binding 1, is a protein of 154 aa and 16 kD. The gene maps to chromosome 20 band q11.
- Id2, inhibitor of DNA binding 2, is a protein of 134 aa and 14 kD. It is expressed in most early fetal tissues but not in the corresponding mature tissues.The gene maps to 2p25.
- Id3, inhibitor of DNA binding 3, also known as Heir-1 is a protein of 119 aa and 12 kD. It is expressed abundantly in lung, kidney and adrenal gland, but lacking in adult brain. The gene maps to 1p36, a region frequently deleted in human cancers including → neuroblastoma.
- Id4, inhibitor of DNA binding 4, is a protein of 161 aa and 16 kD. The gene maps to 6p22-21.

Ideogram

Definition

Ideogram is a diagrammatic representation of the G-banding pattern of a chromosome.

Idiotype

Definition

Idiotype is the sum of the antigenic determinants of epitopes that are encoded in the variable regions of the immunoglobulin heavy and light chain; → B-cell tumours.

IGF-I

Definition

→ Insulin-like growth factor-I.

IGFBP

Definition

IGF-binding protein; → insulin-like growth factors.

IGFR

Definition

Insulin-like growth factor receptor; → insulin-like growth factor-I receptor; → insulin-like growth factors.

IGs

Definition

→ Immunoglobulin genes.

IκB

Definition

IκB is a family of protein inhibitors of Rel/NF-κB complexes.

IκB Kinases

Definition

NF-κB kinases are proteins that are involved in the phosphorylation of nuclear factor kappaB (→ NF-κB; → rel) inhibitors proteins. The activity of the NF-κB family of transcription factors is regulated by phosphorylation and the subsequent degradation of their inhibitory IκB subunits. Site specific serine phosphorylation of IκBs by two IκB kinases (IKKα and IKKβ) targets them for proteolysis. Although IKKα and IKKβ associate together in a heterodimeric complex, they respond to different biological inducers of NF-κB activation; IKKα responds to unknown morphogenic signals and is crucial for NF-κB activation during embryonic development. IKKβ is activated in response to proinflammatory cytokines, such as TNF (tumor necrosis factor) and interleukin-1 (IL-1).

IKBKA

Synonyms

- IKK1 (inhibitor of κ light polypeptide gene enhancer in B cells, kinase 1)
- IKK α (inhibitor of κ light polypeptide gene enhancer in B cells, kinase α)
- NFKBIKA (inhibitor of κ light polypeptide gene enhancer in B cells, kinase α)
- CHUK (conserved helix-loop-helix ubiquitous kinase)

Definition

Inhibitor of κ light polypeptide gene enhancer in B cells, kinase α. See → IκB kinases.

IKBKB

Synonyms

- IKK2 (inhibitor of κ light polypeptide gene enhancer in B cells, kinase 2)
- IKK-β (inhibitor of κ light polypeptide gene enhancer in B cells, kinase β)
- NFKBIKB (inhibitor of κ light polypeptide gene enhancer in B cells, kinase β)

Definition

Inhibitor of κ light polypeptide gene enhancer in B cells, kinase β. The gene maps to chromosome 8p11.2.

IKBKG

Synonyms

- IKK-γ (inhibitor of κ light polypeptide gene enhancer in B cells, kinase γ)
- NEMO (NF-κ essential modulator)

Definition

Inhibitor of κ light polypeptide gene enhancer in B cells, kinase γ. The gene maps to chromosome Xq28; the protein has 419 aa and 48 kD.

IKK1

Synonyms

- IKKα, (inhibitor of κ light polypeptide gene enhancer in B cells, kinase α)
- NFKBIKA (inhibitor of κ light polypeptide gene enhancer in B cells, kinase α)
- IκBKA (inhibitor of κ light polypeptide gene enhancer in B cells, kinase α)
- CHUK (conserved helix-loop-helix ubiquitous kinase)

Definition

Inhibitor of κ light polypeptide gene enhancer in B cells, kinase 1; → IκB kinases.

IKK2

Synonyms

- IKK β (inhibitor of κ light polypeptide gene enhancer in B cells, kinase β)
- NFKBIKB (inhibitor of κ light polypeptide gene enhancer in B cells, kinase β)
- IKBKB (inhibitor of κ light polypeptide gene enhancer in B cells, kinase β)

Definition

Inhibitor of κ light polypeptide gene enhancer in B cells, kinase 2; → IκB kinases.

IKK-α

Definition

→ IKBKA.

IKK-β

Definition

→ IKK2.

IKK-γ

Definition

→ IKBKG.

IL-2

Definition

→ Interleukin-2.

IL-3

Definition

→ Interleukin-3.

IL-4

Definition

→ Interleukin-4.

IL-4-STAT

Definition

→ STAT6.

IL-6

Definition

→ Interleukin-6.

Ileal Conduit

Definition

Ileal conduit is a urinary diversion in which a portion of terminal ileum is removed from continuity with the remainder of the small bowel; a simple conduit for urine, the ileal conduit does not provide continence. The ureters are anastomosed to the proximal end while the distal end is fashioned into a cutaneous stoma to which an appliance is attached to collect urine.

Illegitimate Recombination

Definition

Illegitimate recombination occurs with nonhomologous regions of DNA, each of the polynucleotide strands of dsDNA being religated with DNA from different regions of the same or different chromosomes.

Immediate Early Stress Response

Definition

→ Stress response.

Immortalization

Definition

Immortalization describes the acquisition by a eukaryotic cell line of the ability to grow through an indefinite number of divisions in culture. Cells are capable of indefinite proliferation (or unlimited lifespan) without any other changes in the phenotype necessarily occuring. In long lived multicellular organisms, immortality may be thought of as an abnormal escape from cellular senescence.

Immune Rejection, tumor targeted

Definition

Tumor targeted immune rejection is a cell-mediated immune response directed against tumor antigens, of which virus-induced tumors provide the clearest examples.

Immune Surveillance

Definition

→ Tumor surveillance.

Immunoglobulin

Definition

Immunoglobulins (Igs) are a class of proteins produced by the immune system that serves to protect against specific → antigens.

Immunoglobulin Genes

Definition

Immunoglobulin genes cluster in multimember gene families and are located on chromosome 14 (heavy chain gene segments), 2 (κ light chain) and 22 (λ light chain): V genes (variable region genes), D genes (diversity region genes) and J genes (joining region genes) for the heavy Ig heavy chains, V and J region genes for κ and

λ light chains. Ig gene rearrangement brings together one representative of these gene families (V, D and J for the heavy chain, V and J for κ and λ light chains). Recombination is a remarkable process since it entails double strand DNA breaks, loss or DNA and re-ligation of DNA strands. Joining of the V-D-J segments is imprecise and involves insertion of non-template nucleotides (N-additions) and trimming back of the beginning/ends of the gene segments. Ultimately, the rearrangement leads to a unique sequence of V_H-N-D-N-J_H, which generates diversity for antigen recognition, and which acts a marker for each individual B-cell and its progeny.

Immunohistochemistry

Definition

Immunohistochemistry is the evaluation of protein expression/localization by incubation with antibodies that bind to specific epitopes in the protein.

Immunophenotype

Definition

Immunophenotype refers to the properties of a cell defined by a panel of lineage-associated antibodies. The antibodies, which distinguish clusters of differentiation (CD) groups, recognize the same cellular antigen but not necessarily the same epitope. The highly sensitive markers are, CD19 for B-lineage cells, CD7 for T-lineage cells and CD13 or CD33 for myeloid cells. The highly specific markers are, cytoplasmic CD79a for B-lineage cells, cytoplasmic CD3 for T-lineage cells and cytoplasmic myeloperoxidase for myeloid cells.

Immunoprevention of Cancer

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Definition

Immunoprevention of cancer is an emerging concept that consists of the stimulation of the immune systems of individuals free of an overt neoplasia in order to prevent the development of a foreseeable tumor. In theory, these immune stimulations may be applied to the general population, however individuals carrying a specific risk of cancer or preneoplastic lesions may be considered.

Characteristics

Three set of considerations form the rationale of this new approach:

1. Identification of genes at risk and its mutated or amplified products provide the rational basis for specific and nonspecific immunostimulation of susceptible subjects. Even more appropriately, individuals carrying an early preneoplastic lesion detected occasionally or through cancer screening programs may take advantage of specific or nonspecific immunostimulation that block the progression of the lesion toward an overt neoplasia. Moreover, individuals without a specific genetic risk but are exposed to environmental cancer risks due to their occupation (employees working with asbestos or other carcinogens, etc.) or life style (smokers, consumers of fat rich diets, etc.) may also been considered.
2. Healthy subjects are expected to mount a more effective and powerful response than cancer patients who have already been treated in various ways. Moreover, when the target tissue is still normal or displays no more than a localized preneoplastic lesion, the chances of success should be greater than

when dealing with unresectable or disseminated tumors.

3. Preneoplastic lesions, in contrast to cancer cells, do not display genetic instability or mutations and do not allow the selection of clones that have lost the ability to express the target tumor associated antigen (TAA). By contrast, these events in many cases characterize established tumors. These lesions should also be more permeable to immune mechanisms, since their cells do not markedly remodel the extracellular matrix nor produce suppressive factors, and their vessel endothelium is not yet refractory to leukocyte extravasation.

Advantages

Preventive vaccination or nonspecific immunostimulation may offer alternative options to chemoprevention and preventive surgery. Immunoprevention has many advantages, since in most of the cases it consists in soft, non-invasive manouvres such as injections, gen-gun treatments or vaccinations, all characterized by long intervals of rest. For example, among people at risk, women carrying mutations in the BRCA1 or 2 genes may have the choice of a 'soft' immunoprevention alternative to distasteful prophylactic mastectomy.

Non-specific immune stimulation versus specific vaccination

Non-specific immune stimulation.

Enhancement of non-specific immunity and specific antitumor vaccination are two possible approaches. The advantage of a non-specific antitumor response is that it can be directly applied to a broad range of individuals, irrespective of the type of TAA their foreseeable tumors may eventually express. However, it is unfeasible to imagine healthy persons being non-specifically treated for long periods. The results of the mouse experiments indicate that non-specific stimulation should thus be restricted to patients with a genetic risk of cancer (1), individuals exposed to high carcinogen doses (2), patients with a preneoplastic lesion and those that probably have minimal residual disease after a successful conventional treatment (3).

Specific vaccination.

Specific vaccination of persons at risk and healthy individuals constitutes a very different scenario. Several mutations in oncogene products are required for transformation. By contrast, an alerted immune patrol would detect the initial mutations and be ready to intervene before complete transformation takes place. Although antigens associated to preneoplastic lesions (as well as those for many established neoplasms) have not been yet identified, the product of mutated oncogenes are probable candidates (4). Studies on papillomas induced by methylcholanthrene indicated that TAAs characterizing subsequent, progressing sarcomas are already present at the preneoplastic stage (5). Characterization of specific gene alterations or detection of preneoplastic lesions may indicate which organ and tissue are at risk. In a few cases, more precise information may show which oncogene product will probably be overexpressed or expressed in an altered form and allow vaccination against a single, specific TAA. Molecular characterization of altered gene products predictably destined to become TAA will be the first step towards the engineering of selective vaccines (4). Otherwise, the patient should be vaccinated against the TAA most commonly expressed by the tumors in a given organ. Both CTL (cytotoxic T lymphocytes)(6) and → SEREX techniques (7) allowed identification of several human TAA.

Many new antitumor vaccines that induce an effective resistance to subsequent tumor challenge and inhibit minimal residual disease are already available (8). The question whether specific immunization can be successful once a cell population has been subjected to the initial carcinogenic hit has rarely been examined experimentally. It can, however, be plausibly suggested that cytokines and more conventional adjuvants could induce an effective immune response against ignored or fully tolerated antigens. The specific immunity elicited in mice transgenic for rat → HER-2/neu is a sign that specific vaccination induces strong immune responses against such antigens and may thus inhibit oncogenesis and extend survival (9). An interesting potential universal TAA is the → telomerase catalytic subunit that is markedly

activated in more than 85% of human tumors, whereas it is silent in normal tissues (10).

One can also envisage the even wider application of antitumor vaccines to prevent tumors in the general population, as is done for infectious diseases. This point considers the possibility of preventing the onset of cancers related to an infectious agent by vaccination against the agent itself. This approach is applicable to a sizeable proportion of diverse human tumors including cervical carcinoma (→ human papillomaviruses), hepatocellular carcinoma [→ liver cancer, molecular biology] (hepatitis B and C viruses), and Burkitt lymphoma [→ Epstein-Barr virus] (Epstein-Barr virus). A significant impact of hepatitis B vaccinaton on the incidence of hepatocellular carcinoma has already been reported (11), and promising results being obtained in preclinical models of papillomavirus oncogenesis suggest that human vaccination will eventually be able to prevent cervical carcinoma (12).

Conclusion

Immunoprevention of cancer seems a distant but plausible prospect. Experimental elucidation of its critical issues could provide essential information for its application in humans. Prevention itself would provide a fresh and perhaps conclusive way of winning the long-lasting war against cancer.

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Immunotherapy

Definition

Immunotherapy is the use of a cancer vaccine to actively stimulate the immune system to generate specific immune response against cancer cells.

Imprinting

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Definition

Genomic imprinting describes a phenomenon in which a gene is expressed either from the paternal or form the maternal allele and thus discriminates these genes from the majority of genes that are expressed from both alleles.

Characteristics

Normally genes are expressed from both the maternal and the paternal allele. Genomic imprinting results in allele specific expression of certain genes from either the paternal or the maternal allele. These genes are marked before fertilization in a way that either the maternal or the paternal allele is transcriptionally silenced in the offspring. One of the first indications that certain autosomal regions are subject to genomic imprinting came from mouse genetic studies using Robertsonian and reciprocal translocations. In these studies, uniparental duplications or deficiencies for certain chromosomal regions were analyzed. The failure of a disomy or duplication from one parent to complement a corresponding nullisomy or deficiency from the other parent constituted the genetic evidence for the occurrence of imprinting effects (2). In addition, embryos that contain either two copies of the maternal or the paternal genomes fail to survive in early development indicating the complementary need for both the maternal and paternal genome. More than 25 imprinted genes have been identified in mice and humans and there are estimates for about 100 imprinted genes in the mammalian genome. Certain characteristic features have been identified for imprinted genes (1). Most of the imprinted genes have important roles in early development. Interestingly, imprinted genes tend to occur in clusters suggesting a common regulatory mechanism. One of the best studied cluster of imprinted genes is located on mouse distal chromosome 7 (human 11p15.5), encompassing 1.5 megabases and including the maternally expressed genes *p57KIP2*, *Kvlqt1*, *Mash2* and *H19* as well as the paternally expressed genes *Ins2* and *Igf2*. It is now well accepted that imprinting could be regulated in a tissue specific manner in a way that only some tissues express the gene from one allele while others show biallelic expression. Here, unknown mechanisms exist that allow to by-pass the regulation of imprinting. It is interesting to note that a number of imprinted genes encode for RNAs but do not have an open reading frame and are not translated. It is believed that these RNAs play a role in the regulation of the imprinting process. Interestingly, short GC-rich repeat sequences were identified in the vicinity of many imprinted genes usually located in or near so called differentially methylated sites, → CpG island-like sequences that are methylated on only one allele.

Cellular and molecular aspects

Regulatory mechanisms underlying genomic imprinting are under intense investigations in many laboratories but only incompletely understood. The features of the imprinting signal and the mechanism are unknown, but strong evidence suggests the involvement of DNA → methylation. Several requirements for the underlying mechanisms can be postulated. First, the imprinting signal, or imprint mark, in the imprinted region must be established before fertilization. Second, the imprint mark must be an → epigenetic modification and must directly or indirectly affect the transcription of a gene by silencing one allele and leaving the other active. Third, the imprint mark must be stable in mitosis and must be transmitted during cell division. Finally, the imprint mark must be reversible in a passage through the opposite germline. At present, DNA methylation is the only mechanism that conforms with the above requirements. Several lines of experimental data support the assumption that DNA methylation plays an important role in imprinted regulation. In mammals, DNA methylation oc-

curs only at the cytosine residue of CpG dinucleotides. It was shown that DNA methylation in promoter regions can turn off the transcription of a gene. Most genes are subject to a process of demethylation directly after fertilization with most of the CpG sites unmethylated at the 16 cell stage. However imprinted genes are exceptions in this demethylation process by maintaining small regions that show allele-specific methylation. DNA methyltransferase generates methylation patterns that are transmitted correctly following DNA replication and cell divisions. Studies of the expression of imprinted genes in DNA methyltransferase-deficient mutant mice indicated that normal level of DNA methylation are required for the control of allele specific expression. Studies with transgenic mice suggested that methylation is the epigenetic modification underlying genomic imprinting. A direct correlation between paternal inheritance, transgene hypomethylation and tissue specific expression of the transgene was shown, while the maternally derived copy is methylated and not expressed.

Clinical relevance

Imprinted genes are involved in critical steps during normal embryonic development. A growing body of evidence implicates genomic imprinting in the pathogenesis of certain human disorders, inherited tumor syndromes and sporadic tumors (3). At least ten genetic disorders have been found to be associated with genomic imprinting effects. In some cases, the trait is transmitted exclusively (or mainly) from one parent (either father or mother) or the disease is particularly severe when transmitted from one parent. In other cases the disease is associated with uniparental disomies or parent-of-origin specific aberrations.

The best studied examples of imprinted genetic diseases are the Prader-Willi-Syndrome (PWS) and Angelman syndrome (AS). PWS is characterized by mild to moderate mental retardation, individuals are slow moving and overweight due to severe hyperphangia. Patients with AS show severe mental retardation are thin, hyperactive and show disorders of movement and uncontrolled laughter. Both syndromes are linked to abnormalities on human chromosome 15q11-13. The first hint for a possible imprinting effect in these syndromes came from the finding that the deleted fragments in both syndromes are from opposite parental origins. In PWS the deletion occurred in the paternal copy and in cases of AS the maternal copy was deleted. Additional evidence came from the finding of maternal disomy of chromosome 15 in PWS patients and paternal disomies of chromosome 15 in AS. These data suggest that the *PWS* gene(s) is transcribed from the paternal allele only and the AS gene(s) is expressed from the maternal allele. Several imprinted genes were identified in the critical region for PWS/AS including paternally expressed *SNRPN* and maternally expressed *UBE3A* (4).

There is also evidence that some of the imprinted genes have oncogenic or tumor suppressor function. Loss of tumor suppressor function of an imprinted gene could be achieved by loss of heterozygosity (LOH) involving the usually active copy, as shown for the cyclin dependent kinase inhibitor, $p57^{KIP2}$, in lung cancers, *H19* in → Wilms tumor and *NOEY2*, a member of the *RAS* superfamily, in breast and ovarian cancers. Alternatively, uniparental disomy including the normally silent allele could lead to inactivation of an imprinted tumor suppressor gene. On the other hand, activation of a growth supporting gene such as → *IGF2* could occur by uniparental disomy involving the normally active copy. In addition, relaxation of imprinting control, also called loss of imprinting (LOI), could lead to biallelic expression and thus overexpression of an imprinted oncogene, as shown for *IGF2* in Wilms tumor. The first evidence for the involvement of DNA methylation in LOI came from the finding of complete methylation of the CpG island located immediately upstream of *H19* transcription start site. Usually this CpG island shows allele specific methylation on the maternal allele. This epigenetic change correlated with LOI in *IGF2* and silencing of *H19*.

Another human disease is the → Beckwith-Wiedemann Syndrome (BWS) that is characterized by a number of growth abnormalities including gigantism. Between 5 to 10% of BWS

patients are prone to Wilms tumor, adrenocortical carcinoma, hepatoblastoma or embryonal rhabdomyosarcoma. Wilms tumors have been shown to exhibit preferential loss of maternal alleles at chromosome 11p. A cluster of at least seven imprinted genes was identified in 11p15.5 including the paternally expressed *IGF2* and the maternally expressed *H19*. The most common abnormality in BWS patients is LOI of *IGF2* without any detectable chromosomal abnormalities (5).

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IMT

Definition

→ Inflammatory myofibroblastic tumor.

in situ Hybridization

Definition

In situ hybridization is performed by denaturating the DNA of cells mounted on a microscope slide such that reaction is possible with an added single-stranded RNA or DNA. The added RNA or DNA preparation is radioactively labeled and its hybridization is followed by autoradiography.

Inducible Nitric Oxide Synthase

Definition

Inducible nitric oxide synthase is a protein induced by various inflammatory cytokines that catalyzes the production of nitric oxide from L-arginine.

Inflammation

Definition

Inflammation is a local response of a tissue to injury or infection, caused by invasion of white blood cells that release various local mediators and enzymes.

Inflammatory Myofibroblastic Tumor

Definition

Inflammatory myofibroblastic tumor is a rare yet distinctive pseudosarcomatous inflammatory lesion that primarily occur in the soft tissue and viscera of children and young adults. Histologically these lesions are composed of fascicles of bland myofibroblasts in a variably collagenous stroma and mixed with a prominent inflammatory component of lymphocytes, eosiniphils and plasma cells. Its distinctive histological appearance has given this lesion a wide variety of names including inflammatory pseudotumor, pseudosarcomatous myofibroblastic proliferation, inflammatory sarcome, plasma cell granuloma and inflammatory myohistiocyte proliferation. The diagnosis of IMT is often difficult, depending on the relative proportion of the inflammatory and myofibroblastic components. Some lesions can be difficult to distinguish from a reactive process, whereas other lesions can appear sarcomatous. Whether

the entire histological spectrum represents a single entity and consequently whether consistent chromosomal or gene abnormalities are found throughout the spectrum, has yet to be determined.

Inflammatory Pseudotumor

Definition

→ Inflammatory myofibroblastic tumor.

Inflammatory Sarcoma

Definition

→ Inflammatory myofibroblastic tumor.

Inhibitor of Apoptosis Protein

Definition

Inhibitor of apoptosis protein is a family of anti-apoptotic proteins with one to three → BIR domains.

Inhibitors, molecular and chemical

Definition

Molecular and chemical inhibitors are specific inhibitors that are valuable tools in the study of signalling molecules and pathways. In addition to specific chemical compounds, i.e. chemical inhibitors, so-called 'molecular inhibitors' can also be generated. They could be a dominant negative variant of the molecule, i.e. molecules that could interact with upstream components but are unable to transmit signals, or antisense probes that inhibit production of the molecule or specific antibodies that inhibit protein function.

INI1

Definition

INI1 is a → tumor suppressor gene involved in → rhabdoid tumor.

Initiation

Definition

Initiation is the early steps in the process of carcinogenesis induced by carcinogenic agents, usually causing a mutational change that leads to the development of cancer.

INK4 Family

Definition

INK4 family is one of two groups of cyclin-dependent kinase (CDK) inhibitors (CKIs). The INK4 family currently consists of four members; p15INK4b, p16INK4a, p18INK4c and p19INK4d.

INK4A

Definition

INK4A, also known as → CDKN2A, is the gene for the inhibitor of cyclin dependent kinase 4, p16. By the use of an alternative first exon, the gene encodes for a second protein, p14ARF, which regulates stability of the tumor suppressor protein → p53.

Innate Immunity

Definition

Innate immunity is the basic immune defense, as distinct from the antibody-mediated and cellular immune responses, and depends on the recognition of non-self carbohydrates.

INOS

Definition

→ Inducible nitric oxide synthase.

Inositol Lipids

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Synonyms

Inositol lipids or, more precisely, inositol phospholipids are also referred to as phosphoinositides, and their subclass, where more that one phosphate group is present, as polyphosphoinositides.

Definition

Inositol lipids are a class of phospholipids where inositol is the polar headgroup. The simplest inositol phospholipid is phosphatidylinositol. The inositol moiety can be phosphorylated at several different positions giving rise to a number of other molecular species.

Characteristics

Among different inositol lipids, the importance in → transmembrane signalling and regulation of cell functions are best documented for PtdIns(4,5)P2 and PtdIns(3,4,5)P3. There are several ways in which these low abundance inositol lipids (less that 1% of membrane phospholipids) could provide a signalling link or fulfill other roles in different cellular processes.

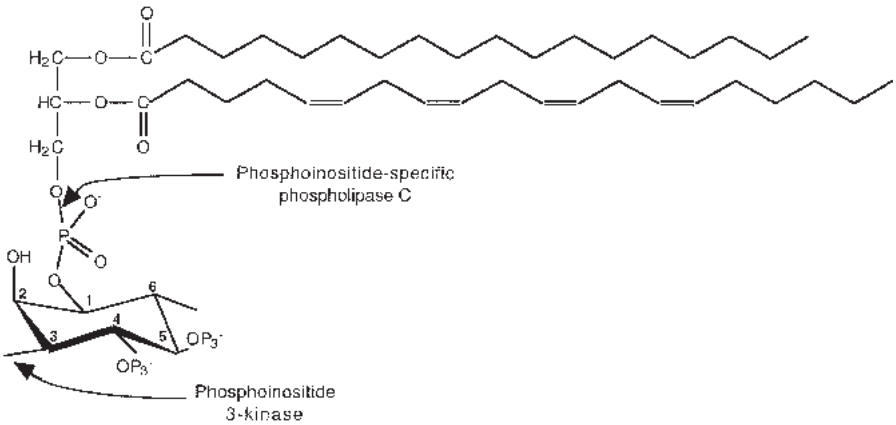
Hydrolysis of PtdIns(4,5)P2 to generate second messenger molecules

Hydrolysis of PtdIns(4,5)P2 occurs in response to a large number of extracellular signals and generates two → second messengers, inositol (1,4,5) trisphosphate [Ins(1,4,5)P3] and diacylglycerol (DG or DAG) molecules. The reaction is catalyzed by phosphoinositide-specific phospolipase C (PI-PLC) (Fig.). There are several → isoforms of this enzymes (PLCβ, PLCγ, PLCδ and PLCε) linked to and activated by different cellular receptors. For example, PLCγ is regulated through tyrosine kinase receptors such as receptors for epidermal growth factor, fibroblast growth factor and platelet-derived growth factor, while PLCε could be a novel target for Ras proteins.

The second messengers generated from PtdIns(4,5)P2 interact with specific intracellular targets and, in turn, cause their activation. Ins(1,4,5)P3 binds to specific receptors in the endoplasmic reticulum causing a release of calcium from this intracellular store into the cytoplasm. Membrane resident DG is required for activation of several isoforms of protein kinase C (PKC). These second messengers act as a common component in different signalling pathways, contributing to diverse cellular responses. Specificity of the pathways is provided at the level of a receptor and downstream components (e. g. calcium regulated proteins and PKC substrates) present in a specific cell type or state.

Binding of PtdIns(4,5)P2 to specific proteins

In addition to its role as a precursor of Ins(1,4,5)P3 and DG, PtdIns(4,5)P2 has emerged as a highly versatile signalling molecule in its own right. These other functions are mediated through direct binding of PtdIns(4,5)P2 to specific protein targets, and include fundamental processes in membrane trafficking and plasma membrane-cytoskeleton linkages. Many proteins that regulate actin cy-



Inositol Lipids. Fig. – Structure of phosphatidylinositol (4,5) bisphosphate [PtdIns (4,5)P2] shows a typical phospholipid containing an inositol ring as a headgroup. The positions on the inositol ring are designated 1-6 and two phosphate groups are present at positions 4 and 5. Phosphatidylinositol (3,4,5) trisphospahate [PtdIns(3,4,5)P3] is generated in a phosphorylation reaction; the third phosphate group is added at position 3 of the inositol ring. Hydrolysis of PtdIns (4,5)P2 at the C-bond separates the hydrophobic part that contains two lipid chains, from water-soluble inositol that contains phospates at the positions 1, 4 and 5.

toskeleton (e.g. gelsolin and profilin) and proteins involved in endocytosis (e.g. dynamin and AP-2 adaptor) bind PtdIns(4,5)P2. The binding involves different positively charged protein surfaces that in some proteins are present within the modular pleckstrin homology (PH) → domain. The result of the binding could be a direct change in the protein function or a regulated membrane targeting. For example, the PH domain of phospholipase Cδ1 associates with membranes of many cell types, but after PLC stimulation and the reduction in PtdIns (4,5)P2 concentrations it translocates to the cytoplasm. Concentration of PtdIns(4,5)P2 is not only regulated at the level of hydrolysis by PLC but also through regulation of several types of inositol lipid kinases and phosphatases.

Generation of PtdIns(3,4,5)P3 and other 3-phosphorylated inositol lipids and their binding to specific intracellular targets

Inositol lipids phosphorylated at the 3-position of the inositol ring [PtdIns(3)P, PtdIns(3,4)P2, PtdIns(3,5)P2 and PtdIns(3,4,5)P3] are generated by phosphoinositide 3-kinases (PI 3-Ks). PI 3-Ks are grouped into three classes on the bases of their structure and according to the inositol lipid they preferentially utilize as a substrate. For example, the class I PI 3-Ks are receptor-regulated → signal-transducer proteins that preferentially phosphorylate PtdIns(4,5)P2 *in vivo* and generate PtdIns(3,4,5)P3 (Fig.). Several target proteins for PtdIns(3,4,5)P3 and PtdIns(3,4)P2 have been described and they include protein kinases such as PKB/Akt, PDK1 and Btk. In the case of PKB/Akt, the direct binding to the PH domain [with high affinity and specificity towards 3-phosphorylated inositol lipids compared to more abundant PtdIns(4,5)P2] results in both membrane targeting and conformational changes that lead to phosphorylation and activation of this protein kinase. Activated kinases, in turn, phosphorylate and regulate downstream targets and thus propagate the signal. As described above for the control of PtdIns (4,5)P2 levels and generation of the second messengers, the 3-phosphorylated inositol lipids also participate in diverse cellular functions including cell survival, proliferation, migration and vesicle budding. In addition to regulation of PI 3-K, the levels of PtdIns(3,4,5)P3 are also controlled by a 3-phosphatase.

Clinical Relevance

There is considerable experimental evidence that the key enzymes involved in the control of inositol lipids, PI-PLC and PI-3 K, play an

important role in processes critical for tumour development and spreading, including cell proliferation, survival and migration. However, oncogenic or constitutively active mutants of either PI-PLC or PI-3 K have not yet been isolated from human tumours. In the case of PI-3 K, an oncogenic form (v-p3k) of the class I PI-3 K has been isolated from a chicken retrovirus that causes hemangiosarcomas. Another oncogenic form of class I PI-3 K (mutation in the regulatory subunit) has been isolated from transformed murine lymphoid cells. Nonetheless, the importance of the control of inositol lipid levels in human cancers have been emphasized by recent findings that the tumor suppressor protein → PTEN is a 3-phosphatase that dephosphorylates PtdIns(3,4,5)P3. The PTEN gene is deleted or mutated in a wide variety of human cancers. Many human tumours have also been found to express increased levels of PI-PLC or PI-3 K.

The role of a signalling protein in generation and spreading of a malignant tumour is not limited to its function as an oncogene or a tumour suppressor gene. For example, it has been documented that the activation of PLCγ is a rate limiting step in breast and prostate tumours that overexpress growth factor receptors. This type of tumour is associated with a poor prognosis. PLCγ seems to be required for cell migration but not for proliferation, and the motility and invasiveness of cancer cells are strongly inhibited either after treatment with a chemical PLC inhibitor (U73 122) or in the presence of molecular inhibitors (dominant negative PLCγ or the antisense of this PLC isoform).

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Insertional Mutagenesis

Definition

Insertional mutagenesis is the alteration of a gene by integration of a foreign, often exogenous, DNA sequence. For instance, a virus DNA can integrate into a gene or in the vicinity of a gene; → retrovirus.

Insertions

Definition

Insertions are identified by the presence of an additional stretch of base pairs in DNA.

Insulin-like Binding Proteins

Definition

Insulin-like binding proteins are large molecular weight proteins that are found in the circulation and in the local tissue environment and bind insulin-like growth factors (IGFs) to regulate their half-life and function.

Insulin-like Growth Factor-I Receptor

Definition

Insulin-like growth factor-1 receptor is a large protein found on the surface of most cells that binds insulin-like growth factors (IGFs) and is stimulated to affect cellular functions.

Insulin-like Growth Factors

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Definition

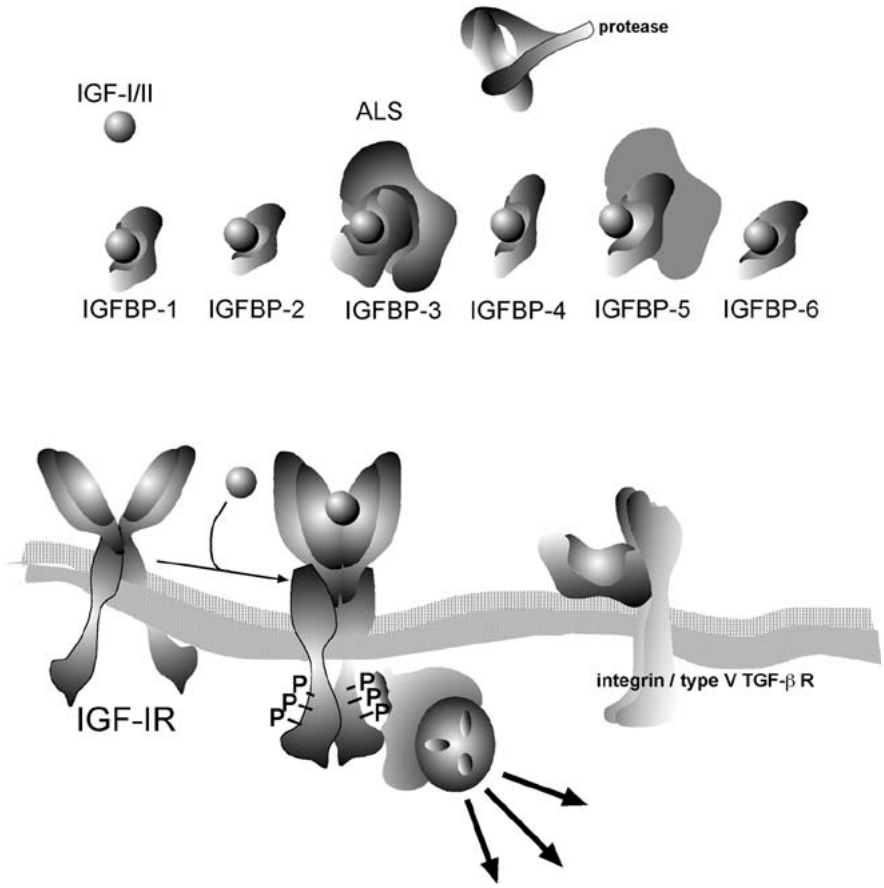
Insulin-like growth factors are a family of insulin growth factor (IGF)-related proteins including growth factors, binding proteins and cell surface receptors. To date, none of these family members have been shown to be oncogenic, i.e., capable of initiating cancer. However, these are potent mitogenic factors that have been implicated in enhancing tumor growth. Furthermore, a body of evidence has recently emerged suggesting that circulating IGF-I levels may represent a risk factor for the development of various types of cancers.

Characteristics

The insulin-like growth factors (IGF-I and IGF-II) are ubiquitously expressed growth factors that are structurally and functionally related to insulin. The IGFs are critical in a wide range of functions including growth and development during the embryonic stages and in normal post-natal growth and development. In adult tissues, these factors support the normal differentiated functions of various tissues and prevent programmed cell death (apoptosis) in certain cell-types. The primary actions of the IGFs are mediated by the IGF-I receptor, which is expressed by all tissues except adult liver. The IGF-I receptor is a cell surface, transmembrane tyrosine kinase receptor and is similar in structure and function to the insulin receptor. The IGF-I receptor is primarily involved in mitogenesis, mediating anti-apoptotic effects and certain other tissue-specific functions. In contrast, the insulin receptor primarily mediates critical metabolic functions. The IGF-II (mannose-6-phosphate) receptor, while it does not have any obvious signaling capabilities, may play an important role in removing IGF-II from the environment, thereby regulating the biological effects of IGF-II. The IGF-binding proteins (IGFBPs), of which there are currently only six known members, regulate the stability and transport of IGFs in the circulation, facilitate transport of the IGFs to the appropriate target tissues and affect the biological actions of the IGFs. Thus, the IGFBPs are critically important in the overall function of the IGFs. Most IGFBPs have higher binding affinities for the IGFs than does the IGF-I receptor, which generally results in a net inhibitory effect on IGF-I action. However, under certain circumstances, the IGFBPs may augment the effects of the IGFs. Proteolytic cleavage or phosphorylation of the IGFBPs or binding of the IGFBPs to the cell membrane instead of the extracellular matrix results in a reduced affinity of these proteins for the IGFs. The IGFBPs also have some IGF-I-independent functions on cells, although these effects are not yet clearly defined.

Mitogenesis and Apoptosis

The IGFs act through the IGF-I receptor to stimulate progression of the cell cycle. This effect is mediated by multiple signaling pathways including the Ras/Raf/MAP kinase and the phosphatidyl inositol 3′-kinase (PI3′-kinase) pathways. Upon binding of IGF-I, the IGF-I receptor undergoes autophosphorylation and activation of its intrinsic tyrosine kinase. Early signaling events of the IGF-I receptor include binding and tyrosine phosphorylation of various substrates including SHC and the insulin receptor substrate (IRS) family of proteins. SHC and the IRS proteins then engage other proteins that lead to activation of the mitogen-activated protein kinase (MAPK) and PI3′-kinase signaling pathways. Activation of the IGF-I receptor also stimulates a pathway involving CrkL, a protooncogene adaptor protein that enhances the action of IGF-I in cells overexpressing CrkL. CrkL interacts with IRS proteins and is phosphorylated on tyrosine residues in response to IGF-I receptor activation. The IGFs increase synthesis of specific cyclin proteins (cyclin D and cyclin E) and enhance the activity of certain cyclin-dependent kinases (cdks), including cdk2, and lead to phosphorylation of the retinoblastoma protein (Rb). There is sig-



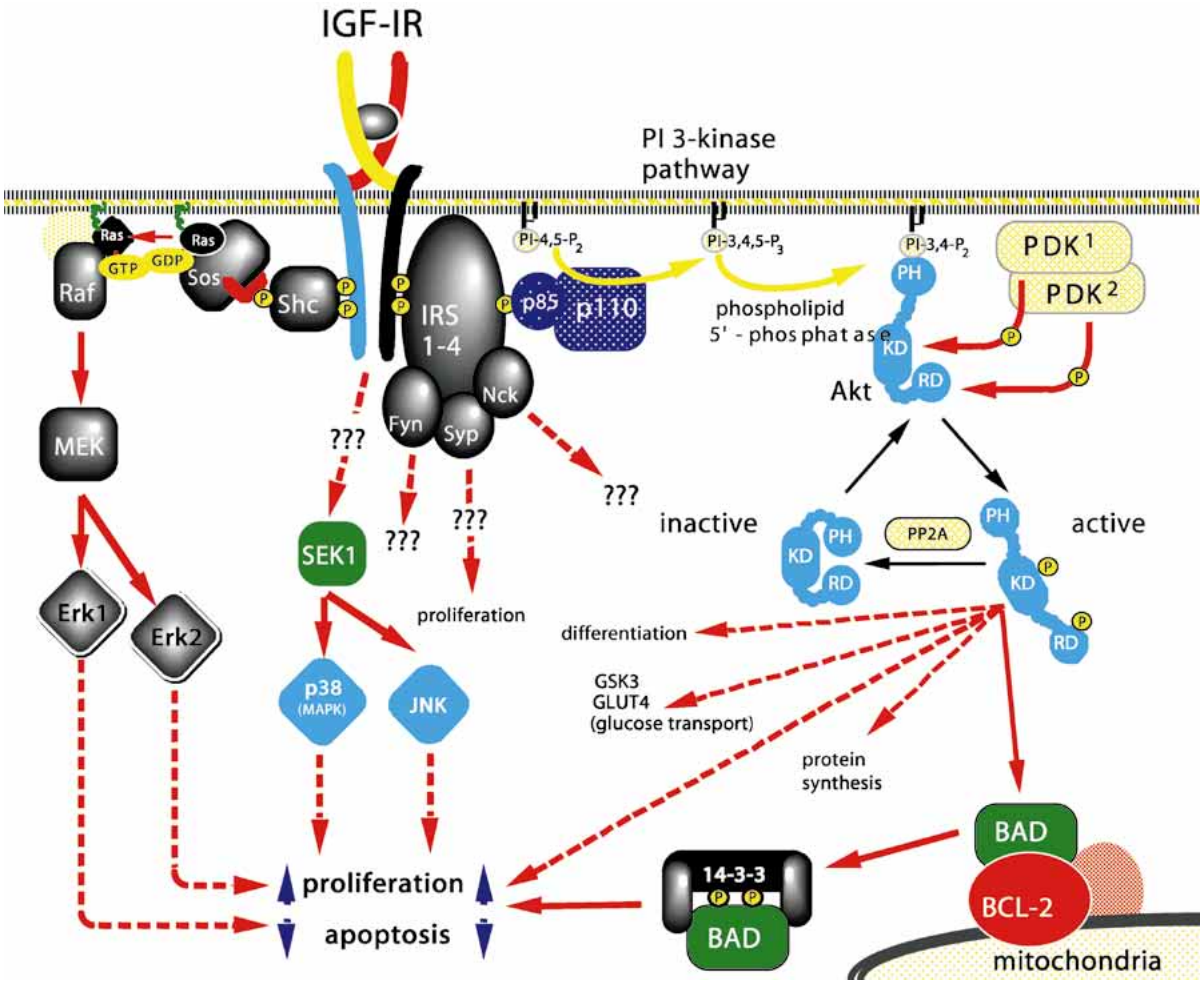
Insulin-like Growth Factors. Fig. 1 – The insulin-like growth factor family is comprised of ligands, IGF-I and IGF-II, six binding proteins (IGFBP 1-6), and the IGF-I receptor, which mediates the cellular responses. IGFBP-3 is the main carrier protein of the IGFs in the circulation and complexes with an acid labile subunit to neutralize the IGFs. IGFs are released from this complex by the action of proteases. The IGFBPs also may affect cellular responses by interacting with cell surface proteins, such as integrins, in an IGF-independent manner.

nificant crosstalk between the IGF-I receptor signaling pathways and other signaling pathways. For example, estrogen receptor activation augments the IGF-I response in MCF-7 (estrogen positive) breast cancer cells at many levels. In addition, estradiol increases the tryosine phosphorylation of the IGF-I receptor and IRS-1, and increases cell cycle cyclin expression and reduces cdk inhibitors.

The IGFs are potent inhibitors of apoptosis, a process mediated primarily via activation of the PI3′-kinase pathway. PI3′-kinase, in turn, activates AKT, a protein kinase that phosphorylates BAD on serine residues. Serine phosphorylated BAD is sequestered by specific scaffolding proteins (14-3-3 proteins) that thereby enables two anti-apoptotic proteins, Bcl2 and Bclxl, to act. Thus, IGF-I induces cell proliferation both by enhancing cell cycle progression and by inhibiting apoptosis.

Expression of various components of the IGF system by tumors

Each of the components of this family are expressed often individually by different tumors. In general, IGF-II is more commonly expressed by tumors than is IGF-I, although IGF-I expression has been found in small lung cancers, ovarian tumors and some CNS tumors. The IGF-II gene is imprinted on one allele by a methylation process. In certain tumors this im-



Insulin-like Growth Factors. Fig. 2 – Activation of the IGF-I receptor results in a number of signaling cascades that are initiated by the autophosphorylation of the receptor and activation of its tyrosine kinase activity. The downstream signaling substrates are numerous and include Shc, insulin receptor substrates (IRS 1-4) and the p85 subunit of PI3′kinase. These lead to activation of signalling cascades, such as the Ras/Raf/MAP-kinase pathway that are involved in cell proliferation and the PI3′Kinase/Akt pathway that inhibits apoptosis.

printing seems to be lost, resulting in increased IGF-II gene expression.

The IGF-I receptor is often expressed at higher levels in tumor cells than in normal tissue. Expression of the IGF-I receptor is regulated by both tumor suppressor gene products and by growth factors. → Wilms Tumor-1 (WT1) and p53 both bind to the IGF-I receptor promoter and inhibit reporter gene expression *in vitro*. Mutations of these genes occurs in various tumors and paradoxically enhances the activity of the IGF-I receptor reporter gene. This may explain the increased IGF-I receptor gene expression observed in Wilms Tumors (a pediatric kidney tumor) and various other cancers, e.g., colon cancer with the most common p53 mutations. Both WT1 and p53 inhibit IGF-II gene expression. However, similar to that of the IGF-I receptor, IGF-II gene expression is increased when these tumor suppressors are mutated. Thus, the autocrine loop between IGF-II and the IGF-I receptor is affected under these circumstances. Basic fibroblast growth factor and platelet-derived growth factor also enhance IGF-I receptor gene expression. Since tumors also express these and other types of growth

factors, this could also facilitate the enhanced IGF-I receptor gene expression observed in tumors. Antisense strategies have been designed to reduce cell proliferation in tumors by reducing IGF-I receptor gene expression. Certain oncogenes can also regulate the IGF-I receptor at the post-translational level. v-Src increases the tyrosine phosphorylation state of the receptor, thereby increasing its tyrosine kinase activity. Tumor growth may be enhanced by injections of recombinant human IGF-I into mice harboring tumors. In these experiments, the latency period before the clinical advent of the tumor is shortened and once the tumors have appeared, their growth is significantly increased. This response is enhanced in those tumors that display higher levels of IGF-I receptor expression.

Various IGFBPs are expressed by most tumors and often in different combinations. For example, estrogen-positive breast cancers release IGFBP-2, whereas estrogen-negative breast tumors release IGFBP-1 and -3. In many cases, the levels of the IGFBPs can be altered by estrogen, tamoxifen or retinoic acid, and these changes in IGFBP levels may explain the altered biological effect of the IGFs on the growth of these tumors. Interestingly, wild type p53 enhances the expression of IGFBP-3, which may then mediate the inhibitory function of p53 on cell growth.

Syndrome of hypoglycemia

An emerging clinical syndrome is tumor-induced hypoglycemia. While this effect is often seen in terminally-ill, poorly nourished patients, it is also seen in patients who have large generally mesenchymal tumors in the abdomen or thorax that secrete large concentrations of IGF-II. The processing of the IGF-II in these patients is incomplete and therefore it is poorly bound to the circulating IGFBP complexes. This allows the IGF-II to interact more readily with insulin receptors and thereby cause hypoglycemia. Clinical diagnosis of tumor-induced hypoglycemia includes normal or elevated circulating IGF-II levels concomitant with suppressed insulin, growth hormone (GH), IGF-I and IGFBP-3 levels at the time of symptomatic hypoglycemia in a patient with a demonstrable tumor. Removal or shrinkage of the tumor is often palliative, while GH and corticosteroid therapy is often helpful.

Future Directions

An increasing body of evidence suggests that the IGF family is a promising target for adjunct cancer therapy. If chemotherapy is inadequate to totally ablate tumor cells, then blocking IGF inhibition of apoptosis may be helpful. The IGF system could potentially be targeted at various levels. It has been suggested that inhibition of circulating GH levels using GHRH antagonists or somatostatin may be useful to reduce circulating IGF-I levels. GH analogues, which block GH action at the level of the GH receptor, may be similarly useful. Another possible site of intervention is at the level of the IGF-I receptor, either by IGF-I analogues that inhibit binding of endogenous IGFs to the receptor, antisense strategies that reduce IGF-I receptor levels or a specific inhibitor of the IGF-I receptor tyrosine kinase. While each of these strategies makes intuitive sense, considerable further studies will be required to bring these approaches into clinical use.

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Integrins

Definition

Integrins are a family of cell surface → adhesion receptors. They are heterodimeric cell surface glycoproteins made of an α and a β subunit that serve as adhesion receptors for extracellular matrix proteins.

Interleukin-2

Definition

Interleukin-2 (IL-2) is a cytokine produced by activated T cells that regulates the magnitude and duration of the T cell immune response following antigen encounter. IL-2 also serves regulatory roles for a number of other hematopoietic cells including B cells and natural killer (NK) cells. Among the signaling pathways activated by IL-2, is the JAK-STAT [→ signal transducers and activators of transcription in oncogenesis] pathway.

Interleukin-3

Definition

Interleukin-3 (IL-3) is a growth factor/survival factor for immunohematopoietic cells. Stimulation of the IL-3 receptor leads to increased formation of diacylglycerol and activation of → protein kinase C. Deprivation of IL-3 leads IL-3-dependent cells to → apoptosis through → Bad activation.

Interleukin-4

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Definition

Interleukin-4 (IL-4) is a prototypic immunoregulatory cytokine secreted by activated CD4 T cells of the → T helper (Th) 2 subset and by basophils and mast cells. IL-4 has an important role in regulating antibody production, hematopoiesis, inflammation and the development of effector T-cell responses.

Characteristics

IL-4 is a 15-20 kD glycoprotein composed of 129 amino acids. IL-4 contains six cysteine residues that are all involved in intramolecular disulfide bridges. The secondary structure of IL-4 was shown to consist of a four-helix bundle with a unique up-up-down-down helix topology. The X-ray structure reveals that IL-4 is a highly compact and globular protein with a predominantly hydrophobic core.

Human IL-4 receptor (IL-4R) is found on T cells, B cells mast cells, basophils, macrophages, fibroblasts, endothelial cells, hepatocytes, keratinocytes, stromal cells and neuroblasts. IL-4 receptor is a heterodimer composed of an α subunit, with IL-4 binding affinity, and the common γ subunit that is also part of other cytokine receptors. It functions to enhance the binding of IL-4 to its receptor, presumably by direct interaction with the ligand, and to induce intracellular signals transduction events such as → JAK-→ STAT signal pathway. Janus kinases JAK1 and JAK3 and signal transducers and activators of transcription 6 (STAT6) have recently been found to play a unique role in IL-4 receptor-mediated intracellular signaling and hematopoietic cell development.

IL-4 is a highly tissue-specific gene that is expressed in the Th2 cell subset and not in the Th1 cell subset. The human IL-4 gene is a single copy gene in the haploid genome. It spans about 10 kb and is located on chromosome

5q23-31 along with the IL-3, IL-5, and GM-CSF genes. Several transcriptional factors such as STAT6, NFAT (nuclear factor of activated T cells), c-Maf and GATA3 also influence gene expression and production of IL-4. For example, STAT6-deficient mice are unable to produce Th2 cytokines. The Th2-specific transcription factor c-Maf promotes IL-4 promoter activity. NFAT1 is involved in termination of the late phase of IL-4 gene transcription, thereby inhibiting Th2 responses.

Generally, antigens and antibodies that cross-link the T-cell receptor induce IL-4 production. The initiation of IL-4 production, a key event in the control of IL-4 production may, in naïve T cells, require limited T-cell receptor (TCR) signaling in co-stimulation with surface CD28 and CD40 ligand. Once IL-4 production is initiated, IL-4 feeds back on the differentiating cells and accelerates Th2 commitment in an autocrine fashion.

Effects of IL-4 depend upon binding to and signaling through IL-4 receptor complex. The IL-4R, which lack intrinsic tyrosine kinase activity, uses multiple intracellular domains to induce proliferative and gene expression responses. The membrane proximal region of IL-4R contains sequences known as Box 1 and Box 2 for binding of Janus family kinases JAK1 (bound to IL-4Rα) and JAK3 (bound to γc). Distal to this region is the I4R motif for binding of insulin receptor substrate (IRS)-1 and IRS-2. IRS-1 and IRS-2 links the IL-4R to downstream signaling cascades including phosphoinositol 3′ kinase (PI-3-K) and mitogen-activated kinase to induce cell growth. A more distal region of the IL-4R, termed the gene expression domain, contains three tyrosine residues, which mediate activation of STAT6 through tyrosine phosphorylation. STAT6 phosphorylation is believed to induce transcriptional activation after the STAT dimers translocate from the receptor to the nucleus, where they bind to specific targeting sequences and influence gene transcription. In short, the JAK/STAT signal pathway connects activation of the receptor complexes directly to transcription of genes. Upon IL-4 receptor oligomerization induced by a polypeptide ligand, JAKs are activated, presumably by trans-auto phosphorylation on tyrosines. Subsequently, JAKs phosphorylate STAT proteins, which form homodimeric or heteromeric complexes via their Src-homology 2 (SH2) domains. These complexes translocate to the nucleus and bind directly to response elements present in the promoters of target genes, thus triggering induction of transcription.

Bioactivity

IL-4 was initially called B cell growth factor-1 because of its role in the early steps of B cell activation. IL-4 has now been shown to regulate a wide spectrum of cellular functions in B cells, T cells, monocytes/macrophages and other haematopoietic and non-haematopoietic cells. IL-4 plays a central role in regulating the differentiation of antigen-stimulated naïve T cells. IL-4 causes such cells to develop into cells capable of producing IL-4 and a series of other cytokines including IL-5, IL-10 and IL-13. IL-4 controls the specificity of immunoglobulin class switching. IL-4 also has an important role in tissue adhesion and inflammation.

IL-4 has been shown to have antitumor and immunomodulatory activity against many human hematopoietic and solid tumor cells *in vitro*. In isolated cases, however, IL-4 was found to have mitogenic activity toward human T-cell leukemia cells. IL-4 receptors are expressed on solid human tumor malignancies. IL-4 inhibits the growth of many solid tumor cell types including malignant melanoma, breast carcinoma, ovarian carcinoma, mesothelioma, neurofibrosarcoma and renal cell carcinoma, non-small cell lung carcinoma and colon carcinoma. Antitumor activity of IL-4 has been explored in various animal models. For example, when injected around tumor draining lymph nodes directly into a solitary tumor nodule or intraperitoneally for systemic metastasis, IL-4 has been shown to have significant antitumor activity. Also, transfection of the IL-4 gene induces killing of many tumor types *in vivo*. In addition, IL-4 potentiates the antitumor effects of TNF or IFN-γ on a variety of tumor cell lines.

Clinical Relevance

IL-4 has diverse effects on health and many pathological states. IL-4-mediated Th2 cell response may avoid the extensive inflammatory tissue injury and prevent autoimmunity by downregulating Th1 cell response. IL-4 has also been shown to have potent antitumor activity *in vitro*. On the other hand, IL-4 and its receptor are the essential mediators in the development of allergic and inflammatory diseases. Inappropriately high IL-4 production has also harmful effects in infectious diseases. In addition, abnormality in IL-4 receptor/JAK/STAT signaling contributes to hematopoietic disorders and tumorigenesis.

IL-4 and allergic and inflammatory diseases.

IL-4 is mainly responsible for Th2-driven tissue inflammation. IL-4 has been implicated in allergic and inflammatory diseases including allergic rhinitis, urticaria, conjunctivitis, food allergies, asthma and systemic anaphylaxis. A dysregulation of IL-4 production or a change in IL-4 responsiveness may be the underlying abnormality in atopy, the tendency to produce excessive IgE in response to allergens. Allergen-specific T cells from atopic subjects preferentially develop into IL-4-producing Th2 cells and their CD4+ cells exhibit high IL-4 production when stimulated by antigens other than allergens. Moreover, IL-4-deficient mice develop attenuated airway inflammation compared with wild type mice. IL-4 plays a significant role in viral, bacterial and parasitic infections. IL-4 may be involved in the progression of acquired immune deficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV-1).

Human severe combined immunodeficiency (SCID).

Human severe combined immunodeficiency (SCID) is a set of primary immunodeficiency diseases characterized by profoundly impaired cell-mediated and humoral immunity. More than 50% of SCID cases are X-linked SCID, which manifests complete or profound defects of T cells and NK cells, but carry normal or slightly increased numbers of B cells, leading to death mostly within a year after birth if not treated with bone marrow transplantation. XSCID is commonly associated with mutations that chromosomally map to Xq13 in the γc receptor gene. Mutations occur throughout the entire γc gene, including the extracellular and cytoplasmic domains, which impair cytokine ligand binding as well as signal transduction. These mutations are manifested as deletions, insertions, splice junction defects, point mutations and premature stop codons in the γc gene. The complete dysfunction of the γc subunit in ligand binding and signal transduction results in the typical phenotype associated with XSCID. Strikingly, a form of autosomal SCID exists with clinical symptoms identical to XSCID in which the gene encoding JAK3 is affected.

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Interleukin-6

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Synonyms

- B-cell differentiation factor (BCDF)
- B-cell stimulating factor-2 (BSF-2)
- hepatocyte stimulating factor (HSF)

Definition

Interleukin-6 (IL-6) is a member of a family of cytokines that includes leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), IL-11 and cardiotrophin-1 (CT-1). These cytokines are functionally redundant and structurally similar. They also share receptor subunit for signal transduction, and thus elicit similar and overlapping physiological responses. IL-6 is a multifunctional cytokine. In addition to its important roles in the immune response, inflammation and hematopoiesis, IL-6 is also involved in other important physiological processes such as promotion of osteoclast resorption of bone and regulation of the growth of many tumor cells.

Characteristics

The human IL-6 gene was cloned in 1986. It contains five exons in its 5 kb genomic DNA, which is mapped to human chromosome 7p21-p14. IL-6 is first synthesized as a precursor protein of 212 amino acids and the mature IL-6 is a glycoprotein composed of 185 amino acids. Different post-translational modifications (e.g., glycosylation and phosphorylation) of the protein results in various molecular masses of 22-28 kD. The promoter of the IL-6 gene contains binding sites for various transcription factors, including binding sites for → NFκB and C/EBPβ (CAATT-enhancer-binding protein β), allowing the transcriptional regulation (induction or repression) of the IL-6 gene in response to various stimuli such as estrogen, interleukin-1 (IL-1) or tumor necrosis factor (TNF). IL-6 is produced in various cell types. Major sources of IL-6 production include stimulated monocytes, fibroblasts and endothelial cells.

Gene

The receptor for IL-6 (IL-6R) is composed of IL-6Rα, an 80 kD IL-6 binding protein and a signal transducer termed gp130. IL-6Rα is similar to other cytokine receptors in the type I cytokine receptor superfamily. Gp130 is a membrane-associated 130 kD glycoprotein, which is shared among the receptors for CNTF, LIF, OSM, IL-11 and CT-1. The sharing of gp130 provides an explanation for the functional redundancy among the IL-6-related family of cytokines. Binding of IL-6 to IL-6Rα triggers the association of IL-6Rα with gp130. The signal is then transduced through activation of tyrosine kinases, Janus kinases (JAKs), and subsequent activation of downstream transcription factors STATs (signal transducer and activator of transcription), resulting in activation of gene expression. There are a few members within the JAK family tyrosine kinases. Among them, JAK1, JAK2 and Tyk2 have been shown to associate with gp130 and to be activated by IL-6. → STATs are tyrosine phosphorylated transcription factors that are activated by JAK tyrosine kinases. There are also a few members in the STATs family. Of these STATs, → STAT1, STAT3 and STAT5 are activated in response to IL-6. Numerous studies indicate that STAT3 is essential in IL-6-induced, gp130-mediated signal transduction, regulating various important physiological processes including cell growth, survival and differentiation. Another pathway activated by IL-6 is the MAP kinase pathway, which is an important pathway involved in regulating cell differentiation, cell growth and death in response to IL-6 and other growth factors. IL-6 receptor is expressed in both immune- and non-immune tissues, such as prostate tissue and neuronal tissue. As a multifunctional cytokine, IL-6 is able to induce differentiation of B cells, T cells, myeloid leukemic cells and even neuronal cells. IL-6 is also able to induce cellular growth of myelomas, keratinocytes, mesangial cells, renal-cell carcinoma, Kaposi sarcoma and tumor cells such as prostate cancer cells. On the other hand, IL-6 is able to inhibit growth of myeloid leukemic cells and some other tumor cells, such as mammary carcinomas, → cervical cancer, human lung cancer cell lines and → melanoma. The biological activity of IL-6 can be enhanced by other factors such as glucocorticoid, which has been recognized to be able to synergize the IL-6 response. In many cases, STAT3 plays essential roles in the synergy between IL-6 and other factors. Soluble form of IL-6Rα can also interact with gp130. Thus, cells lacking IL-6Rα

are not responsive to treatment with IL-6 alone but are responsive to co-treatment with IL-6 and soluble IL-6Rα.

Clinical Relevance

IL-6 is related to various diseases. It has been found that abnormal IL-6 production and abnormal expression of IL-6 receptor are related to autoimmune disease. Serum IL-6 level may be related to various diseases. Low serum IL-6 levels are observed in some diseases (e.g., monoclonal gammopathies), while elevated serum IL-6 levels are associated with other diseases such as human → prostate cancer. A growing number of clinical observations have revealed the frequent association of high serum IL-6 levels with androgen-independent prostate tumors, suggesting the involvement of IL-6 in prostate cancer androgen-independent progression. IL-6 receptor is expressed in both prostate cancer tissues and prostate carcinomas cell lines. In the absence of androgen, IL-6 is able to activate the androgen receptor, which is involved in prostate cancer androgen-independent progression. STAT3 has been shown to be required for IL-6 to activate androgen receptor in prostate cancer cells. In addition to its role in prostate cancer progression, recent studies also indicate that IL-6 could function as a growth factor and could be involved in the oncogenic process of other human tumors such as renal-cell carcinoma, Kaposi sarcoma, lymphoma and mammary carcinoma. IL-6 is also essential for the osteoporosis (accelerated bone loss) caused by estrogen deficiency. Estrogen has been shown to be able to inhibit IL-6 production. Thus, one of the most common forms of osteoporosis is postmenopausal osteoporosis found in women after menopause or ovariectomy, due to overproduction of IL-6 caused by loss of estrogen production. Estrogen replacement therapy has thus been used to prevent postmenopausal osteoporosis.

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Internal Dose

Definition

Internal dose is the amount of a chemical that enters the body as a consequence of ingestion, inhalation or skin absorption; → adducts to DNA.

Internalization

Definition

→ Endocytosis.

Interphase

Definition

Interphase is the period between mitotic cell divisions, which is divided into G_1, S and G_2.

Interphase Cytogenetics

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Definition

Interphase cytogenetics refers to the analysis of chromosomes in non-dividing nuclei without the need for preparing metaphase chromosomes. It is particularly useful to visualize chro-

mosomal aberrations in tissue sections, cytological preparations, and fixed specimen. The technique involves hybridisation probes labeled with either fluorescent or chromogenic tags and microscopic evaluation

Characteristics

Metaphase chromosome analysis

When organs grow or tissues regenerate, or when tumors arise, cells have to undergo numerous rounds of cell division. This process can be divided into several stages. One of them is metaphase which is defined as the state in which cellular structures have been duplicated and the genetic material can be equally distributed to the emerging daughter cells. In this stage of the cell cycle, chromosomes can be visualized using well-established laboratory techniques and microscopic evaluation. The majority of chromosomal aberrations in leukemias and lymphomas has been detected after analysis of chromosomes prepared from mitotic cells in metaphase. For instance, aberrations such as the → Philadelphia chromosome were described when cytogeneticists analyzed stained chromosome preparations from patients with chronic myelogenous leukemia. The Philadelphia chromosome is the result of a translocation between chromosomes 9 and 22. At the molecular level this translocation results in the fusion of two genes (→ BCR-ABL), with the consequence of an overproduction of a tyrosine kinase that can transform hematological cells. The detection of this tumor specific chromosomal aberrations is an integral part of establishing the diagnosis, and can guide the clinician to adapt individualized therapies.

Why interphase cytogenetics?

It seems obvious that the detection of chromosomal aberrations plays an important role in disease management. However, the benefits are not equally distributed for the many different kinds of cancers. In particular in the solid tumors, for example tumors of the breast, lung, colon, prostate and ovary, the detection of chromosomal aberrations as a diagnostic tool is exceedingly difficult when metaphase chromosomes are required for analysis. In many instances, the preparation of chromosomes from these tumors is a rather long and time consuming process, frequently unsuccessful and the interpretation of the result ambiguous. Equally important, many diagnostic specimens for the analysis of genetic changes in solid tumors require fixation of the material. This means that the option of preparing metaphase chromosome, which relies on dividing cells, is lost. It has therefore been a long perceived goal to develop procedures that allow the visualization of chromosomal and genetic changes directly in fixed, non-dividing cells from cancer specimens. This concept is termed interphase cytogenetics (1). Interphase cytogenetics allows one to visualize virtually all types of chromosomal aberrations directly in tissue sections or in cytological preparations of cancer samples. Using interphase cytogenetics, the direct inspection of cytogenetic changes on stained chromosomes is replaced by a targeted visualization of chromosomal aberrations directly in cells. This methodology employs fluorescence *in situ* hybridization, where DNA probes that are tagged with fluorescent molecules, are hybridized to cytological preparations. For instance, a trisomy for chromosome 21 could be visualized with DNA probes that specifically recognize this chromosome. All cells that carry this particular chromosomal aberration would reveal three fluorescent signals, as opposed to two in normal cells. This pattern is independent of the cell cycle since metaphase chromosomes maintain their structural integrity at all stages, and three signals in interphase cells indicate unambiguously the presence of an extra copy of this chromosome.

Chromosomal aberrations in cancer cells occur in different facets. It is estabilised that in many types of leukemias, structural chromosomal aberrations that juxtapose two genes, which are unrelated under physiological conditions, close to each other which results in the functional modification of these genes and trigger transformation. Such translocations comprise the above-mentioned Philadelphia chromosome. The detection of reciprocal translocations in interphase cells can be achieved using

either whole chromosome painting probes or locus specific probes that hybridize adjacent to the breakpoint. Fluorescence *in situ* hybridization techniques allows the labeling of such probes with different colors and hence the design of probe cocktails that indicate specifically the genetic aberration. For instance, the colocalization of red and green labeled probes in interphase cells results in a merged yellow color that would indicate the colocalization of the two probes as a result of a translocation. Similarly, chromosomal deletions of tumor suppressor genes can be assessed. Such deletions appear as a lack of hybridization signals. Tumor cells have in many instances only one cytogenetically visible copy of a tumor suppressor gene left. Another class of relevant chromosomal aberrations in cancer cells are chromosomal trisomies. Chromosomal trisomies are among the early chromosomal changes in the genesis of solid tumors and correspond with poor prognosis in some forms of hematological malignancies. They can be visualized using chromosome painting probes or using chromosome specific probes that label the centromeric or paracentromeric heterochromatin. The latter have the advantage that the signals are more circumscript compared to whole chromosome painting probes. This reduces the possibility of signal overlap and therefore false negative results. At advanced stages of carcinogenesis, tumors can acquire multiple copies of oncogenes. These amplification events correlate in general with poor prognosis, however, in some instances can also stratify patients for specific therapeutic regimens, such as in the case of the her2-neu oncogene amplification in breast cancer. Gene amplifications can be very elegantly visualized and quantified by interphase cytogenetics. A DNA probe that targets the particular oncogene will display multiple copies in interphase cells, and the copy number can be correlated to a non-involved chromosome hybridized with a second fluorochrome.

Application

Cytogenetic characterization of solid tumor genomes using conventional banding techniques has been difficult. However, the application of novel methodologies such as comparative genomic hybridization and spectral karyotyping has revealed a non-random, tumor and tumor stage specific distribution of chromosomal copy number changes. The translation of such chromosomal changes into a diagnostically useful format will be one of the major applications of interphase cytogenetics. One particular advantage of interphase cytogenetics is that the genetic information can be evaluated on individual cells together with such relevant parameters as histo- or cytomorphology. Interphase cytogenetics can therefore become the tool that allows one to combine pertinent genetic information directly with morphological changes in the progression of solid tumors. Since more predictive information can be obtained, it is conceivable that this fusion of genetics and morphology will improve diagnosis, prognosis and therapy planning. Interphase cytogenetics will therefore contribute to a comprehensive phenotype/genotype correlation in solid tumors and hematological malignancies (Ried, 1998).

Future developments of interphase cytogenetics can be envisioned in several areas:

- It would be useful to increase the numbers of chromosomal targets that can be visualized simultaneously. This can be achieved by combining multiple probes, each tagged with a different fluorochrome. Recent developments in fluorescent dye chemistry and in microscope hardware and software along with novel imaging tools, such as spectral imaging, are likely to increase the multiplicity of interphase cytogenetics.
- A combination of genetic and chromosomal markers for specific cancers and cancer stages with morphological markers, for instance markers for cell lineage in hematological malignancies, would increase the value of the respective approaches for cancer diagnostics.
- Refinement of non-fluorescent detection formats for interphase cytogenetics and the development of mutlicolor approaches with permanent dyes would allow for an archiving of tissue samples. This would be particularly useful for applications in pathology,

where it is frequently required to store samples for a certain period of time. In addition, the use of chromogenic dyes helps to overcome one of the inherent problem of fluorescence *in situ* hybridization on fixed samples, namely background fluorescence (Speel et al., 1995)
- Finally, microscopic tools that enable the 3D-reconstruction of tissue sections would minimize the problem of out of focus signals.

In summary, interphase cytogenetics offers a unique approach for the visualization of genetic and chromosomal aberrations in cancer tissues and cytological preparations on a single cell level. The maintenance of the cellular morphology offers great promise that interphase cytogenetics could contribute significantly to the diagnostic enhancement of the mainly morphologically based evaluation of cancer and its precursor lesions.

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Intervening Sequence

Definition

→ Intron.

Intestinal Metaplasia

Definition

Intestinal metaplasia is the loss of cellular differentiation, yielding epithelium that resembles small intestinal epithelium.

Intron

Definition

An intron is a segment of DNA that is transcribed into RNA but removed from within the transcript by splicing together the sequences (exons) on either side of it. Introns have no protein-coding function, but these non-coding regions can include control sequences and other intergenic regions of unknown function.

Intussuception

Definition

Intussuception occurs when one segment of the intestine is sucked into another. It is frequently triggered by the presence of a polyp inside the intestine.

Inv(16)

Definition

Inv(16) is a pericentric inversion of chromosome 16; → AML-1/ETO/CBFβ/TEL in chromosomal translocations.

Invasion

Definition

Invasion is the penetration of an organic matrix (extracellular matrix; → ECM) by actively migrating cells.

Inverse PCR

Definition

Inverse PCR has been used for the amplification of unknown DNA segments that lie outside the boundaries of the known sequence. Genomic DNA is digested with appropriate restriction enzymes and self-ligated at low DNA concentration. The circular DNA is used as a template for polymerase chain reaction (→ PCR), using primers designed against known sequences. The resulting PCR product contains an unknown segment that is flanked by known sequences.

Inverted Repeats

Definition

Inverted repeats comprise two copies of the same sequence of DNA repeated in opposite orientation on the same molecule. Adjacent inverted repeats constitute a palindrome.

Islet Cell Tumor

Definition

→ Gastrinoma.

Isochromosome

Definition

Isochromosome, abbreviated to 'i', is a mirror image chromosome consisting of two identical arms.

Isoform

Definition

An isoform is a structurally and functionally related form of a protein. Isoforms, also referred to as isozymes, are enzyme molecules that catalyse the same reaction but their other properties (e.g. domain organisation or way of regulation) differ.

Isograft

Definition

Isograft is the engraftment of organs, cells or tissues between genetically identical individuals.

Isozymes

Definition

→ Isoform.

ITR

Definition

Inverted terminal repeat (ITR; ends of the → AAV genomes).

J

JAK

Definition

→ Janus kinase protein family. JAK1, Janus kinase 1; JAK2, Janus kinase 2; JAK3, Janus kinase 3.

Janus Kinase

Definition

Janus kinases (JAKs) are a family of tyrosine kinases that function in the signaling by interferons and other cytokines through their ability to phosphorylate STAT [→ signal transducers and activators of transcription in oncogenesis] proteins.

JAK1 is a protein tyrosine kinase of the non-receptor type involved in the interfon α/β/γ signal pathway. It is an intracellular protein of 1141 aa and 131 kD that is a kinase partner for the interleukin-2 (IL-2) receptor. The gene maps to 1p31.

JAK2 is a tyrosine kinase of the non-receptor type, involved in interleukin 3 (IL-3) signal transduction. It is an intracellular protein of 1132 aa and 130 kD. The gene maps to 9p24.

JAK3 is a tyrosine kinase of the non-receptor type, involved in interleukin-2 (IL-2) and interleukin-4 (IL-4) signaling pathways. It is an intracellular protein of 1124 aa and 125 kD that phosphorylates STAT6. The gene maps to 19p13; 3 splice variants exist that are differentially expressed.

JNK

Definition

Jun-N-terminal kinase, also known as SAPK, is responsible for the phosphorylation of c-Jun and ATF-2 proteins in response to cellular stress; → AP-1.

JUN

Definition

Jun is a proto-oncogene homologous to the avian sarcoma virus 17 oncogene. It is an early response gene of mitogenic and radiation stress signals. It encodes the transcription factor jun, which forms heterodimers with fos and related proteins that bind to the → AP-1 consensus site of many gene promoters.

Junctional Complex

Definition

A junctional complex is the collection of various types of junctions, mainly found in epithelial cells. The junctions form a complex of → cell adhesion molecules integrated with the → cytoskeleton. In vertebrates, the most apical junction is the → tight junction, followed by the → adherens junction and the → desmosome.

Kaposi Sarcoma

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Definition

Kaposi sarcoma (KS) is a vascular tumour, often affecting the skin. Four epidemiological forms exist:

- Classic KS occurs mainly in elderly HIV negative male patients of Southern European and Middle Eastern origin.
- Endemic KS: In some African countries, KS has existed for many decades, long preceding HIV. Unlike classic KS, endemic KS also occurs in children, where they often present with lymphadenopathy, rather than skin lesions. Endemic KS is generally a more aggressive disease than classic KS, though less so than African AIDS-associated KS.
- Post-transplant or iatrogenic KS is also known to develop after an organ transplant. Patients of Mediterranean, Jewish or Arabian ancestry are over-represented among immunosuppressed patients who develop KS after a transplant, indicating that those born in countries where classic KS occurs continue to be at risk of developing KS even if they migrate to 'low-risk' countries.
- AIDS-KS: In 1981 the occurrence of two rare diseases in young gay men from New York City (NY, USA) and California were reported: Kaposi sarcoma and *Pneumocystis carinii* pneumonia. This was the beginning of the AIDS epidemic and AIDS-KS is today the most common form of KS. In HIV-infected individuals the underlying immunosuppression can lead to an aggressive disease that starts with skin or mucosal lesions, but without treatment, often develops into disseminated disease affecting various organs including lung, liver, gut and spleen.

Characteristics

Histology

Histologically, KS is a complex lesion: in early KS lesions there are a collection of irregular endothelial lined spaces that surround normal dermal blood vessels and these are accompanied by a variable inflammatory infiltrate (patch-stage). This stage is followed by the expansion of a spindle-celled vascular process throughout the dermis. These spindle cells form slit-like, vascular channels containing erythrocytes (plaque -stage). The later nodular-stage KS lesions are composed of sheets of spindle cells. The spindle cells form the bulk of established KS lesions and are therefore thought to be the neoplastic component. Most of the spindle cells in KS lesions express endothelial markers, including CD31 and CD34. Recently, it was also shown that these spindle cells express markers associated with lymphatic, rather than vascular endothelial cells including vascular endothelial growth factor receptor (VEGFR)-3 and podoplanin. This suggests that these spindle cells might belong to the lymphatic lineage of endothelial cells. Early KS ('patch stage') is probably a non-clonal proliferation of lymphatic endothelial cells or endothelial precursors, with a prominent inflam-

matory and angiogenic response, whereas advanced disease can develop into a true clonal malignancy with metastases of clonally derived spindle cells to different sites.

Studies of AIDS case surveillance support the pre-AIDS data on the existence of a sexually transmissible KS cofactor: KS occurs predominantly in gay and bisexual men with AIDS, less commonly in those acquiring HIV through heterosexual contact and rarely in AIDS patients with hemophilia or in intravenous drug user.

Virus involvement

Sequences of Kaposi sarcoma-associated herpesvirus (KSHV or human → herpesvirus-8) were first identified in Kaposi sarcoma biopsies. KSHV is a gammaherpesvirus and related viruses are found in non-human primates. The KSHV genome consists of an ~140kb long unique coding region (LUR) flanked by ~800 bp noncoding tandemly repeated units. Over eighty five open reading frames (ORFs) have thus far been identified, including nearly seventy with sequence similarity to related gammaherpesviruses. Novel ORFs not present in other herpesviruses were designated K1 to K15, although many of these now appear to be present in related viruses. KSHV encodes for a number of cellular homologs and these include a viral cyclin, bcl-2, IL-6, interferon regulatory factor (IRFs), FLICE inhibitory protein (FLIP) and chemokine homologs (vMIP I, II and III).

Viral strains

ORF-K1 is use to subtype KSHV:subtypes A, B, C and D have been identified which display between 15 – 30% amino acid differences between their ORF-K1 coding regions. These subtypes have close associations with the geographic and ethnic background of individuals. Within these 4 subtypes, over 13 clades have now been described. Subtype B is found almost exclusively in patients from Africa, subtype C in individuals from the Middle East and Mediterranean Europe, subtype A in Western Europe and North America and subtype D has only so far been described in individuals from the Pacific Islands.

KSHV latent proteins

A number of KSHV ORFs are transcribed during latency and these proteins could be involved in oncogenesis:

- Latent nuclear protein (ORF 73 / LNA) of KSHV encodes a latent immunogenic nuclear protein (LNA) detected as nuclear speckling by immunofluorescence using KSHV positive sera on PEL cells. LNA is expressed in all KS spindle cells latently infected with KSHV, in all the immunoblasts in KSHV associated Castleman's disease and in all cells of (primary effusion lymphoma (PEL). Like EBNA-1, LNA is essential to maintain the KSHV episome (extrachromosomal persistence). Furthermore, LNA tethers KSHV DNA to chromosomes during mitosis to allow the segregation of viral episomes to all progeny cells. LNA therefore maintains a stable episome during mitosis. LNA also interferes with p53 andpRb pathways to deregulate the cell cycle. Strategies including the development of small molecules that interfere with the functions of LNA could be useful to abort latent KSHV infection and therefore prevent KSHV associated diseases.
- v-cyclin; cellular cyclins [→ cyclin D] are critical components of the cell cycle: cyclins are regulatory subunits of a specific class of cellular kinases. By physically associating with an inactive cyclin-dependent kinase core (cdk), cyclins lead to the formation of active kinase holoenzymes which recognize and phosphorylate an array of cellular substrate molecules. The phosphorylating activity of these holoenzymes is responsible for regulating the passage of cells through the replication cycle. Cyclins associate with their partners (the cyclin dependent kinases, CDKs) to be fully active. The KSHV cyclin has highest sequence similarity to the cellular D-type cyclins. The KSHV cyclin is expressed during latency inferring a possible role in tumorigenesis. The KSHV-cyclin form active kinase complexes with CDK6

to phosphorylate the retinoblastoma protein (pRb). Furthermore, unlike cellular D cyclin/CDK6 complexes, KSHV-cyclin/CDK6 activity is resistant to inhibition by CDK inhibitors (CKI) p16, p21 and p27. Ectopic expression of v-cyclin prevents G1 arrest imposed by each inhibitor and stimulates cell-cycle progression in quiescent fibroblasts. KSHV cyclin/CDK6 also phosphorylates (inactivates) p27, the CKI known to be an effective inhibitor of cyclin E/CDK2 activity. This suggests that this viral cyclin can activate both pathways necessary for G1/S-phase progression (i.e. cyclin D/CDK6 and cyclin E/CDK2). The expression of the KSHV-cyclin in latently infected spindle and PEL cells, indicate a possible role in either the proliferation or the arrest of differentiation of these cells.

- ORF-K15 latent membrane protein. At the far right-hand end of the KSHV genome, the ORF K15 encodes a putative latent transmembrane protein. Two highly divergent forms of K15 have been identified; the predominant (P) and minor (M) forms. These two alleles have only 33% amino acid homology to each other, yet retain the 12 transmembrane spanning domains and a cytoplasmic signal-transducing carboxyl-terminus. K15 induces NFκB activation and this activity localises within the last 18 amino acids of K15, which contains the putative TRAF-binding motif. K15 is also able to activate JNK. This ability to activate JNK, like that of NFκB activation, is located within the last 18 aa of the K15 extreme C-terminus. The JNK signalling pathway is known to lead to the activation of → AP-1, a pleotropic transcription factor implicated in cellular transformation and phenotypic changes.

Neoplasms associated with KSHV

Three neoplasia are consistently linked with KSHV infection: Kaposi sarcoma, a subtype of Castleman's disease and PEL. KSHV sequences have also been described in squamous carcinoma, multiple myeloma and other vascular tumours, however, most studies could not confirm any association between KSHV and these tumours. The detection of KSHV in some of these reports might be due to PCR contamination.

- Kaposi sarcoma. Four observations link KSHV to Kaposi sarcoma (although none of these findings on their own are sufficient to support a causative role):
  - KSHV DNA is present, by the polymerase chain reaction (PCR), in all four epidemiological forms of KS and in nearly all KS biopsies tested. However, KSHV DNA is rarely, if at all, detectable in other vascular tumors.
  - The detection of KSHV DNA by PCR in the peripheral blood of HIV infected individuals predicts who might subsequently develop KS, indicating that those at risk of KS have a higher viral load than those not at risk.
  - Seroepidemiological surveys show that general populations at risk of developing Kaposi sarcoma have a higher prevalence of KSHV infection. The incidence of classic KS and AIDS-KS in different populations correlates broadly with the prevalence of the virus in these populations.
  - In nodular KS lesions, KSHV is latently expressed in nearly all the tumor (spindle) cells. This is reminiscent of other viral driven cancers e.gs. → Epstein-Barr virus (EBV) latent infection in post-transplant lymphoproliferation or human papillomavirus infection in cervical cancer.
- Plasmablastic variant Multicentric Castleman's Disease; Castleman's disease (CD) is a lymphoproliferative disorder. Recently CD is more often diagnosed in HIV infected patients. A systemic variant of CD is associated with multiple organ involvement, especially spleen and lymph nodes with systemic symptoms such as weight loss and fever. This is called multicentric Castleman's disease (MCD). MCD has been associated with increased circulating IL-6 levels. KSHV DNA is found in some cases of MCD. KSHV is present in plasmablasts in MCD and these plasmablasts are not present in KSHV negative MCD. These KSHV positive plasmablasts belong to the B cell lineage.

KSHV appears also to be present in all tumour cells of plasmablastic lymphoma that develops in patients with the KSHV positive plasmablastic variant of MCD. The development of plasmablastic lymphoma therefore appears to represent a further evolution of this disorder. Unlike KSHV positive primary effusion lymphoma cells, the plasmablasts in MCD are only positive for KSHV, and not for EBV.

Current studies suggest that KSHV positive MCD has a poorer prognosis that the KSHV negative cases. In contrast to KS lesions that can resolve by partly restoring the immune system (e.g. by HAART for HIV positive patients), KSHV positive MCD often continues to progress.

- Primary Effusion Lymphoma (PEL); PEL is a body cavity-based lymphoma that usually presents and persists as an effusion: pleural, pericardial or ascitic. The lymphoma cells in these cases are negative for most lineage-associated antigens, although immunoglobulin (Ig) gene rearrangement studies indicated a B-cell origin. These lymphomas occur predominantly in HIV+ individuals with advanced stages of immunosuppression, but are occasionally seen in HIV negative patients. Like KS which can occur in the same patient, PEL occurs primarily in gay men and not in other HIV-positive risk groups. PEL cells contain between 50 and 150 copies each of the KSHV genome. The majority, but not all, PELs are co-infected with EBV, suggesting that the two viruses may cooperate in neoplastic transformation. Terminal repeat analysis indicates that EBV is monoclonal in most cases, implying that EBV was present in the tumor cells prior to clonal expansion. PEL cells consistently lack molecular defects commonly associated with neoplasia of mature B cells including activation of the proto-oncogenes bcl-2, bcl-6, n-ras, and k-ras, as well as mutations of p53. KS, MCD and PEL have all been described in one patient and up to 30% of HIV infected patients with KSHV positive MCD, will also have or develop KS.

KSVH and immunity

The introduction of aggressive anti-HIV therapies lead to a decline in the incidence of KS in AIDS patients and also in the resolution of KS in those already affected. This suggests that cellular immune responses, compromised in AIDS, but recovering after highly active antiretroviral therapy (HAART), could be important in the control of KSHV infection and in the development of KS. This is further supported by the observation that post-transplant KS lesions can regress when immunosuppressive treatment is stopped.

KSHV, like other herpesviruses, is able to elicit HLA class I restricted cytotoxic T cell (CTL) responses. In one pilot study, KSHV specific CTL responses were not present in most patients with KS, indicating that a decline in cellular immune responses against KSHV may be present in HIV+ patients with KS and could contribute to KS pathogenesis. This would be reminiscent of the lack of EBV specific CTLs seen in immunosuppressed patients which correlates with the onset of EBV driven lymphoproliferation.

Anti-Herpesviral drugs

In vitro, KSHV replication is insensitive to ganciclovir and acyclovir, but is moderately sensitive to foscarnet (phosphonoacetic acid) and sensitive to cidofovir. These agents target lytic herpesviral infection and if lytic infection is necessary to drive tumour formation or to recruit inflammatory cells to form KS lesions, these drugs might prove useful in the management of some patients. Foscarnet has been shown to induce KS lesion regression in one small study and to reduce the onset of KS in other studies. Foscarnet and cidofovir are however associated with significant toxicity and would seem to be inappropriate therapy for most KS patients.

Treatment for KS

In immunocompetent patients with single lesions, excisional biopsy is often all that is required. Patients with a few lesions, especially

if these are confined to a limited region, is best treated with local radiotherapy. Total-skin electron beam therapy has also been used to treat more disseminated skin lesions. Patients with disseminated disease can be treated with systemic chemotherapy. Single agents that often lead to responses include bleomycin, vinblastine/vincristine, oral → etoposide and → doxorubicin. In HIV infected patients, these agents seldom lead to long-term control of the disease. A major disadvantage of doxorubicin and oral etoposide is alopecia. Taxol has also been used, but this agent is associated with significant toxicity. Liposomal doxorubicin is better tolerated and is associated with high response rates. Systemic interferon has also been used and responses have been seen for many months (even years). If tolerated, subcutaneous interferon injections remain a therapeutic option to treat KS. More experimental therapies include thalidomide, retinoic acid and human chorionic gonadotropin. However, these agents only produce transient responses in some patients.

KS often regress with the cessation or modification of immunosuppressive therapy in organ transplant recipients. Effective treatment of HIV infection should prevent KS and has already lead to remission of KS in some patients.

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Karyotype

Definition

Karyotype is the chromosome complement of cells as visualized during mitosis. The total number of chromosomes is given first, followed by the sex chromosome constitution and a description of numerical and structural aberrations. A normal male karyotype is thus written 46, XY and the normal female complement 46, XX.

kb

Definition

1000 base pairs of DNA or 1000 bases of RNA is abbreviated to kb.

KI-67

Definition

Ki-67 is a monoclonal antibody, recognizing the protein → MKI67. The latter is thought to be required for maintaining cell proliferation.

Kinase

Definition

A kinase is a specialized protein possessing enzymatic activity that catalyzes the addition of phosphate groups to amino acids, fatty acids, sugars or nucleic acids. This process is also called phosphorylation. In signal transduction processes, kinases are located at the cell membrane (receptor kinases) or in the cytoplasm. Some cytoplasmic kinases can shuttle into the nucleus and phosphorylate transcription factors.

KIP1

Definition

→ Cyclin-dependent kinase inhibitor 1B.

KiSS1

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Definition

KiSS1 (GenBank Accession No. U43527) is a human → metastasis-suppressor gene that was discovered in human → melanoma cells. The name is derived from SS for putative Suppressor Sequence, the Ki was added to remind people of its discovery in Hershey, Pennsylvania.

Upon introduction into metastatic cells, which normally do not express the gene, the cells retain the ability to form tumors while losing their ability to metastasize.

Characteristics

As melanomas progress toward increasing malignancy, numerous nonrandom chromosomal changes occur. One particular change involving deletions involving the long arm of chromosome 6, tends to occur at a time coincident with acquisition of metastatic potential. This suggested that a metastasis suppressor gene was encoded at that site. This hypothesis was validated upon introduction of chromosome 6 into metastatic human melanoma cells using the technique of → microcell-mediated chromosome transfer. Metastatic potential was lost but tumorigenicity was retained in the chromosome 6-melanoma hybrids. By comparing mRNA expression between parental (metastatic) cells and chromosome 6 hybrids (nonmetastatic) using subtractive hybridization, KiSS1 was identified as being expressed exclusively in the nonmetastatic cells. Transfection of KiSS1 into the metastatic melanoma cells also suppressed metastasis without affecting tumorigenicity.

Does KiSS1 suppress metastasis of other human cancers?

Transfection of KiSS1 into at least two human melanoma cell lines and a human breast carcinoma cell line results in significant suppression of metastasis. Tumorigenicity is not affected by KiSS1 expression.

How does KiSS1 suppress metastasis?

The KiSS1 gene encodes a predominantly hydrophilic protein of 145 amino acids (approximately 15.4 kD). It contains no nuclear localization signal and does not appear to have a trans-membrane domain. It does, however, contain a putative signal sequence, suggesting that it may be secreted. The molecule also contains a → PEST sequence, perhaps explaining why isolation of purified protein has been so difficult.

The KiSS1 gene maps to chromosome 1q32, suggesting that a gene(s) on chromosome 6 regulates its expression. Recently, the regulatory gene has been mapped to a more refined location on the long arm of chromosome 6, but the specific identity remains unknown. Collectively, these results imply that the defect in controlling metastasis lies upstream of KiSS1.

Is KiSS1 a marker of melanoma metastasis?

Clinical data using *in situ* hybridization and immunohistochemical staining has not been performed; therefore, the answer to this question is still unknown. However, using a series of cell lines representing the progression of normal melanocytes to metastatic melanomas, KiSS1 expression is lost as the cells convert from radial to vertical growth phase (benign to malignant transformation). Thus, it is possible the KiSS1 may be useful as a marker of melanoma progression and/or metastasis.

Clinical Relevance

Metastasis is the most life-threatening attribute of cancer cells. Any gene/protein that could block or slow metastasis would benefit long-term survival rates among cancer patients. Likewise, any marker that could allow physicians to more accurately stage the disease would aid in treatment planning and monitoring. KiSS1 offers potential in both arenas.

Of particular note is that suppression of melanoma metastasis in chromosome 6-C8161 cells occurs at the final stage of the metastatic cascade, i.e., during the growth and formation of macroscopic lesions. In theory, therapies directed against metastatic disease would target existing, although potentially occult or dormant, secondary tumors. That is, these cells would have already reached their secondary site by the time treatment was initiated. Thus, therapeutic intervention directed at this final step would seem to be most relevant. To the extent that KiSS1 plays a role in the suppression of metastasis at this late stage, it may provide or lead to therapeutic targets designed to inhibit the ability of the cells to form tumors at a secondary site.

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KIT

Definition

KIT, or v-kit Hardy-Zuckermann 4 feline sarcoma viral oncogene homolog, is a protein of 976 aa and 109 kD. It is a receptor for mast stem cell factor and has tyrosine kinase activity. The binding of ligand to KIT leads to autophophorylation and association with substrates such as → PI3K. The gene maps to 4q12. Defects in KIT result in a developmental abnormality known as piebaldism, an autosomal genetic disorder of pigmentation characterized by congenital patches of white skin and hair that lack melanocytes.

Kit/Stem Cell Factor Receptor in Oncogenesis

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Synonyms

- stem cell factor (SCF) receptor (SCF-R)
- Kit/SCF-R
- c-Kit
- mast cell growth factor (MGF) receptor
- *steel* ligand receptor

Definition

Kit/SCF-R is a receptor protein tyrosine kinase (RPTK) encoded by the → proto-oncogene c-*kit*, which is allelic with the murine dominant *white-spotting* (*W*) locus on chromosome 5, and whose human homologue is located on human chromosome 4 (4 q11-12).

Characteristics

Going from the N-terminus towards the C-terminus, all RPTKs contain an extracellular ligand-binding domain, a single transmembrane-spanning domain and an intracellular domain composed of a hydrophilic juxtamembrane (JM) region, followed by the highly conserved → tyrosine kinase domain and a hydrophilic C-terminus. Based on structural similarities, Kit/SCF-R belongs to the subclass III of RPTKs that includes the receptors for platelet-derived growth factor (→ PDGF) α and β, colony-stimulating factor-1 (CSF-1) and the Flt-3/Flk-2 RPTK. These proteins are ~950 to ~1100 amino acids in length, with ~550 extracellular amino acids and 400-550 intracellular amino acids. Their overall identity is 25 – 40%, but the identity in their intracellular domains is between 45 and 55%, and in the kinase domains proper between 63 and 85%. They are characterized by 5 immunoglobulin-like repeats in the extracellular ligand-binding domain and a long (75-100 amino acids) hydrophilic kinase insert region, which interrupts the tyrosine kinase domain between the ATP-binding region and the conserved catalytic base. The Kit/SCF-R is expressed in hematopoietic, melanogenic and gametogenic precursors and derivatives, and in interstitial cells of Cajal. The development of these cells depends on Kit/SCF-R and its ligand. However, Kit is expressed in numerous other tissues/cell types including mammary duct epithelial cells, thyrocytes and cerebellar basket cells. Two alternative RNA transcripts, resulting from alternate usage of 5′ splice donor sites, generate two forms of Kit/SCF-R in all tissues examined. The only difference is the presence or absence of 4 additional amino acids, GNNK, in the extracellular domain close to the membrane. When overexpressed, the shorter isoform of Kit/SCF-R (ΔGNNK-Kit/SCF-R), which is the most prevalent in most tissues examined, is constitutively tyrosine phosphorylated, causes tumorigenesis in nude mice and greater activation of ERK/MAPK. However, ΔGNNK-Kit/SCK-R has not been directly associated with any tumors in man or mouse.

Molecular and cellular regulation

The general mechanisms of ligand-induced activation of Kit/SCF-R and its downstream signaling have been fairly well characterized, and very similar principles seem to govern ligand-induced signaling by all subclass III RPTKs. In outline, binding of the bivalent ligand, SCF, induces dimerization of Kit/SCF-R leading to activation of its tyrosine kinase domain and subsequent autophosphorylation on specific tyrosine residues. The phosphorylated tyrosine residues and 3-5 amino acid residues immediately N- or C-terminal to each of these, in turn, create specific binding sites for intracellular signaling molecules leading to their recruitment from cytosolic compartments to the membrane, where the activated RPTK is located. The binding of signaling molecules to the phosphorylated tyrosine residues occur through conserved protein-protein interaction domains, such as Src homology 2 (SH2) and protein tyrosine binding (PTB) domains. The SH2 and PTB domains have a defined, conserved primary sequence of ~100 and ~110 amino acids, respectively, and they exist as independently folded domains in a variety of intracellular proteins including proteins possessing a catalytic domain, pure adaptor molecules, structural proteins and translocated transcription factors. The conformational changes induced in the signaling molecules upon binding to an RPTK can unmask other protein-protein interaction domains allowing for interaction, recruitment and activation of yet other intracellular signaling molecules. Several other protein-protein interaction domains that have been identified include proline-rich-binding SH3 and WW domains, phospholipid-binding PH (pleckstrin homology) domains, phosphoserine/phosphothreonine-binding 14-3-3 [→ 14-3-3 proteins] and FHA (Forkhead-associated) domains. The specificity of signaling from different RPTKs is to a large extent determined by the primary sequence surrounding the autophosphorylated tyrosine residues in the receptors. This enables the activated RPTK to ‘select’ a specific subset of signaling molecules within the cell, which, in turn specifically interact with, and/or activate, other sig-

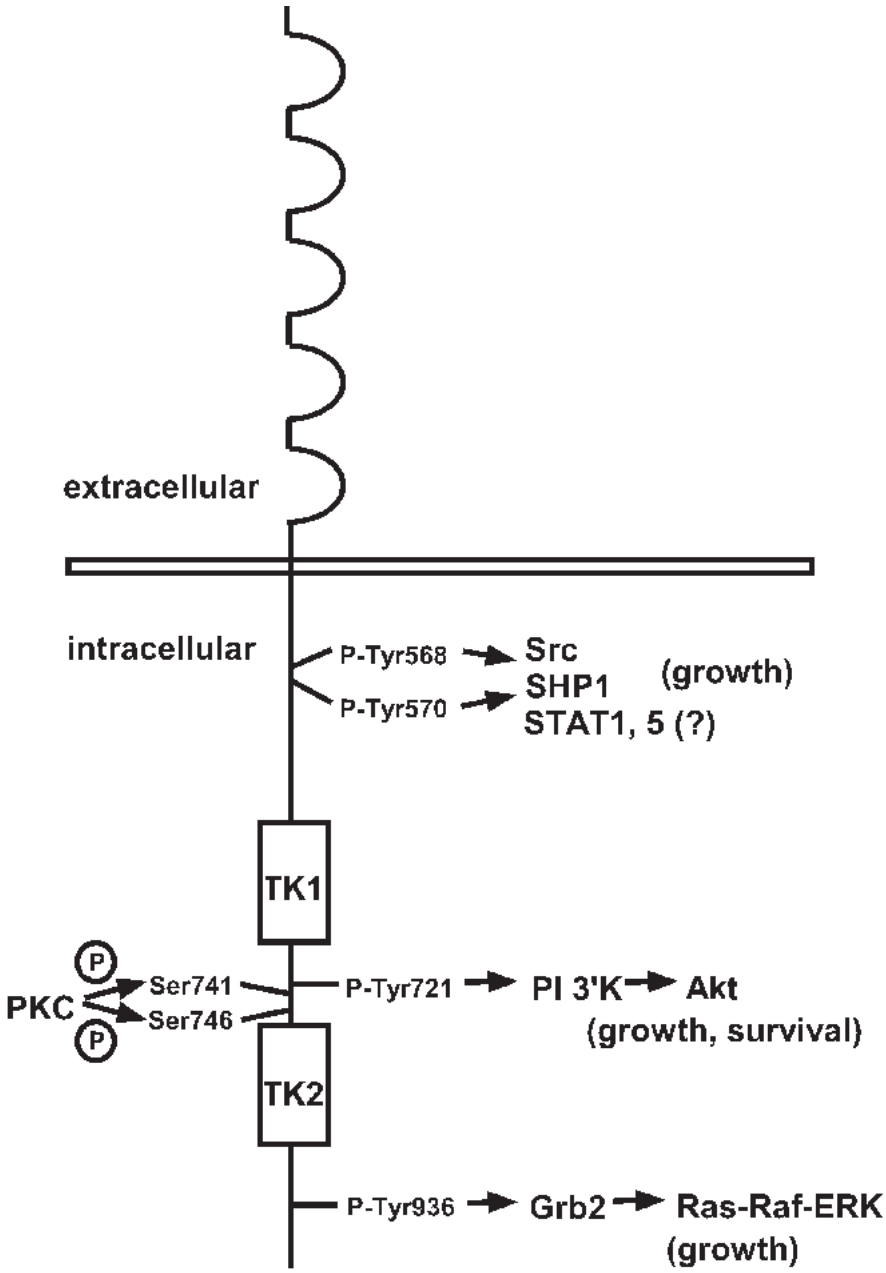
naling molecules, and a network of activated catalytic proteins and of multiprotein signaling complexes result. Examples of Kit/SCF-R-induced signaling molecules and pathways of importance for cell growth control include the Ras-Raf-ERK and Src-activated pathways, which are mainly involved in cell proliferation and the phosphatidylinositol 3′-kinase (PI 3′-K) and JAK/STAT [→ signal transducers and activators of transcription in oncogenesis] signaling pathways involved in cell proliferation and cell survival (Fig. 1). Several signaling components within each of these pathways are able to interact with and/or modify the activity of each other, allowing for so-called → signal transduction cross-talk. This is important for fine tuning and modulation of signaling. It is becoming increasingly clear that such Kit/SCF-R-initiated signaling pathways eventually impinge on and regulate the cell cycle machinery including the → p16-cyclin D-→ Rb pathway, as well as the DNA damage response pathways including p19ARFand p53. It is perhaps exclusively through these latter effects that the signaling pathways ultimately regulate proliferation and/or anti-apoptosis, respectively. For some recent reviews on RPTK-initiated signal transduction and mitogenic/survival signaling pathways (2 3).

Once the appropriate Kit/SCF-R-induced signaling pathways have been activated, it is crucial that further Kit/SCF-R signaling is down-regulated and inactivated to achieve a proper cell biological response. Several mechanisms prevail to achieve this, including negative feedback mechanisms, ligand-induced receptor internalization and degradation, and coordinated activation or organization of negative intracellular regulators including phosphatases and scaffolding proteins. In the case of Kit/SCF-R, SCF stimulation causes activation of → PKC, which acts in a negative feedback loop to inhibit further Kit/SCF-R tyrosine kinase activity by directly phosphorylating two serine residues in the receptor (Fig. 1). SCF stimulation also induces Kit/SCF-R internalization with a $T_{1/2} < 0.5$ hours, and it has been shown that the internalized receptors undergo regulated degradation, in part through → ubiquitin-mediated proteolysis. The ligand-induced Kit/SCF-R down-regulation is likely to play an important regulatory physiological role in development, since SCF-expressing stromal cells interact with Kit/SCF-R-expressing parenchymal cells in gonads, bone marrow and cerebellum during development. In these tissues SCF exists in a mainly transmembrane form, which does not induce Kit down-regulation to the same extent as the soluble form of SCF, thus allowing for more sustained Kit signaling. There is some evidence that there are quantitative and qualitative differences in the activated signaling molucules induced by the two forms of SCF. The transmembrane form of SCF is important for stem cell self renewal and survival, while the soluble form is important for chemotaxis and cell proliferation. As part of a long-term mechanism to ensure proper Kit/SCF-R signaling, the Kit expression level is regulated in a cell- and tissue-specific manner at the transcriptional level as well. The c-*kit* promoter contains Sp1, SCL/Tal1 and AP-2-binding sites and its expression is tightly and positively regulated by each of these transcription factors.

Perturbation of the normal regulatory mechanisms governing Kit/SCF-R-induced signal transduction has serious clinical consequences. Accordingly, both in humans and mice, naturally occurring → loss-of-function mutations and → gain-of-function mutations in Kit/SCF-R have been reported that result in developmental defects or malignancies.

Clinical Relevance

W-mutant and *Steel* mutant mice have naturally occurring loss-of-function (LOF) mutations in Kit/SCF-R or in SCF, respectively, resulting in varying degrees of anemia, mast cell depletion, white-spotted depigmentation, decreased fertility and constipation. A similar phenotype is found in humans suffering from piebaldism, a syndrome caused by LOF mutations in Kit/SCF-R homologous to several of those found in *W* mutant mice. The *W* mutations provided the first reported examples of germ-line mutations in a mammalian proto-oncogene and the first *in vivo* evidence of the importance of RPTKs for not only cellular



Kit/Stem Cell Factor Receptor in Oncogenesis. Fig. 1 – Schematic representation of known tyrosine autophosphorylation sites in human Kit/SCF-R together with the signaling molecules they recruit and activate. Several signaling molecules have been shown to bind to the juxtamembrane tyrosine autophosphorylation sites Y568 and Y570. Concentration differences of these molecules in different cell types, as well as differences in their subcellular distribution, may determine their recruitment to the autophosphorylation sites *in vivo*. Note that Grb2 has been shown to bind to the activated Kit/SCF-R *in vivo*, but that its binding to phosphorylated tyrosine 936 has only been mapped *in vitro*. Grb2 is an upstream activator of the Ras-Raf-ERK cascade. SHP1 is a protein tyrosine phosphatase, expressed in hematopoietic cells.

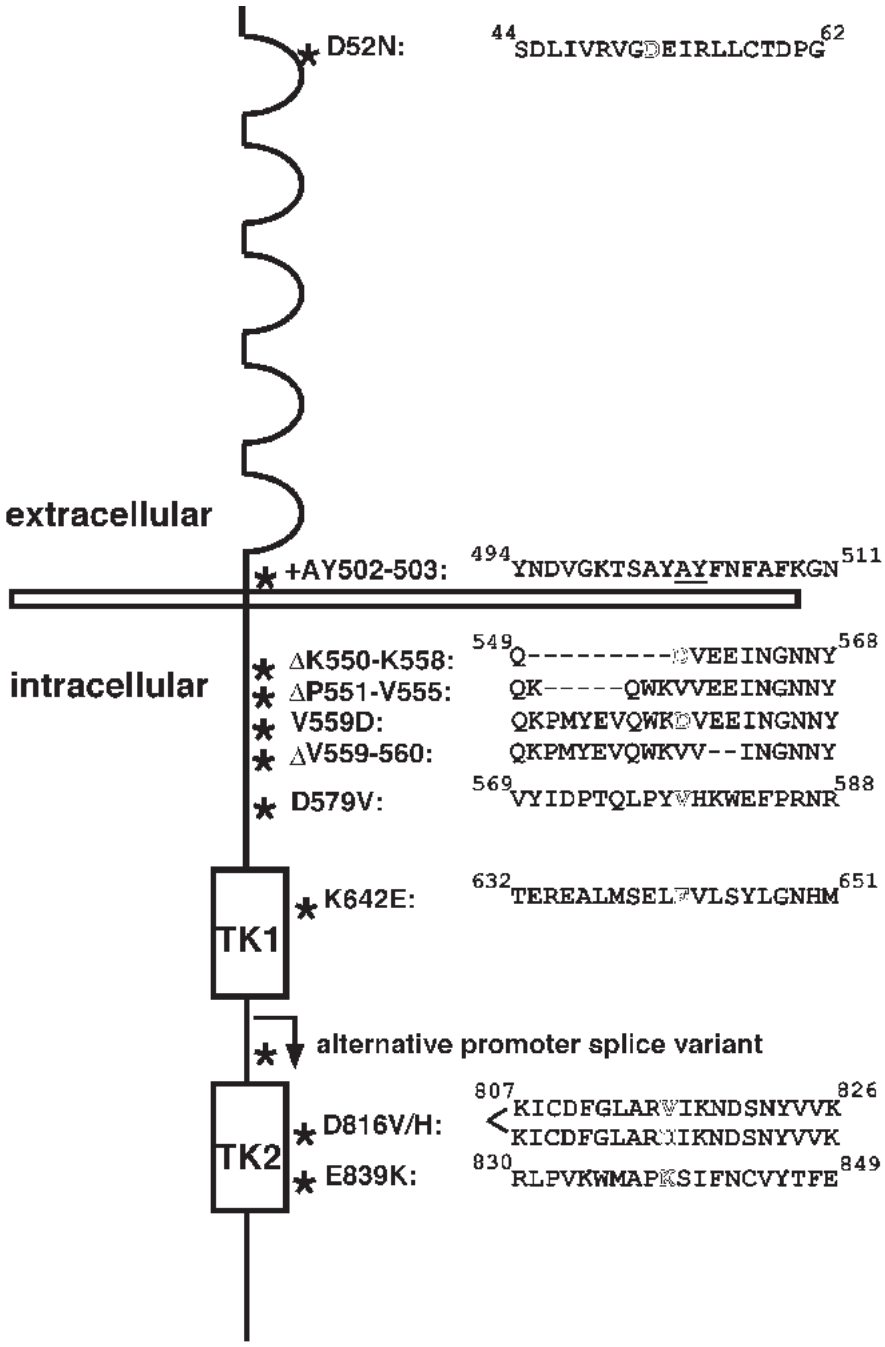
proliferation but also normal development and differentiation in mammals. Many of the *W* mutations are single base c-*kit* substitutions causing in-frame point mutations of highly conserved residues in the kinase domain of Kit/SCF-R, resulting in decreased or abolished kinase activity. Such mutations are dominant negative, which means that the phenotype in afflicted heterozygous individuals is more severe than would be expected from only one allele being mutated. A likely explanation for this is that SCF induces the formation of homo- and heterodimers of wild type (wt) and mutant (mut) Kit/SCF-R, and that only the wt/wt homodimers are fully signaling active, while the mutant receptor suppresses signaling from heterodimers. Since the ratio of wt/wt:wt/mut:mut/mut Kit/SCF-R dimers in a cell heterozygous for mutation in Kit/SCF-R is expected to be 1:2:1, only ~1/4 of all the Kit/SCF-R molecules are engaged in active signaling complexes, which is probably subthreshold levels for most signaling pathways. An increased frequency of germ cell tumors and myeloid leukemias has been reported in some of the *W* mutant mice. This might indicate a possible role for Kit/SCF-R as an anti-oncogene, and again points to the importance of proper regulation of RPTK-induced signaling to ensure normal differentiation and development. For reviews on the physiological roles of Kit (4, 5).

The proto-oncogene c-*kit* was originally identified as the cellular counterpart of the oncogene v-*kit*, which encodes the transforming protein of the HZ4-feline sarcoma virus (FeSV) derived from a feline fibrosarcoma. HZ4-FeSV originated through transduction of feline c-*kit* sequence by feline leukemia virus. The protein encoded by the v-*kit* oncogene is essentially a doubly truncated version of Kit/SCF-R, where the extracellular, transmembrane and ~50 most C-terminal amino acids have been deleted. Its identification from a feline tumor immediately implicated v-*kit* as a tumorigenic → oncogene. More recently numerous gain-of-function (GOF) mutations have been identified in Kit/SCF-R that are strongly implicated in several kinds of human malignancies, including mast cell leukemia/mastocytosis, acute and chronic myeloid leukemias, gastrointestinal stromal tumors, germ cell tumors and possibly thyroid carcinomas. The mutations identified thus far are mainly located in exon 11, which encodes part of the juxtamembrane region, and in exon 17, which encodes part of the C-terminal half of the kinase domain. However, mutations have also been identified in other regions of Kit/SCF-R including the extracellular domain. All the GOF mutations in exons 11 and 17 cause ligand-independent dimerization of Kit/SCF-R resulting in its constitutive kinase activation. Future research into signal transduction by such mutated receptors should reveal more about the mechanisms responsible for transformation. For an overview of all Kit/SCF-R mutations associated with human malignancies identified to date, see Fig. 2.

Mastocytosis/mast cell leukemia. These are relatively rare conditions, characterized by mast cell hyperplasia in bone marrow, liver, spleen, lymph nodes, gastrointestinal tract and skin. There is often clinical evidence of mast cell activation, including urtication, pruritus, abdominal pain, nausea, vomiting, diarrhea, bone pain, flushing, vascular instability and headache. Mastocytosis is classified into 4 groups:

- I indolent forms,
- II mastocytosis with associated hematological disorders, including myeloproliferative/myelodysplastic disorders,
- III aggressive mastocytosis,
- IV mast cell leukemia.

The prognosis is good for patients with indolent mastocytosis but generally poor for the other three groups, with mast cell leukemia having the most fulminant behavior. In 1993, two activating mutations in Kit/SCF-R, D816V in exon 17 and V560G in exon 11 were reported in the human mast cell line HMC 1, which was established from a patient with mast cell leukemia. Subsequent studies confirmed the presence of these mutations, in particular D816V, in Kit/SCF-R in mast cells from patients with mastocytosis. The D816V mutation has been associated with mainly sporadic, systemic mastocytosis in adults, while



Kit/Stem Cell Factor Receptor in Oncogenesis. Fig. 2 – Schematic representation of mutations in Kit/SCF-R, associated with human malignancies and dysplasias. Amino acid residues are denoted with single letters, numbers indicate the residue number in the human Kit protein sequence. Point-mutated amino acid residues are shown in outline, amino acid deletions with a dash and amino acid additions are underlined. The D52N mutation has been associated with myelodysplastic syndromes that include myelofibrosis and chronic myeloid leukemia. The AY duplication, the juxtamembrane mutations and the K642E mutations have mainly been associated with gastro-intestinal stromal tumors, but also with mast cell leukemias. The D816V and E839K mutations have been connected with mast cell leukemias. The D816H mutation has been connected with seminomas and dysgerminomas. Mutations, found in the hydrophilic region between the N- and C-terminal region of the kinase domain result -due to alternative promoter usage- in truncated versions of Kit. These isoforms have been identified in various cell lines which derived from colon carcinomas and hematopoietic malignancies.

the G839K mutation was reported to be typically associated with cutaneous, mainly pediatric cases. Mutations at analogous sites have been identified in mast cells and mast cell lines from other species, including mouse, rat and dog. Mastocytosis is one of the most common tumors in dogs, is usually malignant and as a general rule associated with Kit/SCF-R mutations. The above mutations have been identified in such cases, but the most common mutations involve exon 11 and 12, including tandem duplications and other intragenic rearrangements. The mechanisms for transformation are unknown, but it is possible that a general increase in signaling caused by the Kit/SCF-R mutations is enough to cause mast cell transformation. Accordingly, mice reconstituted with bone marrow cells expressing another constitutively active RPTK v-ErbB, develop malignant mastocytosis with associated acute myeloid leukemia (AML) that can be transplanted to secondary recipients. The V559G and the D814V mutations in murine Kit/SCF-R cause constitutive tyrosine autophosphorylation in retrovirally transduced Ba/F3 and myeloid FDC-P1 cells, and leukemia in nude mice upon injection of the transduced cells. One study showed that in murine mast cells transfected with D816V-Kit/SCF-R there was an enhanced tyrosine phosphorylation of a 130 kD protein and enhanced ubiquitin-mediated degradation of SHP-1, a cytoplasmic protein tyrosine phosphatase that negatively regulates Kit/SCF-R signalling. In conclusion, the D816V-Kit/SCF-R and to some extent exon 11 mutations, are recurrent mutations associated with, and supposedly causally involved in, aggressive forms of mastocytosis and mast cell leukemia.

Gastro-intestinal tumors. A strong association between Kit/SCF-R GOF mutations and gastro-intestinal stromal tumors (GISTs) has been established. GISTs are the most common mesenchymal tumors of which ~1 out of 10 cases is malignant. The primary tumors are located mainly in the ventricle and small intestine, and the highest incidence of new cases is in persons >40 years of age with an even sex distribution. Interestingly, >60% of GISTs are associated with exon 11 mutations in Kit/SCF-R, but none with mutations in exon 17, including of D816. Kit exon 11 GOF mutations occur mainly in malignant GISTs, which tend to be larger, with necrosis, hemorrhage, intra-abdominal spread and liver metastases and frequent recurrences. Hence, the exon 11 mutations portend poor prognosis with a 3 year survival <30% versus >65% for exon 11 mutation negative tumors, and it has been reported that Kit exon 11 mutations are an independent prognostic factor for GIST survival. The Kit/SCF-R (CD117) expression is diagnostic for GI stromal tumors versus leiomyomas and gastric Schwannomas. Most cases examined are also CD34-positive, and ultrastructurally cells look like interstitial cells of Cajal (ICC), which is why it has been proposed that the ICC is the cell of origin for most GISTs. However, tumors phenotypically identical to GISTs ($CD117^+$, most $CD34^+$) occur as primary tumors in the omentum and mesentery as well, which indicates that GISTs might not all originate from ICCs, but rather from a multi-potential precursor cell. A characteristic of GISTs is mitochondrion-rich ICCs, and some GISTs are gastro-intestinal autonomic nerve tumor (GANT)-like stromal tumors. An immunohistochemical and histological re-evaluation of archived paraffin-embedded esophageal tumor samples disclosed that ~25% of these were indeed Kit-positive GISTs with exon 11 mutations rather than leiomyomas or leiomyosarcomas, which was the original classification. This is important, since esophageal GISTs are malignant, while leiomyomas, which are Kit-negative, are benign. Analysis of eight GISTs devoid of exon 11 mutations in Kit/SCF-R revealed exon 9 mutations in 6 and an exon 13 mutation (K642E) in 2, the latter causing constitutive tyrosine autophosphorylation of Kit/SCF-R. Finally, in colon carcinoma cell lines certain intron 15 alternative promoter splice variants, causing either a 78 bp deletion or a truncated Kit/SCFR with 25 unique amino acids fused to the C-terminal 257 amino acids, have been reported sporadically. The functional consequences are unknown. However, a potential autocrine loop of Kit/SCF-R and SCF might exist in colon cancer, since colonic mucosal cells usually express SCF, but areKit/SCF-R-negative. In conclusion,

a majority of GISTs harbor GOF mutations in exon 11 of Kit/SCFR and there is overwhelming evidence that these are involved in the oncogenesis. Kit expression and exon 11 mutations are both of significant diagnostic and prognostic value for GISTs, the prognosis being significantly more severe if both are present.

Acute and chronic myeloid leukemias. A vast number of publications have attempted to address the putative role of Kit/SCF-R in myeloid leukemias. While it is still unclear whether Kit/SCF-R is causally involved in these diseases, it does have an important diagnostic and prognostic value. While only 1-3% of normal mononuclear marrow blasts express Kit/SCF-R, a big European multi-center study concluded that 67% of all acute myeloid leukemia (AML) cases, ~30% of all biphenotypic acute leukemias and all undifferentiated acute leukemias express Kit/SCF-R. Kit/SCF-R is expressed mainly in M4 and M5 AML subclasses, but the highest expression levels are found on blasts in M1 and M2 subclass AML cases. A high proportion of megakaryocytic and erythroid leukemic cells are also positive for Kit expression, as is most blasts in chronic myeloid leukemia (CML). In general, Kit/SCF-R expression is useful in the differential diagnosis between AML (mostly positive) and acute lymphocytic leukemia (ALL; all negative). Negative expression for Kit/SCF-R in AML also identifies with some certainty 2 M5b subgroups. AML blasts express between 600 to 29,000 Kit molecules/cell, but there is no correlation between expression level and prognosis. Despite conflicting reports, it seems to be the consensus from the literature that there is no correlation between Kit expression and prognosis for AML in general, but that expression of Kit/SCF-R in the M1 subclass indicates a worse prognosis. This might be due to a strong correlation between expression of a non-P-glycoprotein multidrug resistance protein and Kit/SCF-R. Recently, mutations in exon 8 of c-*kit* have been identified in a high proportion of AML cases, and all had the inv. 16 re-arrangement. Conversely, ~20% of inv. 16 AMLs had c-kit exon 8 mutations. All exon 8 mutations involved either deletion or replacement of the codon for D419. The functional consequences of these mutations for the kinase activity of Kit/SCF-R are unknown at present. However, retroviral transduction of murine hematopoietic precursors with D816V-Kit and transplantation of these cells into syngeneic hosts resulted in myeloid leukemias in a significant proportion of cases, showing that GOF mutations in Kit is sufficient for leukemic progression in mice. It has been suggested that constitutive association of → Bcr-Abl with activated Kit/SCF-R is responsible for the basophilia and myeloid growth in the chronic phase of CML. However, the ability of SCF to stimulate blast growth has also been utilized to mobilize peripheral $CD34^+/CD38^-$ and other committed progenitors in patients about to undergo bone marrow transplantation for subsequent autologous transplantation. Addition of SCF together with G-CSF and with standard chemotherapy is superior to G-CSF and chemotherapy for this purpose.

Germ cell tumors. All carcinoma-*in-situ* testis, ~90% of seminomas and dysgerminomas, but only ~5% of non-seminomas express Kit/SCF-R. Isochrome 12p is a germ cell tumor marker and loss of heterozygosity on the long arm of chromosome 12 implicates SCF as a tumor suppressor. Furthermore, intersex gonads (45X/46XY and other cases with additional Y chromosome material) have delayed Kit expression and increased testicular cancer risk. This could indicate an anti-oncogenic role of Kit/SCF-R and its ligand for germ cell tumor development under some circumstances. However, other results might indicate that SCF and Kit/SCF-R can drive tumor progression. Hence, ectopic expression of Kit and SCF has been found in cervical and ovarian carcinomas and ovarian teratomas. Furthermore, there is an association between mediastinal germ cell tumors (MGCT) and hematological malignancies (e.g. acute leukemia and malignant histiocytosis), and often Kit-positive areas are found in these mediastinal tumors. This and other results have made it clear that Kit/SCF-R expression is a diagnostic aid for extragonadal seminomas. All classical seminomas are Kit-positive, aneuploid and positive for placental alkaline phosphatase, while 40% of spermatocytic seminomas are Kit-positive, and all are diploid or polyploid and negative for pla-

cental alkaline phosphatase. This indicates that some spermatocytic seminomas might originate from primordial germ cells. In line with this, experimental testicular teratomas can be generated by transplanting E12 male genital ridges to testes of adult mice. Importantly, it was recently found that tumors of seminoma/dysgerminoma type had a D816H mutation in Kit/SCF-R causing its constitutive activation. In conclusion, Kit/SCF-R expression is of diagnostic help for seminomas/dysgerminomas, and GOF mutations in Kit/SCF-R might be oncogenic and involved in the generation of such tumors.

Malignant melanoma. Normal melanocytes depend on bFGF, HGF and SCF *in vitro*. Melanoma cells become independent of these growth factors, in part through autocrine bFGF stimulation. Interestingly, Kit mRNA and protein are down-regulated in human and murine melanoma cell lines. This correlates with *in vivo* findings: While Kit/SCF-R is expressed in normal melanocytes, benign and dysplastic naevi and nontumorigenic melanomas, expression is lost in dysplastic naevi, tumorigenic primary melanomas and metastases. In addition, transfection of Kit/SCF-R into highly metastatic melanoma cell lines, induced slowed growth rate and fewer lung metastases in nude mice. The transcription factor AP-2 controls expression of c-*kit* and the gene for MCAM/MUC18 positively and negatively, respectively. AP-2 is down-regulated in melanomas and this is thought to be the reason for loss of Kit expression, allowing the malignant cells to escape SCF-induced apoptosis. Conversely, enforced AP-2 expression suppresses tumorigenicity and metastatic potential, possibly through c-*kit* transactivation and subsequent SCF-induced apoptosis. It has been proposed that AP-2 loss is a crucial event in malignant melanoma development.

Other neoplastic/malignant lesions. Kit/SCF-R and its ligand have been found co-expressed in cells from small cell lung cancer and → neuroblastoma, and it was reported that it might be involved in malignant progression in these cases. In neurofibromatosis (NF-1) there is infiltration with Kit-positive mast cells in the neurofibroma tissue, which is composed mainly of Schwann cells with an increased SCF mRNA expression compared to normal skin. The mast cells produce collagen VIII, which might contribute to the fibrosis in this disease. There is an abnormally high expression level of Kit in NF-1-derived Schwann cell lines and decreased neurofibromin expression (Ras-GTPase). The proliferation is Kit-dependent. In myelodysplastic lesions, Kit mutations might be involved in the pathogenesis. A recurrent D52N-Kit/SCFR mutation has been reported in these cases. Finally, down-regulation of Kit/SCF-R has been reported in breast cancer and in thyroid carcinomas, despite expression by normal mammary duct epithelial cells and thyroid cells. It has been proposed that the Kit/SCF-R downregulation enables de-differentiation of the cells in these tumor types.

Gene transfer, immunotherapy, vaccination. SCF might be useful in expansion of peripheral blood leukocytes and of hematopoietic progenitors in culture before retroviral transduction or re-introduction in conjuction with autologous bone marrow transplantation. Phase II and III trials are currently being conducted on advanced stages of breast cancer and certain leukemias for this purpose. SCF might also be useful in conjuction with immuno-therapy. For instance, following high-dose cyclophosphamide and IL-3, dendritic cells can be mobilized and expanded *ex vivo* from $CD34^+$ cells in the presence of GM-CSF, TNFα, Flt3 ligand and SCF. Dendritic cells are competent antigen-presenting cells for $CD8^+$ cytotoxic T cells, so they can be used to stimulate the host immune defence against undesirable antigens, including tumor antigens. Ongoing phase II trials are examining the use of such expanded dendritic cells for immunotherapy or vaccination. Finally, relatively specific inhibitors of the Kit/SCF-R tyrosine kinase activity are currently being tested in phase I trials for use in AML. One of these, STi571, was originally identified as an inhibitor of Bcr-Abl, but inhibits Kit/SCF-R as well.Basic research into the role of normal and mutant Kit/SCF-R in oncogenesis will hopefully open up for avenues into rational therapy.

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Klatskin Tumor

Definition

Klatskin tumor is a historical term used to describe perihilar → cholangiocarcinomas, bile duct tumors arising from or near the confluence of the large left and right hepatic ducts as they form the common hepatic duct.

Knockout Mice

Definition

Knockout mice are mice that lack a particular gene function because the gene has been 'knocked out' by mutual exchange (homologous recombination) with an artificial construct that has a defective copy of the gene. Such recombination is manipulated to occur in embryonic stem cells, which are subsequently incorporated in developing embryos (at blastocyst stage). Mice that carry the knocked out gene in their germ cells are mated to produce homozygously affected 'knockout' pups.

Kringle Domain

Definition

Kringle domain is a triple-disulfide-loop structure spanning approximately 80 amino acids and playing a role in protein-protein interactions. This domain was first found in bovine prothrombin; → macrophage-stimulating protein.

L

Laminectomy

Definition

Laminectomy is the surgical removal of the posterior arch of a vertebra.

Large Cell Medulloblastoma

Definition

Large cell medulloblastoma is a recently acknowledged variant of medulloblastoma, accounting for approximately 4% of cases. It is characterized by more abundant cytoplasm than seen in classic medulloblastoma and large areas of necrosis.

Large Dense Core Vesicles

Definition

Large dense core vesicles (LDCVs) are electron dense granules of neurons and neuroendocrine cells with a diameter ranging from 80 to 400 nm. The dense matrix contains granins, neuropeptides and hormones, the membrane cytochrome b_{561} and dopamine-β hydroxylase.

Laryngeal Carcinoma, genetic changes

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Definition

The vast majority of malignant neoplasms of the larynx arise from the surface epithelium and are therefore classified as keratinizing or non-keratinizing squamous cell carcinomas (SCC). The other rare malignant forms include verrucous carcinoma, adenocarcinoma, fibrosarcoma and chondrosarcoma. Histopathologically, laryngeal SCC can further be classified into: well differentiated (more than 75% keratinization), moderately differentiated (25 – 75% keratinization), poorly differentiated (<25% keratinization).

Characteristics

Laryngeal carcinoma accounts for a small fraction (less than 2%) of all human malignancies, but the incidence varies among different countries. It is most common between the ages of 45 and 75 years. Men are four or five times more frequently affected than women. The etiology is unknown, but exposure of the mucosa to a wide variety of ingested and inhaled exogenous carcinogenic agents, such as tobacco smoke and alcohol, greatly increases the risk of developing these tumors.

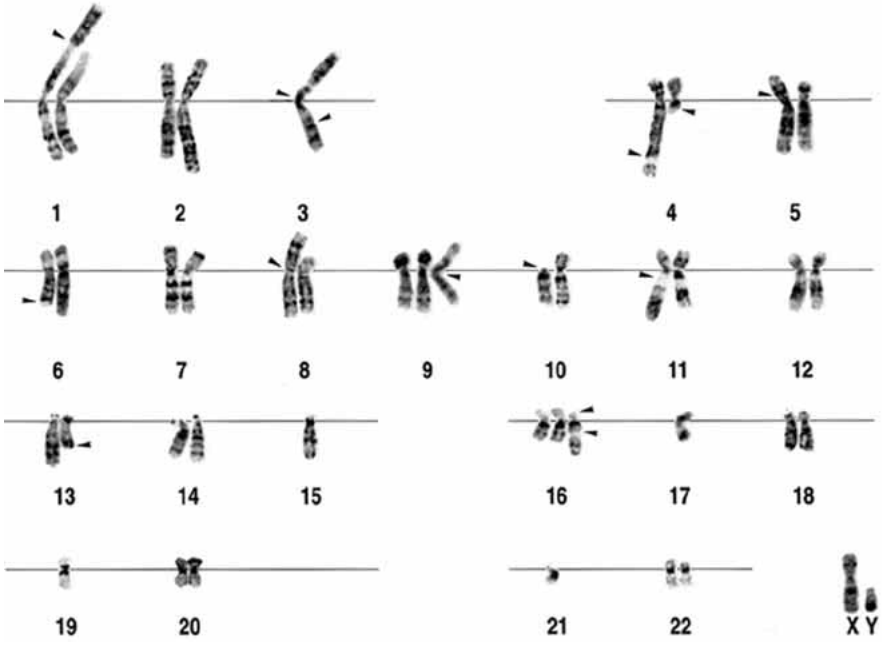
Laryngeal carcinoma infiltrates locally in the mucosa and beneath the mucosa and could me-

tastasize via the lymphatic system and the bloodstream. According to their anatomical localization, laryngeal carcinomas could be subdivided into supraglottic carcinomas, confined to the supraglottic space and spreading interiorly into the preepiglottic space, glottic carcinomas, rarely spreading into the supraglottic area but rather into the subglottic space, and subglottic carcinomas, often showing an infiltrative growth pattern unrestricted by tissue barriers.

Carcinoma of the supra- and subglottic larynx are more likely to be non-keratinizing and poorly differentiated and, in general, they have a more aggressive behavior and tend to metastasize early (20 – 40% of the cases). In contrast, lesions of the true vocal cords are more often moderately to well differentiated, rarely metastasize and tend to be associated with a better prognosis.

Genetic changes in laryngeal carcinomas

Chromosome abnormalities. Less than 100 laryngeal carcinomas with clonal chromosome abnormalities have been reported. In general, the → karyotype is complex with a non-random pattern of deleted and amplified chromosome segments. This is in line with the notion that laryngeal carcinoma, like most other malignancies, develops through the accumulation of multiple genetic changes. The chromosomes most frequently involved in structural rearrangements are chromosomes 1 to 5, 7, 8, 11, 12 and 15, with breakpoints clustering to the pericentromeric regions, i.e., the centromeric bands p10 and q10 and the juxtacentromeric bands p11 and q11, accounting for 43% of the total breakpoints. The most common imbalances brought about by numerical and unbalanced structural rearrangements are loss of chromosomal region 3p21-pter, part of or the entire chromosome arms 4p, 6q, 8p, 10p, 13p, 14p, 15p, and 17p, and gain of chromosomal regions 3q21-ter, 7q31-pter, and 8q. A total of 17 recurrent structural aberrations, mostly in the form of whole-arm translocations, → isochromosomes (i), and deletions (del), have been identified. The most common among them were i(8q), i(3q), i(5p), del(3)(p11), and homogeneously staining regions (hsr), a cytogenetically detectable sign of gene amplification, in band 11q13 (Fig. 1).

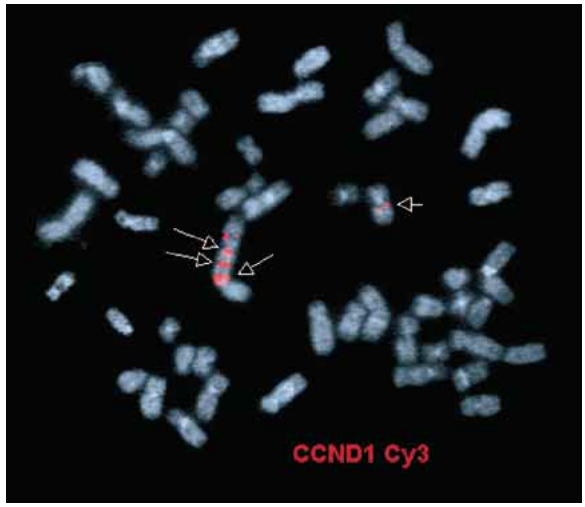


Laryngeal Carcinoma, genetic changes. Fig. 1 – Representative karyogram of a laryngeal SCC. Arrowheads indicate breakpoints in clonal aberrations.

A subgroup of laryngeal SCC has multiple, unrelated abnormal clones, with simple, often balanced structural rearrangements or numerical changes. These clones have always had near-diploid chromosome numbers. The finding of such cytogenetic polyclonality could be interpreted as evidence of 'field cancerization' but it can not be ruled out that the cytogenetically unrelated clones are united by a submicroscopic, pathogenetic mutation; the cytogenetic differences would then only reflect differences in clonal evolution. The third alternative is that some of the near-diploid clones actually represent preneoplastic lesions or genetically damaged, non-neoplastic epithelial or stromal cells in the tumor surrounding.

Fluorescence in situ hybridization (FISH). FISH analysis has been undertaken to verify and in detail characterize the most common recurrent chromosomal changes in head and neck SCC, including laryngeal SCC. FISH has demonstrated that cytogenetically detectable hsr in these tumors almost always corresponds to amplification of DNA sequences originating from 11q13, that → *CCND1* is always included in the amplicom (Fig. 2), and that the amplification of 11q13 is often concomitant with deletion of distal 11q. The latter finding indicates that not only the amplification of one or more dominantly acting oncogenes in 11q13, but also loss of a tumor suppressor gene in the distal part of 11q are critical for the development of laryngeal SCC. Detailed FISH characterization of pericentromeric rearrangements, in particular for chromosomes 1 and 8, with the use of YAC clones spanning the pericentromeric region of chromosomes, suggest that the essential outcome of these rearrangements at DNA level is the resulting genomic imbalances, i.e., loss or gain of neoplasia-associated genes, and not rearrangement of genes in the euchromatin near the centromere. Furthermore, more precise mapping of breakpoints on chromosomal arms 1p and 8p has delineated critical regions, for deletions within 1p11-p13 and the subtelomeric region of 8p.

Molecular genetic findings. Loss of heterozygosity (→ LOH) studies have pointed out the frequent loss of alleles from 3p, 8p, 9p, 13q and 17p in laryngeal SCC. A number of recent studies



Laryngeal Carcinoma, genetic changes. Fig. 2 – FISH using a CCND1 cosmid. Short arrows indicate normal signal, and long arrow indicate amplified signal.

based on allelotyping or comparative genomic hybridization (CGH) indicate that head and neck SCC, including laryngeal SCC, display massive and widespread genomic imbalances and certain chromosome segments are lost more often than others. Apart from LOH from 3p, 9p, 13q, and 17p in more than 50% of the cases, deletions in 3q, 4p, 4q, 6p, 6q, 8p, 8q, 11q, 14q, 17q, 19q, and 20p have been found in 30 – 50% of the cases. Some candidate tumor suppressor genes in frequently deleted regions, e.g., → *CDKN2A* in 9p, → *RB1* in 13q, and → *TP53* in 17p, have been investigated with regard to homozygous deletions or inactivating mutations. Except for *TP53*, which has been reported to be structurally rearranged in about 40% of the cases, evidence of functional inactivation of these particular genes has been detected in only 10 – 20% of the tumors. The most frequently amplified DNA sequences are located in chromosomal band 11q13; reported frequencies vary between 15% and 60%, with an average of 30% in primary head and neck SCC, including laryngeal SCC. FISH and molecular studies have implicated *CCND1* as the prime target in this amplification process. Several attempts have been made to correlate cytogenetic or molecular genetic data with clinical outcome in laryngeal carcinoma patients, and it has been shown that 11q13

rearrangements and amplification/overexpression of *CCND1* seem to be associated with a poor prognosis.

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Laser

Definition

Light amplification by the stimulated emission of radiation (laser) is a coherent light source used to deliver light energy of a single wavelength and high intensity; → CALI.

Laser Diagnostics

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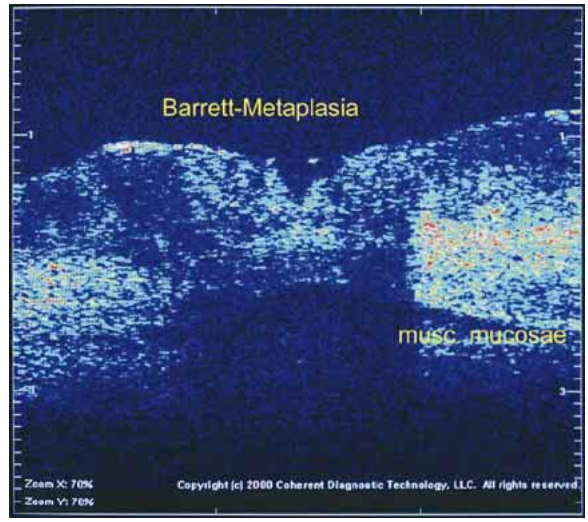
Definition

Laser diagnostics are procedures designed to detect or to differentiate neoplastic tissues based upon various forms of interaction of laser-emitted photons with tissues.



Laser Diagnostics. Fig. – Optical coherence tomography of esophageal mucosa registered *ex vivo* with a clinical OCT-prototype (Coherence Diagnostic Technologies / Carl Zeiss). An island of Barrett esophagus epithelium is surrounded by normal squamous cell epithelium. The actual depth limitation enables imaging of the mucosal layer only (dimensions are not calibrated; the vertical scale represents about 3 – 4 mm, the lateral scale 12 mm).

Description

The interaction of light with tissue may result in absorption or scattering of the photons. These phenomena may be directly observed for diagnostic purpose in a transillumination approach. Actual research in → optical mammography focuses on time-resolved systems able to separate scattering from absorption. The thermal expansion of a tissue area absorbing more light that the surrounding tissue may be used for an acoustic recognition in so-called photoacoustic diagnostic systems (still at an early experimental phase).

Furthermore, fluorescence may result from absorption. Natural fluorophores are rare, thus, fluorescence spectroscopy may sensitively detect the presence of an exogenous or a specific endogenous fluorophore accumulating in malignant or premalignant lesions (→ fluorescence diagnostics).

The phenomenon of backward scattering of incident photons is exploited for imaging of superficial tissues by optical coherence tomography (OCT). The backscatter intensity of

any point in the tissue is determined by interferometry using very short coherence light sources, one of which are short pulsed lasers. Scanning vertical and lateral dimensions, tissue may be imaged at almost microscopic resolution. The vertical resolution of OCT-systems depends on the medium coherence length of the light source and is actually limited at 4-10 µm, which is at least 10-fold better than the limits of highfrequency ultrasonography. However, the strong absorption and random scattering of tissues limits OCT to superficial tissue layers with a maximum depth of about 2 mm. In oncology, OCT may thus be suitable for the recognition of early neoplastic lesions of the aerodigestive tract, the urinary tract and eventually of the skin.

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Latency, viral

Definition

Latency, as related to viruses, is viral persistence without a productive cycle. In the Epstein-Barr virus (→ EBV) field, for instance, three types of latency (I, II, III) have been defined according to the different sets of EBV genes expressed. They can be described as followed.

Latency I is characterized by the expression of the EBV protein EBNA-1 and the EBERs, as is the case in Burkitt lymphoma [→ malignant lymphoma, hallmarks and concepts].

Latency II is characterized by the expression of EBNA-1, LMP-1 (in some cases LMP-2A/B) and EBERs, as is the case for NPC (nasopharyngeal carcinoma).

Latency III is characterized by the full set of the so-called 'latency genes'. This is the case of most EBV-immortalized cell lines in culture, as well as the EBV-associated B lymphomas of immuno-depressed patients.

Latent Viral Infection

Definition

Latent viral infection is where the virus persists in the infected host throughout life in a non-replicative state. Upon reactivation the virus replicates productively and may cause recurrent infections; → latency, viral.

Lats in Cell-cycle Regulation and Tumorigenesis

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Definition

The *lats* (*large tumor suppressor*) family of → tumor suppressor genes were discovered as a result of work in the *Drosophila melanogaster* fruit fly model system. The *lats* genes encode Ser/Thr kinases. Flies mosaic for mutations in the *lats* gene (also known as warts) produce large tumorous outgrowths. The fly gene was used as a probe to identify mammalian homologs, and it was discovered that mice and humans each have two homologs of the fly gene (*LATS1* and *LATS2*). When either the human *LATS1* or the *LATS2* cDNA is expressed in the fly, they are both capable of rescuing the developmental defects and tumor phenotypes caused by lats mutations. The Lats1 gene has been knocked out in mice, and the knockout

mice develop soft tissue sarcomas and ovarian stromal cell tumors. Biochemical experiments with the human *LATS1* gene and genetic experiments in the fly indicate that one of the ways lats impacts the cell cycle is by negatively regulating the Cdc2/Cyclin A complex.

Characteristics

Drosophila lats

lats was discovered in a → mosaic screen. Because many mutations in cancer related genes often cause embryonic lethality before the animals can be scored for overproliferation phenotypes, many important cancer related genes would be missed by such a screen. Rather than requiring an animal homozygous for a lethal mutation to be examined, mosaic screens make use of animals which carry isolated clones which are homozygous for the mutation of interest. Since most of the animal carries a wild type copy of the gene, the lethality problem is circumvented. A conventional genetic screen requires the establishment of mutant lines and the dissection of dead larvae or pupae to reveal overproliferated tissues. Mosaic screens have the advantage of being much quicker than conventional screens because the phenotype of the mutant clones can be examined in the first generation after the mutations are induced.

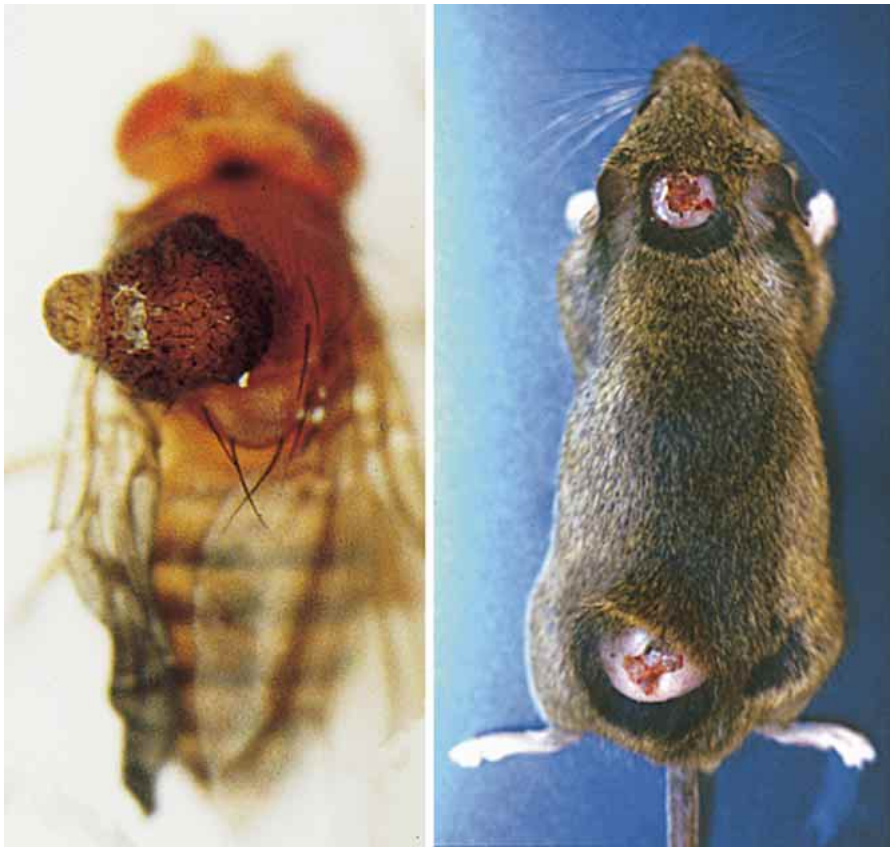
lats was identified on the basis of the overproliferation phenotype, observed in → mosaic animals (Fig. 1A). Somatic cells mutant for lats overproliferate dramatically, to form large tumors in a wide variety of tissues in mosaic adults. The most dramatic tumors produced by lats clones can be as large as 1/5 of the body size. Sectioning of lats tumors in mosaic flies revealed unpatterned overproliferated tissues with many lobes and folds and the size and shape of the mutant cells are heterogeneous and irregular. Clones, containing the lats mutation differed in their behavior when compared to clones that were mutant for other *Drosophila* tumor supressors such as *l(1)discs large-1(dlg)*. Cells of the tumor suppressor mutants proliferate slower than wild type cells; mutant clones for these genes induced during the first instar larval stage are competed away during growth and do not form detectable clones in the adults. lats mutant clones induced at similar developmental stages form dramatically overproliferated tissues in adults. This suggests that *lats* mutant cells proliferate faster than wild type cells. Cells in overproliferated mutant clones on the body are capable of differentiation and produce bristles and hairs, although the morphologies of these structures are not entirely wild type. Careful examination of numerous marked mutant clones verifies that only *lats* mutant cells are overproliferating and that wild type cells are unaffected. Thus, the *lats* overproliferation phenotype is not caused by prevention of differentiation and the phenotype is cell autonomous.

The *lats* mutations were genetically mapped to the 100A1-5 → polytene chromosome region and the lats locus was further defined by a single complementation group of over fifty alleles. Homozygous mutants for the various *lats* alleles display developmental defects ranging from lethality (embryonic, larval, or pupal) in stronger alleles to a wide variety of phenotypes in weaker alleles including held out wings with broadened blades, rough eyes with ventral outgrowths, and outgrowths on the dorsal-anterior region of the head. Some pupal lethal alleles cause giant animal phenotypes with dramatically overproliferated neural and epithelial tissues.

The *lats* transcriptional unit resides in a complicated genomic region where it is located within the intron of another gene and is flanked by transcripts from two other genes. Rescue experiments were required to definitively determine that *lats* is responsible for the observed phenotypes. Expression of the lats cDNA under the control of the heat shock-inducible promoter rescues the lethality of *lats* alleles and suppresses tumor formation in lats mosaic flies. Sequence analysis revealed that *lats* encodes a putative Ser/Thr kinase with a large N-terminal domain.

Mammalian Lats

Mouse and human homologs of the *Drosophila* *lats* gene were identified by screening cDNA li-



Lats in Cell-cycle Regulation and Tumorigenesis. Fig. 1 – Lats has a tumor suppressor function in flies and mice. **A** The pictured adult fly contains a clone of lats mutant cells, which have overproliferated to form a large tumor outgrowth. **B** The homozygous *Lats1* knockout mouse also developed a tumor in the form of a soft tissue sarcoma.

braries. The mouse *Lats* and human *LATS* genes are nearly identical. The kinase domains of the fly and mammalian genes are highly conserved. The N-terminal of the mammalian *Lats* genes is more diverged from the fly gene, but they share scattered homologous motifs. Despite any sequence differences, expression of human *LATS1* or *LATS2* cDNA under the control of the heat shock-inducible promoter completely suppresses tumor formation in lats mosaic flies, and rescues all of the developmental defects in homozygous lats mutants including embryonic lethality. This demonstrates that mammalian *Lats* genes are authentic homologs of the *Drosophila lats* tumor suppressor. The functional conservation between both molecules provides the opportunity to use the strengths of each experimental organism to explore the biochemical and genetic properties of the Lats protein.

Several methods were used to investigate the function of *Lats* in mammals, To learn more about mouse *Lats1*, *Lats1* knockout mice (*Lats1*$^{-/-}$) were generated. These *Lats1*$^{-/-}$ mice exhibit several developmental and hormonal defects including a lack of mammary gland development, infertility, and growth retardation, but more importantly, these mice develop soft tissue sarcomas (Fig. 1B) and ovarian stromal cell tumors and are highly susceptible to carcinogenic treatments.

Although mapping data for mouse *Lats1* has not yet been published, *Lats2* has been mapped to mouse chromosome 14 by interspecific backcross analysis. The human *Lats* homologs have also been mapped:

- *LATS1* (h-warts) was mapped to human chromosome 6q24-25.1 by PCR mapping using somatic cell hybrid analysis.
- *LATS2* was mapped to human chromosome 13q11-q12 by fluorescence *in situ* hybridization (→ FISH).

Study of human LATS1 focused on biochemical analysis. Imunoprecipitation of human LATS1 protein from Hela cells reveals that LATS1 is phosphorylated in a cell cycle-dependent manner. The LATS1 protein exists in a phosphorylated form during late prophase and remains phosphorylated during metaphase. Dephosphorylated LATS1 becomes detectable during entrance into anaphase, and by the beginning of telophase most of the LATS1 protein is dephosphorylated. LATS1 remains dephosphorylated during G1, S, G2, and G0 phases. Further immunoprecipitation experiments were used to investigate if LATS1 is directly involved in the regulation of the cell cycle. Cdc2 coimmunoprecipitates with LATS1 in mitotic cells. The amount of Cdc2 co-precipitated was most abundant during early mitosis, but the amount progressively decreases in subsequent stages of the cell cycle and is absent in quiescent cells. The variation in co-precipitated Cdc2 levels is not due to fluctuations in overall Cdc2 levels during the cell cycle as the total Cdc2 levels remain at a near constant level in cycling cells. This suggests that LATS1 preferentially associates with Cdc2 during early mitosis, which is a period when LATS1 is in its phosphorylated form. Experiments utilizing the baculovirus expression system revealed that Cdc2 and LATS1 proteins could be co-immunoprecipitated using either anti-human LATS1 or anti-Cdc2 antibodies suggesting that the *in vivo* LATS1/Cdc2 complex may result from direct binding of the two proteins.

The → yeast two-hybrid assay was used to examine the binding between LATS1 and Cdc2 and the results corroborated the co-immunoprecipitation data. Full length LATS1 and the N-terminal region of LATS1 interacted with Cdc2 in the assay. The C-terminal kinase domain, however, did not interact with Cdc2 indicating that LATS1 associates with Cdc2 through its N-terminal domain. Neither full length LATS1 nor the N-terminal region of LATS1 revealed any interaction with the two G1 cell cycle kinases, CDK2 and CDK4, indicating that the association between LATS1 and Cdc2 is specific. The specific association of LATS1 and Cdc2 suggests that LATS1 may act as a tumor suppressor through negative regulation of Cdc2 activity. Histone H1 kinase assays using immunoprecipitated LATS1/Cdc2 and Cdc2/Cyclin B complexes show that in contrast to the Cdc2/Cyclin B complex, the LATS1/Cdc2 complex exhibits no detectable kinase activity for histone H1. Neither Cyclin A nor Cyclin B proteins could be detected in the LATS1/Cdc2 immunocomplex when tested using anti-Cyclin A and B antibodies.

An integration of the lats data

The human biochemical data was further supported by genetic interactions between lats, cdc2, and cyclin A, in *Drosophila*. Reducing the dose of cdc2 resulted in suppression of the lats late pupal lethal alleles leading to a rescue of the lethality and other morphological phenotypes. Reduction of cdc2 activity is also able to suppress the overproliferation phenotypes of moderate lats alleles. In agreement with the results of the yeast two-hybrid assay for human LATS1 and CDC2, reduction of Cdc2c, the *Drosophila* homolog of CDK2, does not modify the lats mutant phenotypes. cyclin A and cyclin B were tested in a similar manner, and while cyclin B failed to show a genetic interaction with lats, cyclin A was able to suppress the lats mutant phenotype in a manner similar to cdc2. The specific genetic interactions between lats, cdc2, and cyclin A support the biochemical data suggesting that LATS regulates cell proliferation by negatively regulating Cdc2/Cyclin A activity.

The role of LATS as a negative regulator of Cdc2/Cyclin A is consistent with the Drosophila lats phenotype as a tumor suppressor. Ectopic activation of Cdc2/Cyclin A in G1 arrested cells by overexpression of Cyclin A can drive the G1/S transition in cells that lack Cyclin E and this G1/S activity is greatly enhanced when both Cyclin A and an activated form of Cdc2 are overexpressed. Evidence from two other *Drosophila*

mutants, roughex (rux) and fizzy-related (fzr), support the notion that increased Cdc2/Cyclin A activity in *Drosophila* can result in overproliferation. rux mutants cause cells to accumulate Cyclin A in early G1 and the cells progress into S phase precociously. Loss of fzr, a CDC20-related gene, results in the accumulation of mitotic cyclins in G1 cells and drives progression through an extra division cycle in the embryonic epidermis. Consistent with this data, Cyclin A has been shown to accumulate abnormally in lats mutant cells, and lats mutant phenotypes can be suppressed by Cdc2 and cyclin A mutations.

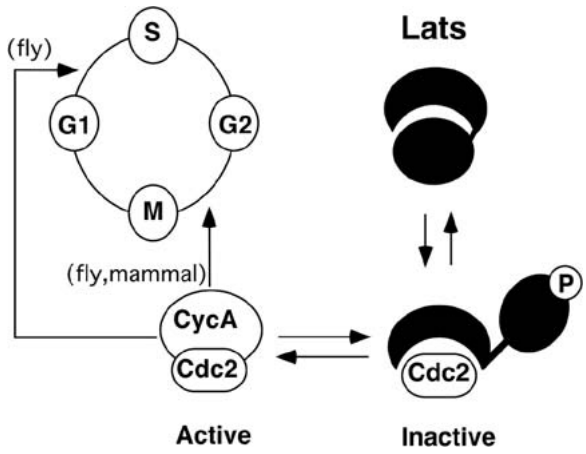
The lats phenotype is unique in several respects. lats mutants deregulate Cdc2/Cyclin A activity which impacts both the G1/S and G2/M checkpoints. This deregulation of Cdc2/Cyc A by *lats* mutants appears to be specific, whereas other mutations such as *rux*, *fizzy* (*fzy*), and *fzr* accumulate multiple mitotic cyclins which affects the activities of several Cdc2/Cyclin complexes. LATS affects a CDK/Cyclin complex that participates at two different cell cycle stages as opposed to other mutations that either activate a CDK/Cyclin complex during a single cell cycle stage or throughout the entire cell cycle. The combination of these properties provides an explanation for the severity of the overproliferation phenotype in *Drosophila lats* mutants.

Although inactivation of *lats* causes tumors in both *Drosophila* and mice, the correlation between genotype and phenotype varies. Every $lats^{-/-}$ cell overproliferates in mosaic flies while only restricted tissues in $Lats^{-/-}$ mice develop tumors. There is an important difference between the cell cycle in mammals and in *Drosophila*. In mammals, the Cdc2/Cyclin A complex is involved in the regulation of G2/M, but in *Drosophila*, Cdc2/Cyclin A functions at G1/S in addition to its function at G2/M. In mammals, Cdc2 is not responsible for G1/S regulation and this function is instead provided by a different CDK, CDK2, which complexes with cyclin A for its G1/S activity. It is likely that such complexities and redundancies of the mammalian genome are responsible for the phenotypic differences between mice and flies.

An additional function for the LATS C-terminal kinase domain has been suggested by yeast two-hybrid experiments which have shown that the N-terminal region of LATS1 interacts much more strongly with Cdc2 than full length LATS1 does. This indicates that the C-terminal kinase domain of LATS1 has a negative effect on the binding between the LATS1 N-terminal region and Cdc2. One possible explanation for this phenomenon is that the LATS1 C-terminal kinase region exerts this effect by binding intramolecularly to its own N-terminal region (Fig. 2). This explanation is supported by the positive interaction between the LATS1 C-terminal kinase domain and the N-terminal region of LATS1 in the two-hybrid assay. Since the phosphorylation of LATS1 seems to be prerequisite for binding to Cdc2, a potential model for LATS1 function can be proposed. Phosphorylation of LATS1 may change its conformation and disrupt the intramolecular association between the N-terminus and C-terminus of LATS1, freeing the N-terminal domain of LATS1 for Cdc2 binding. If this model is correct, it will be necessary to identify any protein kinases and phosphatases that modulate the phosphorylation state of LATS1 during the cell cycle to gain a full understanding of the regulation of LATS1 activity.

In both flies and mammals, Cyclin A and Cyclin B are degraded over different time courses during mitosis, although the precise mechanism of such differential inactivation of Cdc2/Cyclins is unknown. Our biochemical and genetic data indicates that Lats specifically modulates Cdc2/Cyclin A activity, but not Cdc2/Cyclin B activity (cyclin A but not cyclin B interacts with lats genetically, and lats mutant cells abnormally accumulate Cyclin A but not Cyclin B) and Lats may therefore be an important player in the differential inactivation of Cdc2/Cyclin complexes during mitosis.

Yeast two-hybrid assays demonstrate that Lats specifically interacts with Cdc2 but not other CDKs and this finding is supported by genetic data in Drosophila which shows that lats interacts with cdc2 but not the fly CDK2 homolog, cdc2c. Given that p16- and p21-like CDK inhibitors have not been found for Cdc2, it is possible that Cdc2 and the rest of the CDKs are negatively regulated by different families of proteins. Alternatively, the activity of each



Lats in Cell-cycle Regulation and Tumorigenesis. Fig. 2 – A model for Lats function. Phosphorylation of Lats at early mitosis might change its conformation to free the Lats N-terminal region from the Lats C-terminal kinase domain. This may then allow the Lats N-terminal region to bind to Cdc2, leading to the inactivation of the mitotic kinase activity of Cdc2. Cdc2/Cyclin A is involved in the regulation of G2/M in both flies and mammals, but the Cdc2/Cyclin A complex is only involved in the regulation of G1/S in the fly.

CDK could be modulated by both types of negative regulators.

Overexpression of Cdc2 and Cyclin A has been reported in multiple types of human tumors and negative regulators of CDK/Cyclins (e.g. → p16) have been shown to be tumor suppressors in mammals. The finding that Lats negatively regulates the Cdc2/Cyclin A complex, and the fact that Lats1 behaves as a tumor suppressor in the mouse, suggests that inactivation of LATS1 will be involved in tumor development in humans as well. *Drosophila* genetics is currently being used to identify genes in the lats pathway. Characterization of these lats interacting genes will not only provide mechanistic insight into how lats functions, but it will also provide candidate genes which may be involved in human tumorigenesis.

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LCL

Definition

→ Lymphoblastoid cell lines.

LDCVs

Definition

→ Large dense core vesicles.

Lectins

Definition

Lectins are a family of carbohydrate-binding proteins found in animals and plants. Different types of lectins recognize specific carbohydrate ligands.

Leimyosarcoma

Definition

Leimyosarcoma is a malignant tumor consisting of smooth muscle cells and small cell sarcoma tumor; leimyomata [→ uterine leiomyoma, clinical oncology].

Lentiginosis

Definition

Lentiginosis is brown to black pigment spots on lips, around the mouth and in the buccal mucosa and less frequently around nose and eyes, and on the fingers and feet.

Lentiginosis Polyposa Peutz

Definition

→ Peutz-Jeghers-syndrome.

Leucine Zipper

Definition

A leucine zipper is an amphipathic α-helical motif in which leucine residues are present every seven amino acids, forming a hydophobic stretch on the α-helix surface. Dimerization of two leucine-zipper containing proteins is mediated by hydrophobic interactions; → E-box.

Leukemia

Definition

Leukemia is the malignant proliferation of hemopoetic cells with abnormally high cell count in the hemopoietic tissues, other organs and usually in the blood. It is classified by the dominant cell type and by the duration from onset to death. In acute leukemia this occurs within a few months in most cases. The duration of chronic leukemia exceeds one year, with a gradual onset of symptoms of anemia or enlargement of the spleen, liver or lymph nodes; → hematological malignancies.

LFAs

Definition

Lymphocyte functional antigens (LFA) are cell adhesion molecules originally found on the surface of lymphocytes and responsible for their specific homing properties.

LFS

Definition

→ Li-Fraumeni syndrome.

LHRH Agonists

Definition

LHRH agonists are analogues of gonadotropin releasing hormone; (LHRH= Luteinizing Hormone Releasing Hormone, also called: gonadothrophin-releasing hormone (GnRH)). They act as potent inhibitors of gonadotropin secretion, when given continuously, and ultimately results in near castrate testosterone levels; → prostate cancer.

Library

Definition

A library is a set of cloned fragments representing the entire genome.

LIFE

Definition

→ Light-induced fluorescence endoscopy.

Li-Fraumeni Syndrome

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Definition

Li-Fraumeni syndrome (LFS) is an inherited neoplastic disease with autosomal dominant trait. It is characterised by multiple primary neoplasms in children and young adults, with a predominance of soft tissue sarcomas, osteosarcomas, breast cancer and an increased incidence of brain tumours, leukemia and adrenocortical carcinoma.

Characteristics

Diagnostic criteria

The clinical criteria used to identify an affected individual in a Li-Fraumeni family are:

- Occurrence of sarcoma before the age of 45.
- At least one first degree relative with any tumour before age 45.
- A second (or first) degree relative with cancer before age 45 or a sarcoma at any age.

Criteria for the diagnosis of LFS variant are somewhat less stringent.

- Three separate primary cancers, the first tumour diagnosed under the age of 45.
- or the combination of (i) childhood cancer or LFS-associated neoplasm under the age of 45, and (ii) first or second degree relative with LFS-associated tumour at any age and (iii) first or second degree relative with any cancer diagnosed under age 60.

Genetics

In approximately 70% of Li-Fraumeni cases, affected family members carry a germline mutation of one allele of the → *TP53* tumour suppressor gene. Conversely, approximately 50% of families with *TP53* germline mutations meet the criteria of the Li-Fraumeni syndrome. However, the extent of the overlap may be greater, as in some families with *TP53* germline mutations only one tumour was analysed or data were available for only one generation. Some of the families classified as Li-Fraumeni or Li-Fraumeni variant that lack a *TP53* germline mutation have recently been reported to carry a heterozygous *hCHK2* germline mutation. This gene codes for a protein involved in G2 checkpoint control, which prevents cells with damaged DNA from entering mitosis.

TP53 germline mutations

The current understanding of the molecular basis of LFS is largely restricted to *TP53* germline mutations. From 1990 to 1998, a total of 143 families with a *TP53* germline mutation were reported. For an update, see the IARC database http://www.iarc.fr/p53/germ.htm.

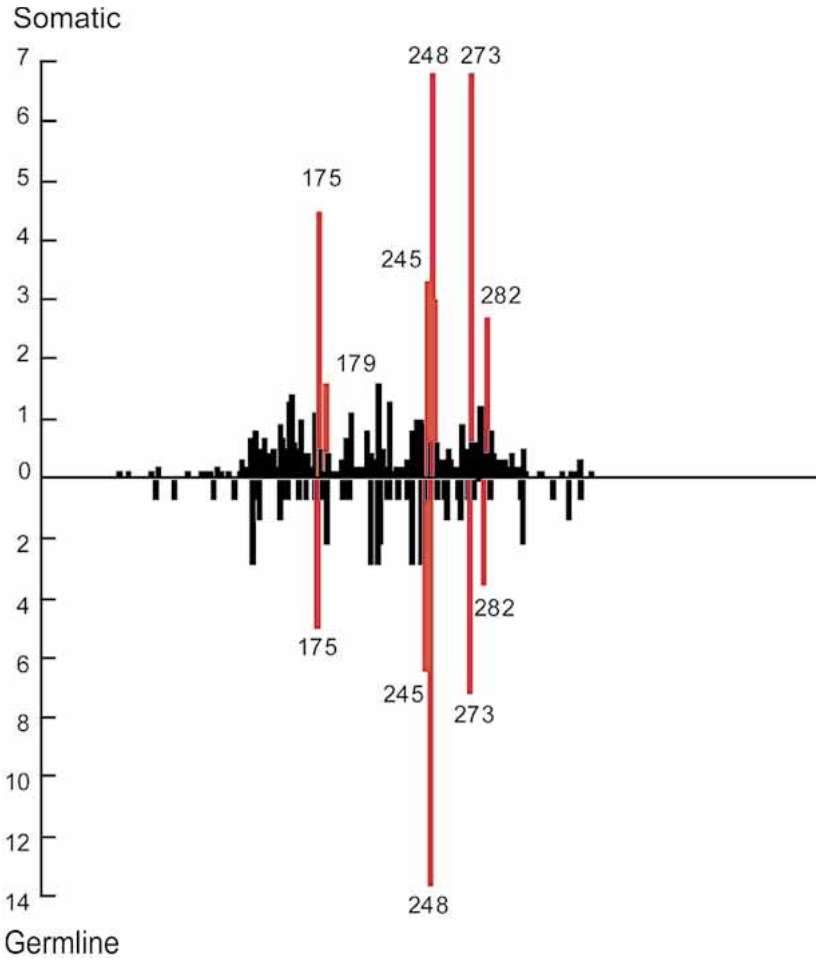
The *TP53* gene on chromosome 17p13 has 11 exons that span 20 kb. Exon 1 is non-coding and exons 5 to 8 are remarkably conserved among vertebrates. The *TP53* gene encodes a 2.8 kb transcript encoding a 393 amino acid protein, which is widely expressed at low levels. This protein is a multi-functional transcription factor involved in the control of cell-cycle progression, of DNA integrity and of the survival of cells exposed to DNA-damaging agents as well as several non-genotoxic stimuli. TP53 mutant proteins differ from each other in the extent to which they have lost suppressor function and in their capacity to inhibit wild type TP53 in a dominant-negative manner. In addition, some TP53 mutants appear to exert an oncogenic activity of their own, but the molecular basis of this gain-of-function phenotype is still unclear. The functional characteristics of each mutant TP53 protein may depend, at least in part, on the degree of structural perturbation that the mutation imposes on the protein.

Type and origin of *TP53* mutations

In the 143 families reported, point mutations (85%) were most frequent, followed by deletions (9%), splice mutations (3.5%) and insertions (2%). Among the point mutations, G:C→A:T transitions at CpG sites prevail. A similar pattern is observed in sporadic brain tumours, colon carcinomas and malignant lymphomas. G:C→A:T transitions at CpG sites are considered to be endogenous, i.e. formed as a result of deamination of 5-methylcytosine, a reaction that occurs spontaneously but which is usually corrected by DNA repair mechanisms. The occurrence of such mutations may be increased by factors that enhance the rate of methylation or the rate of deamination of 5-methylcytosine, as well as by defects in mismatch repair. Compared to somatic mutations, *TP53* transversions are relatively rare in the germline. Overall, the spectrum of *TP53* germline mutations is consistent with a formation through endogenous mutagenesis, rather than with causation by exposure to exogenous mutagenic carcinogens.

Distribution of germline mutations

As with somatic *TP53* mutations,*TP53* germline mutations are located in highly conserved re-



Li-Fraumeni Syndrome. Fig. 1 – Similar to sporadic cancers, TP53 germline mutations preferentially occur in hotspot regions. Germline mutations prevail in codons 245 and 248. From: H. Ohgaki et al., In: Pathology and Genetics of Tumours of the Nervous System, P. Kleihues and W.K. Cavenee eds., pp. 231-234, IARC Press: Lyon 2000).

gions of exons 5 to 8, with major hotspots at codons 175, 248, and 273 (Fig. 1). However, for germline mutations there seems to be a certain preference for codons 245 and 248. Although most mutations at codons 248 and 273 may convey a relatively mild phenotype, the substitution of arginine for histidine at codon 175 has been shown to have a strong dominant-negative phenotype in several experimental systems. Three mutations, Cys176, His179 and Arg249, which are commonly mutated in tumours with somatic *TP53* mutations, were not found in tumours associated with *TP53* germline mutations.

Target organs

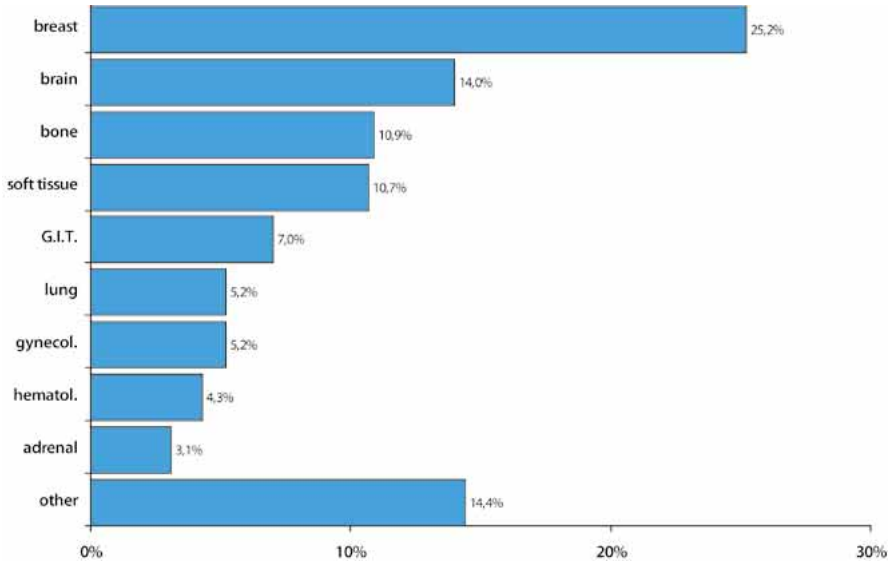
Although inherited *TP53* mutations are present in all somatic cells of affected family members, neoplastic transformation occurs with a remarkable organ and cell type specificity. Breast carcinomas are most frequently observed, followed by brain tumours, bone sarcomas and soft tissue sarcomas (Fig. 2). Leukemia and adrenocortical carcinomas are not frequent (<5%), but if present add considerable weight to the diagnosis of LFS.

Sex distribution of patients with *TP53* germline mutations

Assuming an autosomal dominant mode of inheritance, it would be expected that males and females would be equally affected. A statistically significant deviation from an assumed male/female ratio of 1:1 was found for brain tumours (2.3:1) and bone sarcomas (2.1:1). These ratios are similar to those of the respective sporadic tumours, which show a ratio of 1.6:1 for brain tumours and 1.5:1 for sarcomas. Thus, this gender bias does not reflect genomic imprinting but is likely to be caused by inherent susceptibility factors that similarly determine gender preference in the evolution of the respective sporadic neoplasms.

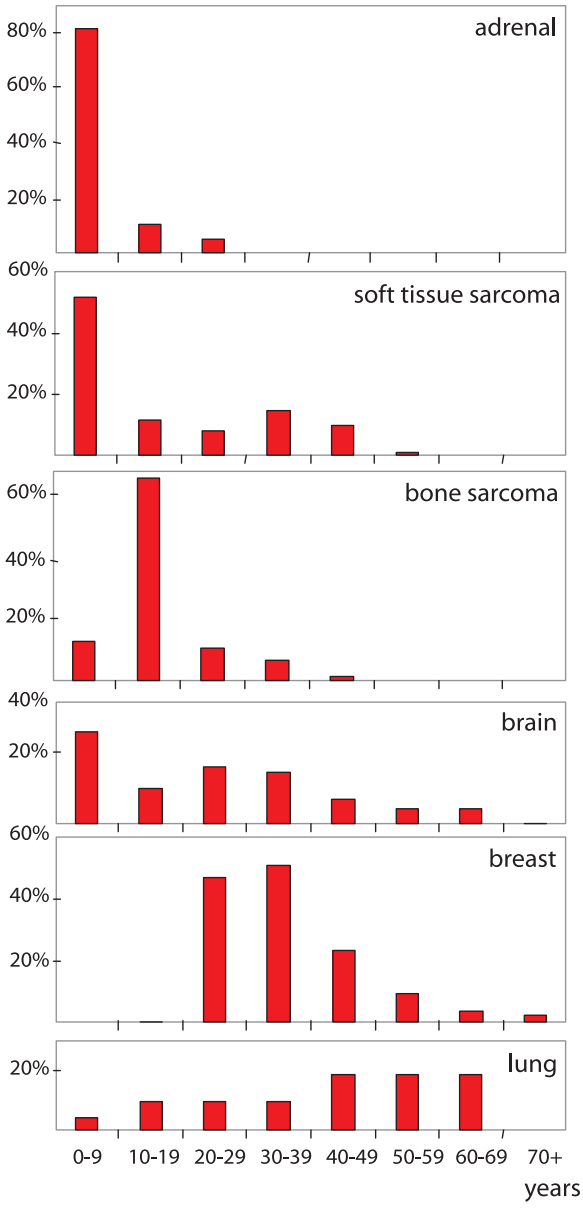
Age distribution in relation to tumour type

In addition to a general tendency for an occurrence earlier in life, there are remarkable organ- and cell-specific differences regarding the age at which tumours associated with *TP53* germline mutations clinically manifest. For each type of neoplasms there appears to exists a distinct time span during which neoplas-



Li-Fraumeni Syndrome. Fig. 2 – Target organs for malignant transformation in patients with Li-Fraumeni syndrome. Breast cancer, soft tissue and bone sarcomas, and brain tumours are most frequent. Reproduced from Ref. 6.

tic transformation is most likely to occur (Fig. 3). For example, adrenocortical carcinomas associated with a *TP53* germline mutation develop almost exclusively in children (mean age, 5.5 years). This is in contrast to sporadic adrenocortical carcinomas, which have a broad age distribution with a peak beyond age 40. This age difference is so consistent that any child with adrenocortical carcinoma should be considered as potentially carrying a *TP53* germline mutation.



Li-Fraumeni Syndrome. Fig. 3 – Age distribution of Li-Fraumeni patients according to tumour type. Reproduced from Ref. 6.

Familial tumour clustering

Most tumour types occur between 1.25 and 1.6 times per family carrying a *TP53* germline mutation. Among the families with at least one case of breast cancer, the mean number of breast carcinomas per family was 2.4, whereas among the families with at least one case of brain tumour, the mean number of CNS tumours per family was 1.6. However, there are families with a remarkable familial clustering of breast cancer, sarcomas or brain tumours. There is currently little evidence for the hypothesis that certain mutations carry an organ- or cell-specific component, except for the observation that of a total of four cerebral choroid plexus tumours reported in LFS families, three had mutations in the same codon 248. The familial clustering of certain tumour types may be influenced by the genetic background of the affected families, e.g., polymorphisms in transformation-associated genes other than *TP53*, or by gene-environmental interactions, e.g. exposure of families to similar environmental carcinogens or life-style factors.

Role of environmental and non-genetic host factors

TP53 mutations are an early and frequent event in sporadic carcinomas of the oesophagus, but there are only two reported cases of oesophageal carcinomas, among a total of 599 tumours associated with *TP53* germline mutations. This suggests that additional factors play a role, e.g. excessive alcohol consumption and smoking or chronic reflux oesophagitis. This is similarly true for lung carcinomas in which *TP53* mutations, often G:C→T:A transversions, constitute an early event in carcinogenesis but which are infrequent in LFS families. In the stomach, *TP53* mutations also occur in an early stage of carcinogenesis but may by themselves not be sufficient to initiate carcinogenesis in the absence of → *H. pylori* infection and/or a high-salt diet. This view is supported by the observation

that familial clustering of stomach cancer associated with a *TP53* germline mutation has only been observed in Japan, i.e. a country that still has a high gastric cancer incidence.

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Ligand

Definition

A ligand is a protein or chemical that binds to a specific receptor and stimulates its enzymatic activity and/or function.

Light Receptor

Definition

Light receptors are → G-protein-coupled receptors in the retina that are activated by photons. The light receptors, rhodopsin and opsin, respond to light of a specific range of wavelengths and convey this signal through G-proteins (transducins) to a cGMP-phosphodiesterase. Light activation results in a decrease of intracellular cGMP levels.

Light-induced Fluorescence Endoscopy

Definition

Light-induced fluorescence endoscopy is a bronchoscopy system for neoplasia detection based upon differential autofluorescence properties of early and invasive lung cancer; → laser diagnostics.

LINE Element

Definition

Long interpersed nuclear elements (Line), or LI elements, are a class of moderately repetitive sequences found in the human genome. Approximately 100,000 copies are interspersed in the human genome. Full length LI are about 6 kilobases, but most copies are truncated at the 5′ end or otherwise rearranged. Intact Line ele-

ments, of which there are an estimated 30-60 different ones in the human genome, have a promoter and two open reading frames that provide the capacity for movement by a process called retrotransposition [→ retroposon]. LI insertions have been found to be the cause of de novo mutations in a number of human genes, including genes involved in cancer.

Linkage

Definition

Linkage describes the tendency of genes to be inherited together as a result of their location on the same chromosome. It is measured by percent recombination between loci.

Linkage Disequilibrium

Definition

Linkage disequilibrium describes a situation in which some combinations of genetic markers occur more or less frequently in the population than would be expected from their distance apart; this implies that a group of markers has been inherited coordinately. It can result from reduced recombination in the region or from a founder effect in which there has been insufficient time to reach equilibrium since one of the markers was introduced into the population.

Linkage Group

Definition

A linkage group includes all loci that can be connected (directly or indirectly) by linkage relationships; equivalent to a chromosome.

Liver Cancer, molecular biology

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Definition

Primary liver cancer includes four major histological tumor types. The most frequent form is hepatocellular carcinoma (HCC), a malignant epithelial neoplasm that develops from hepatocytes, the basal liver parenchymal cells. Hepatoblastoma is a rare embryonic liver tumor arising from immature hepatocytes, mainly in children under two years of age. Cholangiocarcinoma (CC) develops from the epithelium of intrahepatic biliary ducts, and angiosarcoma is a rare malignant mesenchymal (vascular) tumor. This chapter presents only the molecular biology of HCC.

Characteristics

Epidemiology

Liver cancer is one of the most common human cancers worldwide, with an estimate of 315, 000 new cases diagnosed each year. It ranks fifth in frequency in the world in terms of relative cancer incidence rates, but it shows heterogeneous geographical distribution, with the highest rates in Asia and Africa. HCC develops more frequently in males than in females, sex ratios ranging between 1.5 and 3 in most countries. It occurs predominantly in the second half of life, with increasing incidence between the ages of 40 to 80. Generally, HCC arises in the context of extensive liver lesions, including liver cirrhosis in 80% of cases, or chronic hepatitis.

Etiology

HCC is one of the rare human neoplasms seroepidemiologically related to viral infections. More than 70% of HCC cases worldwide are associated with chronic infection with → hepatitis

B virus (HBV) or → hepatitis C virus (HCV). Other major risk factors include alcoholic cirrhosis, dietary intake of aflatoxin B1, a fungal metabolite that contaminates crops in some tropical areas, and inherited metabolic disorders such as tyrosinemia, hemochromatosis, and α1-antitrypsine deficiency. Increased risk of HCC development is associated with combined infections with HBV and HCV, with aflatoxin intake in chronic HBV carriers, and with alcohol abuse in HCV infected patients.

Role of viral factors

Chronic HBV infection plays a complex role in liver carcinogenesis, involving both direct and indirect mechanisms.

Direct mutagenic role

HBV DNA frequently integrates into host cell chromosomes. HBV integrations seem to occur randomly over the entire human genome, with no preferential site. Viral DNA integration is frequently associated to gross genetic alterations such as chromosomal translocations, deletions or amplifications of large chromosomal regions. Therefore, integration of HBV DNA may promote genomic destabilization, in turn favoring the accumulation of genetic mutations at early steps of HCC development. In occasional cases, viral integration has been shown to induce insertional mutagenesis of cellular genes. In four independent HCC cases, viral DNA integration sites have been identified within cellular genes that play important roles in the control of cellular growth, differentiation or viability: *retinoic receptor-beta*, *cyclin A2*, *mevalonate kinase* and *SercA1* genes. These tumors produce viro-cellular chimeric proteins endowed with transforming capacities. The recent development of PCR-based technologies for rapid isolation of viral integration sites will probably allow a better evaluation of the exact prevalence of such oncogenic insertional events in a near future. Another argument favoring a direct tumorigenic role of HBV comes from studies of animal models for virally-induced liver cancer. Woodchucks chronically infected with the woodchuck hepatitis virus (WHV), an hepadnavirus closely related to HBV, develop frequent and early onset HCC, while uninfected animals rarely show any neoplasm. WHV DNA is found integrated in the vicinity of an oncogene of the *MYC* family (either → *MYC* or → *MYCN*) in more than 80% of woodchuck liver tumors. → *MYC* genes act as important regulators of cell growth, death and differentiation, and their abnormal expression has been implicated in the genesis of multiple human neoplasms. Integration of WHV DNA induces aberrant overexpression of the targeted *MYC* oncogene, which in turn initiates the malignant process. The strong oncogenic impact of these mutagenic insertions has been demonstrated in transgenic mice recapitulating the multistep process of liver carcinogenesis.

Oncogenic potential of the HBX transactivator

The HBV regulatory protein X (HBX) has pleiotropic functions and it can interfere with multiple cellular pathways controlling cell cycle, proliferation, DNA repair and apoptosis. In transgenic mouse strains, liver expression of HBX can either induce liver cancer or act as a co-factor and a tumor promoter in liver carcinogenesis.

Immunopathogenesis

There is a general consensus that hepatocellular damage in human hepatitis B is caused by the host immune response and not by the virus itself. Evidence for an indirect role of HBV in cancer formation has been provided by transgenic mouse models. Sustained viral replication and expression of most viral genes in the liver can be achieved in HBV transgenic mice with no pathological consequence. However, sustained expression of the large envelope protein of HBV in the mouse liver induces a process of necrosis and regeneration that ultimately leads to malignant transformation. Moreover, chronic hepatitis induced by the cellular immune responses against the HBV surface antigen (HBsAg) has been associated with frequent emergence of liver tumors. Thus, a potentially important factor in tumorigenesis is the accelerated turnover of infected hepatocytes, which results from continuous cell death triggered by the host immune response ad subsequent cell

proliferation in the context of inflammatory disease. Hepatocyte DNA lesions during persistent HBV infection may result from chronic cellular dysfunction characterized by exposure to mutagenic products secreted by inflammatory cells, impaired detoxification pathways or DNA repair mechanisms. Moreover, the endogenous production of mutagens such as oxygen free radicals and nitrosamines during chronic liver inflammation leads to the formation of pro-carcinogenic DNA lesions.

Genetic alterations in HCC

Cytogenetic analysis of hepatoma cell lines and primary liver tumors has revealed that most HCCs are aneuploid and harbor multiple different chromosomal abnormalities, with recurrent deletions of the short arm of chromosome 1. During the last decade, extensive studies have utilized two novel methodologies, the PCR-based microsatellite marker analysis (MSA) and a molecular cytogenetic method called Comparative Genomic Hybridization (CGH). These studies have provided a global evaluation of the major gross genetic changes in HCC and of their relative importance in tumors of various geographical and etiological origins. In MSA studies, the highest percentages of allelic deletions (LOH: → loss of heterozygosity) were found at chromosomes 8p23, 4q22-24, 4q35, 17p13, 16p13-15, 16q23-24, 6q27, 1p36 and 9p12-14. No obvious correlation was found between LOH at specific chromosomal regions and viral hepatitis infection, HBV DNA integration or clinico-pathological data of the patients. LOH at 1p was prevalent in small, well-differentiated HCCs. In contrast, LOH at 16p and 17p occurred more frequently in advanced, metastatic tumors. LOH at chromosomes 4q, 16q and 17p was frequently associated with HBV infection of the patients. A significant correlation was found between LOHs at chromosomes 1p, 4q and 16p, and between LOH at 4q and the presence of p53 mutations, suggesting that the corresponding putative tumor suppressor genes may collaborate in liver tumorigenesis. Notably, loss of 8p has been associated with metastatic potential, as also found in colorectal and renal carcinomas. In CGH studies, the finding of prevalent losses at chromosomes 4q, 8p, 16q, 17p, 13q and 6q is in good agreement with the results of MSA. In addition, these studies demonstrated frequent DNA copy gains at 8q, 1q, 6p and 17q. Over-representation of these chromosomal arms has also been described in other types of cancer. Amplification of the terminal region distal to 8q24 correlates with frequent overexpression of the *c-myc* oncogene in liver tumors. The putative oncogenes located on other chromosomal arms have not been identified so far.

Together, these studies suggest that non-random chromosomal gains and losses may occur in an orderly fashion in liver cancer. Importantly, the genes implicated in most genetic alterations have not been identified so far.The complexity of genetic changes likely reflects the heterogeneity of risk factors and underlying liver disease, as well as the long latency for tumor development. The relative frequency of allelic losses may vary with the associated risk factors, implying that different genetic pathways may be triggered by viral and chemical agents implicated in liver cancer.

Inactivation of Tumor Suppressor Genes

The *p53* gene [→ p53 gene family] is probably the most common molecular target involved in human carcinogenesis. The p53 tumor suppressor protein is activated in response to DNA damage, inducing either cell-cycle arrest to permit DNA repair, or apoptosis. Loss of p53 function occurs mainly through allelic deletions at chromosome 17p13 where the gene is located, and missense mutations within the DNA-binding domain. In liver cancer, LOH at chromosome 17p13 has been observed in 25 to 60% of tumors in different studies, and the worldwide prevalence of *p53* mutations can be estimated to around 28%, with however important geographical variations. It is now well established that a mutation at codon 249 of the *p53* gene is frequent in some regions of Africa (Mozambique, Senegal) and the southeast coast of Asia (Qidong, Vietnam) where chronic HBV infection is highly endemic and the aflatoxin B1 content of the diet is high. Thus, the specific 'hot spot' 249 mutation appears to be a hallmark

of dietary exposure to aflatoxin B1. In other countries, p53 mutations are rarely seen in liver cancer, and they are distributed all over the coding exons.

Allelic deletions at chromosome 13q14 have been associated with the inactivation of the → RB tumor suppressor gene. RB has been implicated in cell-cycle control, and disruption of the RB pathway renders cells insensitive to antiproliferative signals. In liver cancer, LOH at the *RB* locus has been found in 25 – 48% of cases, and RB expression is strongly down-regulated in 30 – 50% of tumors, correlating with genetic alteration of the *p53* gene. While mutations of the *RB* gene itself have not been documented so far, inactivation of the RB pathway is achieved mainly by methylation-dependent silencing of p16INK4, an inhibitor of cyclin-dependent kinases which blocks the cell cycle by dephosphorylation of RB. In addition, the over-expression of gankyrin, a new oncogene homologous to a subunit of the 26S proteasome, promotes RB degradation by the ubiquitin-proteasome pathway.

Allelic deletions at chromosome 6q26-27 are associated to the inactivation of the mannose-6-phosphate/IGF-II receptor (*M6P/IGF2R*), a gene implicated as a tumor suppressor by its ability to activate TGF-ß signaling and to promote the degradation of IGF-II, a potent growth factor for liver cells. In liver tumors harboring a 6q LOH, mutations of the *M6P/IGF2R* gene on the remaining allele have been detected in 25% of cases, leading to major amino acid substitutions or premature truncation of the protein.

Genetic Alterations in Oncogenes

β-catenin gene alterations have recently been implicated in liver oncogenesis. β-catenin is an important multifunctional protein involved in cell-cell adhesion and in transduction of differentiation signals during embryogenesis. Mutations in the β-*catenin* gene have been detected in 22% of liver tumors. All mutant forms of β-catenins harbor missense mutations or short deletions in the amino-terminal domain (so-called 'destruction box') and they are resistant to degradation. Thus, whereas β-*catenin* is expressed at the cell membrane in normal adult epithelial cells, the mutants accumulate in the cytoplasm and nucleus of tumor cells. It has been shown recently that deregulated β-catenin can induce neoplastic transformation of normal epithelial cells, demonstrating that β-catenin is a bona fide oncogene. In HCC, β-catenin mutations are less frequent in HBV-related than in HCV-related tumors. Activation of β-catenin is also achieved in liver cancer by loss-of-function mutations of Axin, a tumor suppressor protein known to bind β-catenin and promote its degradation. In contrast with colorectal cancer, the adenomatous polyposis coli (APC) tumor suppressor gene is not mutated, and activating mutations in *ras* family oncogenes occur infrequently in hepatocellular carcinoma. Curiously, β-catenin aberrations in hepatoblastoma consist mainly in interstitial deletions in about one half of cases, and no β-catenin mutation has been evidenced in cholangiocarcinoma.

Conclusions

Presently, the identification of candidate oncogenes and tumor suppressors in the most frequently altered chromosomal regions in liver cancer is a major challenge. Great insights will come from integrating the signals from different pathways operating at pretumoral and tumoral stages. This search might, in term, permit an accurate evaluation of the major targets for therapeutic treatments.

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Liver Cirrhosis

Definition

Liver cirrhosis is an irreversible chronic injury of hepatic parenchyma, including extensive fibrosis in association with formation of regenerative nodules.

Liver Flukes

Definition

Liver flukes are trematodes capable of parasitizing the human biliary tract, usually of the species *Opisthorchis viverrini* and *Clonorchis sinensis*, which are typically acquired by eating infected fish. They are endemic in some areas of Asia and chronic infestation leads to a high incidence of hepatolithiasis and → cholangiocarcinoma.

L-JAK

Definition

→ Janus kinase 3.

L-MYC

Definition

→ MYCL.

LOD Score

Definition

A LOD score is a measure of genetic linkage, defined as the $\log_{10}$ ratio of the probability that the data would have arisen if the loci are linked, to the probability that the data could have arisen from unlinked loci. The conventional threshold for declaring linkage is a LOD score of 3.0, that is a 1000:1 ratio (compared with the 50:1 probability that any random pair of loci will be unlinked).

LOH

Definition

→ Loss of heterozygosity.

LOI

Definition

The gene encoding insulin-like growth factor 2 (IGF2) has been demonstrated to be imprinted, with the paternal allele being expressed and the maternal allele being silent. Loss of imprinting (LOI) of IGF2 has been demonstrated in a number of tumor types including → Wilms tumour (WT), with increased expression of IGF2 thought to lead to tumour development.

Long Interspersed Nuclear Elements

Definition

Long interspersed nuclear elements (→ LINE element).

Loss of Heterozygosity

Definition

Loss of heterozygosity (LOH) or → allelic loss can be experimentally demonstrated in cases in which the two alleles differ. It is the loss of an allele in tumor DNA compared to matched normal DNA from the same individual. LOH is a very frequent somatic genetic

change in human tumors. When LOH is found to occur at high frequency in a particular chromosome region, it is generally considered indicative of the location of a → tumor suppressor gene whose loss/inactivation occurs by a 'two-hit' mechanism (i.e., physical loss of one copy of an allele and mutation or other genetic/epigenetic alteration of the other copy of the same allele).

Loss of Imprinting

Definition

→ Imprinting of one of the parental alleles can be lost leading to increased or reduced transcription/expression of the imprinted gene. Loss of imprinting (LOI) is often seen in genes associated with tumorigenesis or with particular genetic disorders.

Loss-of-function Mutation

Definition

A loss-of-function mutation is any mutation of a gene that causes decreased or abolished function and/or activity of its encoded protein or of a protein that is directly or indirectly regulated by the mutated gene.

LPD

Definition

→ Lymphoproliferative disease.

LTR

Definition

LTR is an abbreviation for long-terminal repeat, a sequence directly repeated at both ends of retroviral DNA [→ retrovirus]. LTR's contain strong promoters as well as transcription termination signals.

LYL Family

Definition

Lyl family is a → bHLH protein family, comprising mammalian Tal-1, Lyl-1, Nscl1, Nscl2 and Tal-2, and implicated in development of hematopoetic lineages.

Lymphoblastoid Cell Lines

Definition

Lymphoblastoid cell lines (LCL) are EBV-immortalised B cell lines established by *in vitro* infection or culture of peripheral blood lymphocytes (PBL) from EBV-infected individuals. These cells do not develop into tumors when injected into nude mice.

Lymphoid Follicles

Definition

Lymphoid follicles are aggregations of lymph tissue.

Lymphoma

Definition

Lymphoma is the general term for malignant neoplasms of lymph and reticuloendothelial tissues. Lymphomas present as apparently circumscribed, solid tumors that are composed of cells which appear primitive or resemble lymphocytes, plasma cells or histiocytes. Lymphomas appear most frequently in lymph nodes, spleen or other sites of lymphoreticular cells.

When disseminated, especially lymphomas of the lymphocytic type may invade the peripheral blood and manifest as leukemia. → Hodgkin disease and Burkitt lymphoma are special forms; → malignant lymphoma.

Lymphoproliferative Disease

Definition

Lymphoproliferative diseases are due to the outgrowth of → Epstein-Barr virus-infected B cells, which would normally be controlled by EBV-specific T cells.

Lynch Syndrome

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Synonyms

- hereditary nonpolyposis colorectal cancer (HNPCC)

Definition

Lynch syndrome is an autosomal dominantly inherited cancer susceptibility syndrome, characterized by cancers of multiple anatomic sites, of which colorectal cancer (CRC) is the most common. Mismatch repair (MMR) genes, inclusive of *hMSH2*, *hMLH1*, *hPMS1*, *hPMS2* and *hMSH6*, in their mutant form are causal for the cancer phenotype. Lynch syndrome appears to show genotypic and phenotypic heterogeneity. *hMSH2* mutations may predispose to a greater frequency of extracolonic cancers while mutations in *hMSH6* may result in a predominance of gynecologic cancer, particularly endometrial carcinoma, so that CRC may not pose the primary basis for Lynch syndrome diagnosis. Lynch syndrome is the most commonly occurring hereditary CRC disorder.

Molecular genetic findings have enabled hereditary CRC to be divided into two groups: 1) tumors that show microsatellite instability (MSI), occur more frequently in the right colon, have diploid DNA, harbor characteristic mutations such as transforming growth factor β Type II receptor and *BAX26* and behave indolently, of which the Lynch syndrome is an example; 2) tumors with chromosomal instability (CIN), which tend to be left-sided, show aneuploid DNA, harbor characteristic mutations such as *K-ras*, *APC*, *p53*), and behave aggressively, of which familial adenomatous polyposis is an example.

Characteristics

Characteristics (clinical, molecular and pathology): Affected individuals inherit a mutation in one of the MMR gene alleles. When a second mutation is acquired in the wild-type allele, the target cell is less able to repair DNA mismatch errors. Tumors composed of such cells characteristically manifest microsatellite instability and are said to have replication error phenotype (RER^+). Most of the tumors arising in the Lynch syndrome are MSI^+, while about 15% of apparently sporadic CRCs are MSI^+. This is exceedingly interesting in that those sporadic MSI^+ tumors have clinical pathologic features similar to those observed in the Lynch syndrome (1-3).

Prior to molecular genetic breakthroughs, one was required to depend upon the cardinal features of the Lynch syndrome, since there were no premonitory signs or biomarkers to guide diagnosis. These cardinal features are as follows:

- the inheritance pattern is autosomal dominant;
- gene penetrance is ~ 85 – 90%;
- gene carriers develop colorectal carcinoma at an early age (~ 45 years);
- most (~ 70%) cancers arise proximal to the splenic flexure;
- multiple CRCs, both synchronous and metachronous, are common;

- accelerated carcinogenesis is present;
- the prognosis is better than for sporadic colon cancer;
- the pathology features of CRC are often distinguishable (but not pathognomic) and include poor differentiation, increased signet cells, medullary features, peritumoral lymphocytic infiltration, Crohn's-like reaction, and an increased frequency of tumor infiltrating lymphocytes (TILs) admixed with tumor cells.

These clinical features characterize Lynch syndrome I. Lynch syndrome II is characterized by all of these same features and, in addition, shows an increased risk for malignancy at certain extracolonic sites, including the endometrium, ovary, stomach, small bowel, hepatobiliary tract, pancreas, ureter, renal pelvis and breast.

History

The history of the Lynch syndrome dates to an observation of Dr. Aldred Warthin, pathologist at the University of Michigan School of Medicine. He became deeply moved when his seamstress, in 1895, told him that she would likely die of cancer of the colon, stomach or her female organs, because of the enormous proclivity to these cancers in her family. Warthin listened intently, developed her pedigree, and along with other similar cancer prone families published this work in 1913. He updated the family in 1925. The seamstress's family has since been known as Family G. Lynch et al.(4) described the natural history and genetics of two large Midwestern kindreds (Families N and M) which subsequently were found to have features similar to Family G. Dr. A. James French, Warthin's successor as chairman of pathology at the University of Michigan, heard about Families N and M and recalled that his predecessor, Aldred Warthin, had discovered a similar family in 1895. Dr. French invited Dr. Lynch to take custody of all the detailed documents and pathology specimens which the meticulous Dr. Warthin had investigated, catalogued and published over a span of more than 30 years. Family G was then updated and published in 1971 (5). A detailed review of the history of the Lynch syndrome has been published by Lynch et al.(6).

Management of the Lynch syndrome is predicated upon the cardinal features of its natural history, discussed above. Most importantly, given the proximal predilection for CRC, colonoscopy is mandatory. In fact, evidence is already in hand that colonoscopy will significantly reduce morbidity and mortality in Lynch syndrome patients(7,8). Approximately one-third of the cancers occur in the cecum, so that colon cleanout is necessary for good visualization of the cecum. Given its early age of onset, and accelerated carcinogenesis, we recommend colonoscopy be initiated between ages 20-25 and repeated every 1-2 years. In the Lynch syndrome II variant, in addition to the colon, attention for screening is focussed on the endometrium and ovary. At age 30, transvaginal ultrasound of the endometrium and ovary is performed and endometrial aspiration is considered.

The search for a germ-line mutation should be performed only on families with substantial evidence of a hereditary cancer syndrome. Therefore, to establish a syndrome diagnosis, collecting the patient's cancer family history is mandatory and may potentially constitute the most cost-beneficial component of a patient's medical workup. Once the Lynch syndrome diagnosis is established, high-risk patients are then presented with opportunities to search for the germ-line cancer-prone mutation. Herein, genetic counseling prior to DNA collection and at the time of disclosure of DNA test results is recommended. Once a Lynch syndrome MMR germ-line mutation is identified within a family, those who are positive for the mutation are provided genetic counseling at the time of disclosure of results and are afforded an opportunity to follow highly targeted surveillance and management recommendations, while those who are negative for the mutation will revert to general population guidelines.

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Lysosome

Definition

The lysosome is a catabolic organelle in the cytoplasm of eukaryotes. It is characterised by a membrane proto-ATPase that maintains the low pH of 5 to 6. Lysosomes contain hydrolytic enzymes like proteases, nucleases, phosphatases, glucosidases and lipases. Proteins and other cellular material that is to be degraded enter the lysosome by endocytosis or autophagy. Together with the ubiquitin/proteasome system, the lysosome is the main site of intracellular protein degradation and also fulfills storage functions for ions and small molecules.

M

Macropain

Definition

→ Proteasome.

Macrophage-stimulating Protein

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Synonyms

- hepatocyte growth factor-like protein (HGFL)
- macrophage stimulating protein (MSP)
- MST1
- HGFL
- DNF15S2

Definition

MSP is a plasminogen-related growth and motility serum protein that was discovered as a macrophage chemotactic factor.

Characteristics

Structure

MSP is a heterodimeric protein (total mass 78 kD, 711 aa) composed of two chains, a 53 kD α and a 25 kD β chain that are linked together by a disulfide bond. Features of the α chain include an N-terminal domain corresponding to the plasminogen preactivating enzyme (PAP or hairpin loop), four kringle domains and a segment that terminates in the cleavage site for activation. The β chain has the serine protease-like domain that is devoid of enzymatic activity because of catalytic triad mutations. MSP is most closely related to hepatocyte growth factor/scatter factor (HGF/SF), to which it has 45% sequence similarity, and the human gene maps to 3p21.

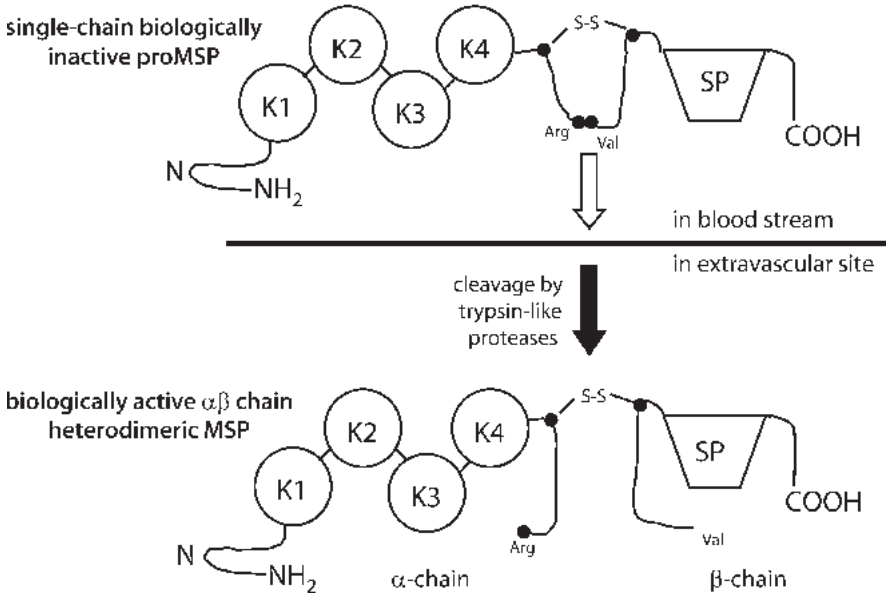
Biological activities and targets

- Murine resident peritoneal macrophages: involved in shape changes, chemotaxis and inhibition of inducible NO-synthase (iNOS).
- Epithelial cells: involved in enhanced adhesion to extracellular matrix, chemotaxis, proliferation, protection from apoptosis and increased beat frequency of ciliated epithelium.
- Hematopoetic cells: involved in proliferation, apoptosis and cytokine production.
- Osteoclast-like cells: involved in bone resorption.

Cellular and molecular regulation

MSP is synthesized by hepatocytes as a biologically inactive single chain pro-MSP and is converted at extravascular sites to active MSP by trypsin-like proteases, which cleave at Arg483/Val484 to generate a disulfide-linked α/β chain heterodimer. The enzymes involved in the conversion of pro-MSP to MSP are:

- purified coagulation system enzymes *in vitro*: factors XIa and XIIa, serum kallikrein;
- a trypsin-like proteolytic activity on the membrane of peritoneal macrophages;
- a trypsin-like protease in human wound fluid.



Macrophage-Stimulating Protein. Fig. – Structure and conversion of pro-MSP to MSP. MSP comprises an N-terminal domain (N) which includes a hairpin loop, four kringle domains (K1-K4) and a serine proteinase-like domain (SP). The interchain disulfide bridge is formed by the α chain Cys468 and the β chain Cys588 (solid circles connected by -S-S-). MSP circulates in the blood as single-chain biologically inactive pro-MSP. At extravascular sites, trypsin-like proteases convert pro-MSP to biologically active, disulfide-linked and αβ heterodimeric MSP by cleavage of the peptide bond between Arg483 and Val484 (shown as solid circles).

MSP receptor signalling

MSP mediates its biological activities by activating a cell receptor tyrosine kinase known as RON (**R**ecepteur d'**O**rigine **N**antaise) in humans and STK (**S**tem cell-derived **T**yrosine **K**inase) in mice. RON/STK is a classical transmembrane receptor belonging to the Met-receptor tyrosine kinase family. The *RON* gene maps to 3p21, in close vicinity to the *MSP* gene. MSP-induced receptor activation involves, most likely, dimerization of receptors followed by autophosphorylation occuring in trans (between two receptors) of specific tyrosines in the cytoplasmic portion of the receptors. RON/STK receptors transmit a signal from the cell surface to the inside, which causes the cell to respond to MSP in a specific manner. MSP-activated signals and effects are cell type specific. MSP mitogenic effects are mediated via focal adhesion kinase (FAK), SRC and mitogen-activated protein kinase (MAPK). Phosphatidylinositol-3-kinase (PI-3K) mediates MSP-induced adhesion and motility. The PI-3K/AKT and the MAPK pathways transduce MSP anti-apoptotic effects, whereas activation of JUN N-terminal kinase (JNK) may induce apoptosis in selected hematopoietic cells.

Clinical Relevance

Active MSP is found in wound fluid, and a high level of RON receptor expression has been detected on the surface of epithelial cells in burn wound epidermis, suggesting a possible role for MSP in wound healing. Overexpression and constitutive ligand-independent RON activation are found in some breast carcinomas, suggesting a possible RON contribution to breast cancer progression. Transfection of a RON cDNA carrying activating mutations in the cytoplasmic domain at Asp 1232 or Met 1254 converts cells to tumorigenicity, raising the possibilty that mutated RON may contribute to tumor development in humans, although a primary role for mutated RON in causing human cancer has not been identified.

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Macrophage Stimulating 1 Receptor

Definition

The macrophage stimulating 1 receptor (MST1R) belongs to the tyrosine kinase family of receptors. It is a protein of 1400 aa and 152 kD that has tyrosine-protein kinase activity. It is the receptor for → macrophage-stimulating protein (MSP) and is phosphorylated in response to ligand binding. The gene maps to 3p21.3, in close vicinity to the *MSP* gene.

MAD

Definition

MAD encodes a nuclear protein (221 amino acids and 25 kD) that belongs to the basic region helix-loop-helix [→ BHLH] family of proteins. It dimerizes with → MAX and antagonizes transcriptional activity of the → MYC protein; MAD/MAX dimers bind to DNA through the CAC GTG sequence (→ E-box). MAD competes for MAX/MYC dimerization. The gene maps to chromosomal position 2p13. Mad family are a group of → bHLH proteins comprising MAD, MAD3, MAD4, MNT and MXI1.

MAGE

Synonyms

- MAGE-like Xp
- melanoma antigen, family B, 1
- MAGEL1

Definition

MAGE stands for melanoma antigen. → HUGO nomenclature symbol is MAGEB1. The → melanoma antigen encoded by the *MAGE-1* gene (chromosome Xp21) was the first tumor antigen described in human cancer. MAGE-1 protein has 347 aa 39 kD, its funtion is unclear. Normally the MAGE-1 protein is expressed only in the testis, but due to de-→ methylation it is also expressed in melanoma and other tummors. MAGE-1 is used as a vaccine in the immunotherapy of melanoma.

Magnetic Resonance Imaging

Definition

Magnetic resonance imaging (MRI) is magnetic resonance principles coupled with data processing that is used for cross-sectional imaging of the human body.

Major Histocompatibility Antigens

Definition

Major histocompatibility antigens are polymorphic proteins between individuals of a given species or different species that can be directly recognized by recipient T lymphocytes, eventually leading to severe and rapid rejection reactions.

Major Histocompatibility Loci

Definition

Major histocompatibility loci are a class of proteins used to distinguish cells as self and to avoid immune reactions against the organism itself; → HLA.

Major Late Promoter

Definition

The major later promoter is a strong viral promoter that directs the expression of several of the viral structural proteins, including the capsid proteins hexon, penton base and fiber; → adenovirus.

Malignant Lymphoma, hallmarks and concepts

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Definition

Malignant lymphomas are defined as neoplasms of the lymphoid tissue. Myeloid neoplasms comprising chronic myeloproliferative disorders, myelodysplastic syndromes and acute myeloid leukemias are excluded, although primary pathogenetic events involve the pluripotent hemopoeitic stem cells and their early differentiation steps similar to some of the malignant lymphomas. Also excluded are primary non-lymphoid tumors (e. g. benign and malignant tumors of soft tissue components) of the lymphoid tissue, whereas histiocytic and accessory/dendritic cell tumors traditionally are listed among the neoplasms of the lymphoid system. Contrary to most organs and tissues, and in spite of the fact that lymphomas show a wide range of aggressiveness from localized, indolent to highly aggressive, rapidly metastasizing and incurable disease, no *bona fide* benign lymphomas have been defined. Distinct lymphoma entities in general show a characteristic clinical and biological behavior, but within these entities a broad continuum of malignancy grades may be observed in individual cases, either due to disease progression in a multi-step process of lymphomagenesis and/or the involvement of specific risk factors.

Characteristics

Classification

The WHO classification of tumors of hematopoietic and lymphoid tissues follows the rules of the REAL classification, and substitutes all earlier classification systems that are now considered obsolete. According to clinical rules, Hodgkin lymphomas are distinguished from non-Hodgkin lymphomas. Distinct clinical and pathological entities are recognized by a combination of morphological, immunophenotypic, genetic and clinical features required for their specific diagnosis. Within each entity, morphological and clinical variants may be recognized that are important for diagnostic and/or prognostic reasons. Specific biological features may characterize a subtype of a disease entity (e.g. mediastinal B cell lymphoma in the category of diffuse large B cell lymphoma) where morphological and clinical features overlap, but molecular data suggest a separate category.

The classification is organized according to the functional differentiation pathways of lymphoid and immune regulatory cell types and correlated to a suggested normal counterpart of lymphoma cells, and thus requires their exact immunological phenotypic characterization. The initial clinical features such as nodal or extranodal disease localization and presence or absence of leukemic dissemination are important, whereas primary genetic abnormalities or specific etio-pathogenetic factors apply for only certain entities, and within these entities for a

greater or lesser part of cases. Malignant non-Hodgkin lymphomas and their major variants are listed according to the main differentiated cell type as precursor or mature peripheral cell type tumors and their relationship to the B cell, T/NK cell or histiocytic/dendritic cell systems (Tables 1-3).

In the Hodgkin lymphomas (HL), two entities are recognized; nodular lymphocyte predominant HL and classical type HL, where the

Malignant Lymphoma, hallmarks and concepts. Table 1 – B cell neoplasms.

Precursor B cell lymphoblastic leukemia/lymphoma
(Precursor B cell acute lymphoblastic leukemia)
Peripheral B cell Neoplasms
B cell lymphocytic leukemia/small lymphocytic lymphoma
B cell prolymphocytic leukemia
Lymphoplasmacytic lymphoma
Mantle cell lymphoma
Follicular lymphoma
Cutaneous follicle center lymphoma
Marginal zone B cell lymphoma of mucosa-associated lymphoid tissue (MALT type)
Nodal marginal zone B cell lymphoma (± monocytoid B cells)
Splenic marginal zone B cell lymphoma (± villous lymphocytes)
Hairy cell leukemia
Diffuse large B cell lymphoma Variants: Centroblastic; Immunoblastic; T cell or histiocyte-rich; Anaplastic large B cell Subtypes: Mediastinal (thymic) large B cell lymphoma; Intravascular large B cell lymphoma; Primary effusion lymphoma
Burkitt lymphoma
Plasmacytoma
Plasma cell myeloma

Malignant Lymphoma, hallmarks and concepts. Table 2 – T cell neoplasms.

Precursor T cell lymphoblastic leukemia/lymphoma
(Precursor T cell acute lymphoblastic leukemia)
Peripheral T cell and NK cell neoplasms
T cell prolymphocytic leukemia
T cell large granular lymphocytic leukemia
Aggressive NK cell leukemia
NK/T cell lymphoma, nasal and nasal type
Sezary syndrome
Mycosis fungoides
Angioimmunoblastic T cell lymphoma
Peripheral T cell lymphoma (unspecified)
Adult T cell leukemia/lymphoma (HTLV 1+)
Anaplastic large cell lymphoma (T and null cell types)
Primary cutaneous CD30 positive T cell lymphoproliferative disorders Variants: Lymphomatoid Papulosis (Type A and B); Primary cutaneous ALCL; Borderline lesions
Subcutaneous panniculitis-like T cell lymphoma
Enteropathy-type T cell lymphoma
Hepatosplenic γ/δ T cell lymphoma

Malignant Lymphoma, hallmarks and concepts. Table 3 – Histiocytic and dendritic cell neoplasms.

Macrophage/histiocytic neoplasm Histiocytic sarcoma
Dendritic-cell neoplasms
Langerhans cell histiocytosis
Langerhans cell sarcoma
Interdigitating dendritic cell sarcoma/tumor
Follicular dendritic cell sarcoma/tumor
Dendritic cell sarcoma, not otherwise specified

latter has several variants (nodular sclerosis, mixed cellularity, lymphocyte-rich and lymphocyte-depleted variants of CHL) (Table 4).

Molecular genetic basis of NHL

Primary genetic alterations are known for many B cell NHL, as well as some T cell NHL (Table 5). The transforming event often involves activation of proto-oncogenes by their translocation into strongly constitutional active or cell-specific expressed cellular genes, forming fusion transcripts that retain the oncogenic or transcriptional active part of the proto-oncogenes. The functional consequence in some B cell lymphomas, characterized by a protracted and indolent course, seems to confer resistance to apoptosis (follicular lymphoma, marginal zone B cell lymphoma of MALT type). Apoptosis is considered to be the most important regulatory mechanism in the clonal evolution of B cells, which is active in selection processes establishing anti-self tolerance during early development as well as during antigen driven selection occurring in germinal centers of B cell follicles. Immunoglobulin rearrangements and heavy chain switching, as well as immunoglobulin gene related hypermutation processes during these steps of evolution, may cause a state of temporary active genomic destabilization that carries an increased risk for the formation of translocations involving the immunoglobulin genes in the long arm of chromosome 14. Hereby, activated B cells destined to undergo apoptosis are rescued to survive.

Malignant Lymphoma, hallmarks and concepts. Table 4 – Hodgkin lymphoma (Hodgkin disease).

Nodular lymphocyte predominance Hodgkin lymphoma
Classical Hodgkin lymphoma
Hodgkin lymphoma, nodular sclerosis
Classical Hodgkin lymphoma, lymphocyte-rich
Hodgkin lymphoma, mixed cellularity
Hodgkin lymphoma, lymphocyte depletion

Some of these translocations involving strongly activating genes or genes altering cell-cycle regulation are enough to achieve the fully malignant phenotype (e. g. activation of the c-myc gene in Burkitt lymphoma by the translocation t(8;14)) while other translocations (e. g. the activation of the anti-apoptotic principle of the bcl-2 gene by translocation t(14;18) in follicular lymphoma) are found also in normal hyperplastic lymphoid tissue. Less than 10% of follicular lymphomas show this translocation as the only genetic abnormality. Neoplastic transformation is achieved by (multiple) secondary genetic events and/or the specific antigen dependent activation of the altered cell clone. An acquired increase of genomic instability along the LOH or tumor suppressor pathway, leading to loss or functional inactivation of important regulatory and anti-oncogenic genes, are an important phenomenon in many lymphomas and may be responsible for the progression of indolent to highly aggressive lymphomas. Altered gene activities frequently found in diffuse large B cell lymphomas progressed from indolent low grade lymphomas involve deletion or inactivation of the TP53 gene, inactivation of p16 INK4A on chromosome 9p, amplification and activation of the c-myc as well as inactivation of putative tumor suppressor genes by deletions in the long arm of chromosome 6.

Antigen receptors in NHL

Antigen receptors provide the most important cellular signaling of lymphocyte differentiation and selection during ontogenesis and antigen related reactions, and may also be of great importance in the pathogenesis of malignant lymphomas.

Immunoglobulin receptor of B cells:

The unique clonal antigen receptors in B cells are formed by the rearranged VDJ and constant regions of heavy and light chain genes. The demonstration of a single rearranged receptor is used to prove clonality of lymphoproliferation. V-gene usage and organization of VDJ gives important clues to aspects and differentiation of HL. For extranodal B cell lymphomas, a biased

Malignant Lymphoma, hallmarks and concepts. Table 5 – Primary genetic events and their effects in malignant lymphoma.
Abbreviations: *IGH* Immunoglobulin heavy chain gene; *IGL* Immunoglobulin light chain gene; *NFKB2* Nuclear factor kappa B2; *BCL2* B-cell lymphoma/leukemia gene 2; *API2* Apoptosis inhibiting protein 2; *MALT1* Mucosa-associated lymphoid tissue gene 1; *BCL10* B-cell lymphoma/leukemia gene 10; *CDK6* Cyclin-dependent kinase 6; *IGK* Immunoglobulin kappa light chain gene; *PAX5 (BSAP)* B-cell specific activator protein; *BCL6* B-cell lymphoma/leukemia gene 6; *MYC* c-MYC oncogene; *TCRA* T-cell receptor alpha chain gene; *TCO1* T-cell lymphoma/leukemia gene 1; *NPM* Nucleophosmin gene; *ALK* Anaplastic lymphoma kinase; *TFG TRK*-fused gene; *TPM* Tropomyosin gene; *ATIC* Aminoimidazole carboxamide ribonucleotide; formyltransferase/IMP cyclohydrolase gene.

NHL entity	Chromosomal aberration	Genes involved	Function
B-CLL	t(14;19)(q32;q13)	*IGH* *BCL3*	?
	t(10;14)(q24;q32)	*IGH* *NFKB2*	Induction of proliferation
Follicular lymphoma	t(14;18)(q32;q21)	*IGH* *BCL2*	Resistance to apoptosis
Mantle cell lymphoma	t(11;14)(q13;q32)	*IGH* *Cyclin D1*	Dysregulation of cell-cycle control
Marginal zone B-cell lymphoma of MALT-type	t(11;18)(q21;q21)	*API2* *MALT1*	Resistance to apoptosis
	t(1;14)(p22;q32)	*IGH* *BCL10*	Resistance to apoptosis
Splenic marginal zone B-cell lymphoma	t(2;7)(p12;q32)	*CDK6* *IGK*	Dysregulation of cell-cycle control
Lymphoplasmacytic lymphoma	t(9;14)(p13;q32)	*IGH* *PAX5 (BSAP)*	Enhanced response to proliferation signals
Diffuse large B-cell lymphoma	t(3;14)(q27;q32)	*IGH*	Transcriptional induction of proliferation
		BCL6	Resistance to differentiation
Burkitt Lymphoma	t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11)	*IGH, IGL* *MYC*	Transcriptional induction of proliferation (G1)
Prolymphocytic leukemia of T-type	Inv(14)(q11q32) t(14;14)(q11;q32) t(X;14)(q28;q11)	*TCRA* *TCL1* *TCRA* *MTCP1*	Enhancement of cellular proliferation and survival
Anaplastic large cell lymphoma (T/0)	t(2;5)(p23:q35) t(1;2)(q25;p23) t(2;3)(p23;q21) Inv(2)(p23;q35)	*NPM-ALK* *TPM3-ALK* *TFG-ALK* *ATIC-ALK*	Constitutional activation of ALK tyrosin kinase
Hepatosplenic γ/δ T-cell lymphoma	i(7)(q10)	?	?

V-gene usage (with respect to the normal repertoire) has been detected. Furthermore, the demonstration and distribution of mutations in the complementary determining regions (CDR) and framework regions (FR) show the influence of antigen on the selection of neoplastic B cell populations. The presence of mutations in Ig-genes is an important clue to assess the prefollicular B cell or follicular and post-follicular B cell origin of lymphoma cells. Whereas chronic lymphocytic leukemia shows either non-mutated or mutated Ig-receptors, most lymphomas show either non-mutated receptors (precursor B cell lymphoblastic lymphoma and mantle cell lymphoma) or mutated receptors (follicular lymphoma, marginal zone B cell lymphoma and most aggressive B cell lymphomas including diffuse large B cell lymphoma and Burkitt lymphoma). Ongoing mutations indicating a still active process of immunoglobulin-related hypermutations was found in follicular lymphoma, some marginal zone B cell lymphoma and diffuse large B cell lymphoma. Therefore, most lymphomas originate in the antigen-driven germinal center or by an extrafollicular post-germinal center lymphocyte activation process.

The specificity of antigen receptor idiotypes of malignant non-Hodgkin lymphoma, as far as known, is related to different autoantigens including anti-immunoglobulin and anti-idiotype activities in chronic lymphocytic leukemia, plasmocytoma and marginal zone B cell lymphoma of MALT. Antigen receptor based clonal activation is considered to be important during early lymphomagenesis, whereas later stages of lymphoma progression are clearly independent of antigenic challenge.

T cell receptors in peripheral T cell lymphomas:

The specificity of α/β and γ/δ types of T cell receptors as well as the expression and functional activity of NK cell receptors on T/NK cell lymphomas is still unknown.

Etiology

Among the etiological factors for malignant lymphoma, viral and chronic bacterial infections play a major role. Insertional mutagenesis, transcriptional activation by viral genes as well as viral homologues of cellular oncogenes and growth factors may cause lymphoma transformation directly or indirectly by increasing the risk of further cellular mutagenic events.

The Epstein-Barr virus (EBV) plays a major role in the pathogenesis of Hodgkin lymphoma (HL), endemic Burkitt lymphoma and many immunodeficiency related malignant lymphomas. It is also found in a variety of peripheral T cell lymphomas, especially in angioimmunoblastic T cell lymphoma and extranodal T/NK cell lymphoma of nasal type as well as in about 5% of diffuse large B cell lymphomas in the Western hemisphere. Epstein-Barr virus in all these cases is clonally integrated in lymphoma cells, suggesting that the virus was already present in the tumor stem cell. The transformation processes appear to be various in different lymphoma types and are still not completely understood.

HTLV-I is invariably linked to adult T cell lymphoma/leukemia, which is endemic in South Japan, the Caribbean and certain regions in Africa. The transformation is related to the transcriptional activation of cellular genes by the viral transcription factor TAX in CD4-positive T-helper lymphocytes and further mutagenic events.

The human immunodeficiency virus (HIV) is associated with increased occurrence of mostly aggressive B cell lymphomas, either of Burkitt lymphoma [→ Epstein-Barr virus] subtype or immunoblastic-plasmablastic lymphomas. 50% of Burkitt lymphomas and 80% of immunoblastic and plasmablastic diffuse large B cell lymphomas in HIV-infected individuals are caused by → EBV, and is related to the status of immunodeficiency. Rare specific subtypes of NHL seen in AIDS patients are the primary effusion lymphomas that are related to HHV8 (the human Kaposi Sarcoma-associated Herpes virus) frequently with co-infection of EBV. Whether HIV plays a direct role in EBV- and HHV8-negative lymphomas, or are induced by other viruses or sporadic mutagenic events in hyperproliferative B lymphocytes, is unknown.

→ *Helicobacter pylori* (H.p.) infection is a causative factor of extranodal marginal zone B cell

lymphomas of MALT type in the stomach. Early stages of lymphoma development may be successfully treated by *H.p.* eradication. The molecular genetic analysis of *H.p.*-associated lymphomagenesis in the stomach shows that different factors are effective in the transformation processes.

- An indolent form of gastric MALT-type lymphoma does not show progression and is associated with the translocation t(11;18)(q21;q21). No transformation to high grade lymphomas and no further genetic imbalances are found in these cases.
- Low grade MALT type lymphomas show characteristic genetic imbalances on 3q;11q and 18q.
- Diffuse large B cell lymphomas show similar chromosomal imbalances but additional and more frequent changes on chromosomes 6q and 7q as well as → LOH in the loci of the → *APC* gene, → $p16^{INK4A}$ gene, the retinoblastoma gene → *RB1* and → *p53* tumor suppressor gene.
- *De novo* diffuse large B cell lymphomas show genetic imbalance losses and deletions as in the afore mentioned aggressive B cell lymphomas, but without the alterations seen in low grade tumors.

The scenario reflects the different pathways of lymphoma transformation: stable accumulative disease, a progressing genetically unstable disease or aggressive *de novo* lymphomas, which are similar to sporadic non-*H.p.*-associated lymphomas of the B cell type.

Cell biological features of Hodgkin lymphoma

All cases of nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) and 98% of classical Hodgkin lymphoma (CHL) are B lymphocyte derived monoclonal lymphoproliferative disorders of young adults. For the pathological diagnosis it is typical that scattered tumor cells (called Hodgkin cells and Reed-Sternberg cells in CHL and L&H cells in NLPHL) are found in a non-neoplastic background infiltrate of various cell types forming the lymph node tumors. A rosette-like arrangement of T lymphocytes around tumor cells is often characteristic, suggesting the functional interaction of tumor cells and T lymphocytes in the production of multiple cytokines and chemokines, which are responsible for the pleomorphic histological picture and clinical paraneoplastic features (so-called B symptoms). The tumor cells of HL correspond to germinal center-derived activated B lymphocytes, differing in their phenotype and receptor status between NLPHL and CHL (Table 6). Rare cases of HL may show a T cell phenotype and genotype.

The B cell nature of L&H-, HD- and RS cells has been elucidated by single cell microdissec-

Malignant Lymphoma, hallmarks and concepts. Table 6 – Immunophenotype of classical Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma.
+ = positive; (+) = often positive; (-) = often negative; - = negative

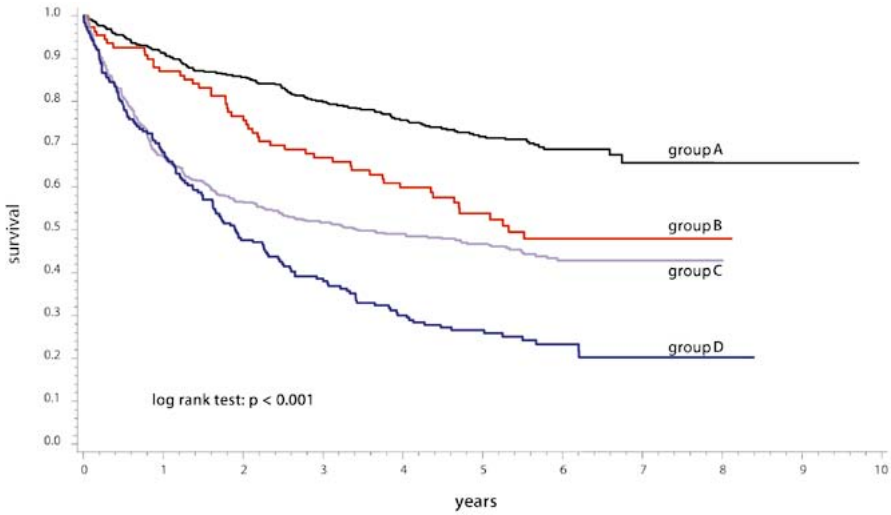
	Classical Hodgkin lymphoma	NLPHL
CD30	+	-
CD15	(+)	-
CD20	(-)	+
EMA	(-)	+
J-chain immunoglobulin production	-	(+)
EBV association	Mixed cellularity: 75 % nodular sclerosis: 25 %	-
Background composition	Mainly T cells (TIA1+)	B cells rosettes by CD57+ T cells

tion from tumor tissue. L&H cells show transcription of intact immunoglobulin receptor genes, whereas in HD and RS cells of CHL the transcription of immunoglobulin genes is blocked. In an earlier hypothesis, it was postulated that this feature is due to the 'crippling' of immunoglobulin genes due to hypermutation often leading to stop codons and deletions. However, this view has recently been challenged by the demonstration of defective co-activators for immunoglobulin gene transcription (Oct 2 and BOB 1), considered to cause this basic cellular defect.

The constitutive activation of the NF-κ pathway by different molecular events (i. e. inhibitor inactivation of IκBα-2, hyperexpression of Traf-1 signaling and unknown reasons) appears to be a central defect of cell regulation in Hodgkin lymphoma. The hyperactivity of this important transcription factor leads to resistance of apoptosis and increased transactivation of cytokine and chemokine genes and their receptors, stimulating hyperproliferation. This may result in survival of activated germinal center cell populations normally destined to die by apoptosis.

Basic clinical features of malignant lymphoma

Malignant lymphomas occur at a frequency of about 12:100,000 per year in the Western population, equivalent to about 10,000 new cases in Germany per year. Hodgkin lymphoma, with a frequency of 4:100,000 per year, has been stable over the last decades, whereas non-Hodgkin lymphoma show a continuous increase over time. This increase is still evident if absolute data are corrected for the increase of mean lifetime expectancies. The reason for the increase of non-Hodgkin lymphoma is unclear. For most NHL and NLPHL there is a slight to marked male predominance, whereas classical Hodgkin lymphoma and mediastinal B cell lymphoma occur more frequently in young females. The age range of malignant lymphoma is broad from young children until the highest age



Malignant Lymphoma, hallmarks and concepts. Fig. – Clinical groups of the International Lymphoma Classification Project according to different five year survival. *Group A:* 5 year Overall Survival > 70%: Indolent slow decline and/or few disease related deaths. (Anaplastic large cell lymphoma, marginal zone B cell lymphoma of MALT-type, follicular lymphoma).
Group B: 5 year Overall Survival 50 – 70%: Continuous slow decline of survival. (Nodal marginal zone lymphoma, lymphoplasmacytoid lymphoma, small lymphocytic lymphoma).
Group C: 5 year Overall Survival 30 – 50%: Rapid initial decline of survival plateau and few disease related deaths after 2 years. (Primary mediastinal large B cell lymphoma, diffuse large B cell lymphoma, Burkitt lymphoma, Burkitt-like lymphoma).
Group D: 5 year Overall Survival < 30%: Rapid decline and low level plateau or rapid continuous decline. (Precursor T lymphoblastic lymphoma, peripheral T cell lymphoma, mantle cell lymphoma).

groups, with characteristic age distribution for certain subtypes in young patients (Burkitt lymphoma, lymphoblastic precursor cell lymphoma, diffuse large B cell lymphoma of centroblastic type and anaplastic large cell lymphoma of T type). Other lymphomas are more frequent in the young adult population (Hodgkin lymphoma, mediastinal B cell lymphoma, anaplastic large cell lymphoma T type, extranodal diffuse large B cell lymphoma) or higher age groups (indolent lymphomas, which apparently do not exist under the age of 20, diffuse large B cell lymphoma and peripheral T cell lymphomas).

With the introduction of modern multiagent chemotherapy regimens the prognosis of HL and aggressive non-Hodgkin lymphoma was greatly improved, whereas other non-Hodgkin lymphomas still show an incurable, indolent or aggressive course (Fig.).

Modern classifications of lymphoma entities may better define and find new and more specific treatment options for the incurable diseases. A better definition of molecular events in malignant lymphoma (e. g. by gene expression profiling) may also help to define specific risk factors and prognostic features that can be used to modify present treatment protocols on a rational basis and adapt more specific treatment regimens to individual diseases.

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Malignant Tumor

Definition

A malignant tumor is a cancer that invades adjacent tissues and metastasizes to other organ sites.

MALT Lymphoma

Definition

Mucosa associated lymphatic tissue (MALT) lymphoma is a low grade B cell gastric non-Hodgkin lymphoma [→ Hodgkin disease]. In contrast to the lymph nodes or spleen it is not as anatomically distinct, but is embedded in the mucosae of various organs. Gut associated lymphatic tissue is called GALT and bronchus associated lymphatic tissue BALT.

m-AMSA

Definition

Amsacrine, or m-AMSA, is an acridine compound that inhibits → topoisomerase II.

Map Distance

Definition

Map distance is measured as cM (centiMorgans) = percent recombination (sometimes subject to adjustments).

MAP Kinase

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Definition

Mitogen activated protein (MAP) kinases are a subfamily of protein kinases, enzymes which carry out the chemical transfer of phosphate from ATP to protein acceptors. Such phosphorylation events regulate protein function by inducing changes in conformation and/or molecular recognition. MAP kinases are components of signal transduction pathways that allow extracellular signals such as hormones and growth factors to modulate cellular responses as varied as growth, differentiation, motility and → apoptosis. MAP kinases are ubiquitous and members of the family have been identified and characterized in many eukaryotic organisms including mammals, frogs, fruit flies, nematodes, slime mold, yeast and plants.

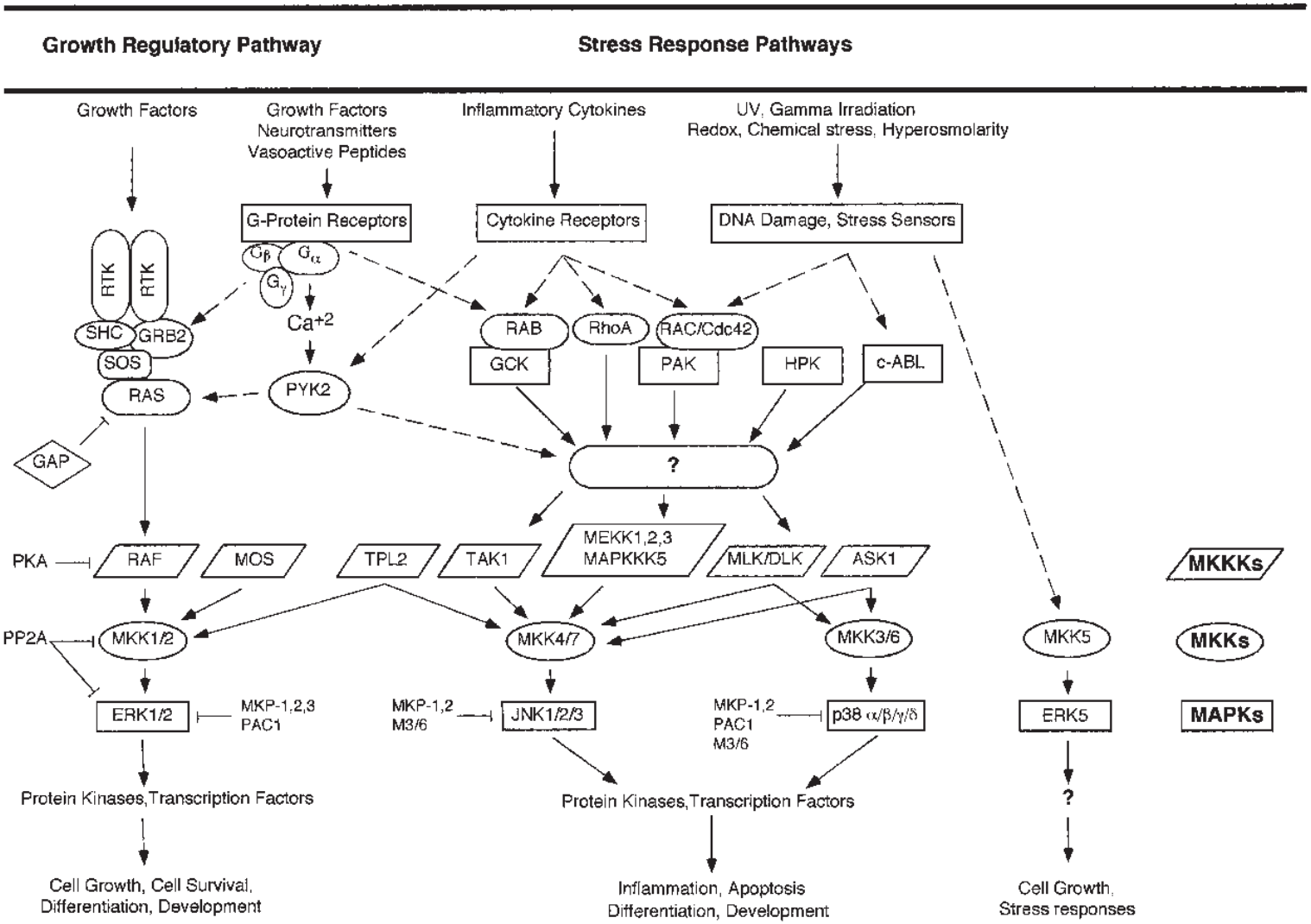
Characteristics

MAP kinase pathways are distinguished by their regulatory module consisting of three components:

- MAP kinases,
- MAP kinase kinases (MKKs, also referred to as MEKs), which are direct activators of MAP kinases,
- MAP kinase kinase kinases (MKKKs), which are direct activators of MKKs.

Each regulatory step involves activation by protein phosphorylation, thus the components form three tiers of a protein kinase cascade. In mammals, 11 distinct MAPK genes and 7 MKK genes have been identified to date, related by sequence similarity within each class. Four distinct families of MKKKs exist, which include members of the Raf family of protein kinases, MEK kinases (MEKKs), multilineage protein kinases (MLKs) and the germ cell specific kinase, Mos.

MAP kinases are multifunctional enzymes that phosphorylate serine or threonine residues within many cellular proteins. Target recognition almost always involves proline residues C-terminal to the site of phosphorylation. In contrast, MKKs have a rather narrow substrate specificity restricted to MAP kinases, and different MKKs recognize specific MAP kinases for phosphorylation and activation. Several MKKKs can recognize and activate different MKKs, in many cases through mechanisms facilitated by small GTP-binding proteins. Finally, the MKKKs can be activated by many mechanisms, not all of which are understood. This enables each



MAP Kinase. Fig. – Mammalian MAP kinase pathways. Examples of pathway signaling components are illustrated, highlighting the MAPK, MKK and MKKK enzymes that make up the MAP kinase module. Activating steps are represented by solid pointed arrows (→) and inhibitory steps are represented by blunted arrows (⊣). Signaling events that are indirect or not described mechanistically are denoted by dashed arrows.

MAP kinase module to be activated in response to a variety of signaling events.

Cellular and molecular regulation

MAP kinase pathways generally fall into growth regulatory or stress-activated categories based on sequence, regulation and function. In mammalian systems three pathways are best understood, although additional MAP kinases are known whose functions are less well characterized. Homologous MAP kinase pathways in other eukaryotic systems such as yeast, flies and worms are known to function in differentiation and development.

Growth regulatory MAP kinase pathways are potently activated by growth factors and mitogenic stimuli. Enzymes in the main growth regulatory MAP kinase pathway were the first to be discovered in mammalian cells and led to their designation as mitogen activated protein kinases (MAP; a name now applied to all pathways). These enzymes function to facilitate cell-cycle progression, enabling cells to undergo DNA replication. Components of the pathway include extracellular signal regulated kinases, ERK1 and ERK2 (MAPKs), which are activated by MAP kinase kinases, MKK1 and MKK2 (MKKs). These MAP kinase kinase are in turn activated by Raf-1 or B-Raf (MKKKs). The mechanism of pathway activation usually involves ligand binding to a cell surface receptor, recruitment of guanine nucleotide exchange factors to the receptor, activation of the small GTP-binding protein, p21-Ras, by an exchange factor, recruitment of Raf-1 to activated p21-Ras, and enhancement of Raf-1 activity towards MKK1/2.

How does the ERK pathway regulate growth?

Upon activation in response to mitogenic signaling, ERKs 1 and 2 phosphorylate and activate nuclear factors that function in transcription. This promotes the synthesis of messenger RNAs, which encode proteins that promote cell-cycle progression and synthesis of DNA. In one well established example, ERK phosphorylates a nuclear factor named Elk1, which enhances transcription of cyclin D1, a subunit of cell cycle-regulatory kinases needed for the onset of DNA replication. Other targets for ERK include factors that facilitate protein translation as well as synthesis of metabolic intermediates. Inhibition of the ERK pathway can be achieved using cell permeable inhibitors or by expression of inhibitory mutants or antisense constructs of ERK. Such experiments show that blocking this pathway arrests the cell-cycle prior to DNA replication. In many cell types, blocking this pathway also facilitates cell death, revealing its importance in cell survival mechanisms. Thus the ERK pathway regulates growth in most mammalian systems by promoting cell-cycle progression and cell survival.

Paradoxically, activation of the ERK pathway in certain cell types results in growth inhibition. Examples include neuronal and blood cell precursors, where the pathway arrests cell growth and induces expression of cell lineage-specific proteins, thus promoting differentiation. How the same pathway promotes growth in some cells while blocking growth and inducing differentiation in others is still poorly understood. Most likely, cells respond in different ways because they express proteins unique to each cell type that lead to distinct cellular responses when phosphorylated by the pathway. It has also been proposed that cells respond differentially to variable amplitudes and kinetics of MAP kinase pathway activation, as well as cross-regulation by other signal transduction pathways. For example, in the PC12 rat pheochromocytoma cell line, transient activation of the ERK pathway in response to epidermal growth factor promotes cell proliferation. Whereas prolonged activation of the pathway for several hours in response to nerve growth factor promotes neuronal cell differentiation and cell growth arrest. After induction of neuronal differentiation, inhibition of ERK leads to cell death, illustrating that MAP kinases promote cell survival in both nonproliferating and proliferating cells. ERK pathways also regulate embryonic development and is necessary for vulval development in the nematode, *Caenorhabditis elegans*, eye development and body patterning in the fruit fly, *Drosophila melanogaster*, mesoderm induction during embryo de-

velopment in the frog, *Xenopus laevis*, and positive T cell selection in mice.

Stress-regulated MAP kinase pathways are triggered by exposure of cells to environmental stresses such as hypoxia, osmotic stress, oxidation, antibiotics and radiation, and by signaling from cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1). Three major stress regulated pathways can be found in mammalian systems: One pathway was identified in studies of a nuclear transcription factor, c-Jun, thus the associated MAPKs are named c-Jun N-terminal kinases 1, 2 and 3 (JNKs, sometimes referred to as stress-activated protein kinases α, β and γ, or SAPKs). JNKs are activated by MKKs 4 and 7 (sometimes referred to as JNKKs or SEKs). A second stress-regulated pathway is composed of MAPKs named p38 MAPKs α, β, γ and δ, which are activated by MKKs 3 and 6. A third pathway, composed of ERK5 (MAPK) and MKK5, is poorly understood but appears to combine regulation and function of both growth regulatory and stress-activated MAPK pathways. The stress-regulated MKKs (3, 4, 6 and 7) can be activated by different MKKK families, including MEK kinases (MEKKs) and multilineage protein kinases (MLKs). Mechanisms involved in activating MKKKs by cytokine receptors or environmental stresses are not clearly defined and appear to be quite varied under different conditions.

An important function of the JNK family of stress-regulated MAP kinase pathways is to regulate cellular responses to apoptotic signals, by facilitating apoptosis or by enabling cells to survive during development. The pathway is strongly activated by cytotoxic signals including redox stress, antibiotics, UV and gamma irradiation, as well as TNF-α and IL-1. The assembly of signaling complexes through interaction of MKKKs, MKKs and MAPKs with scaffold proteins appears to be a key requirement for activation of the JNK pathway. Cellular targets for JNKs include nuclear factors c-Jun and ATF2, thus gene transcription is a key mechanism by which JNKs control cell responses. Deletion of JNK1 or 2 in mice by homologous recombination leads to defects in T cell differentiation, but otherwise normal development. However, eliminating both JNK1 and 2 causes severe defects in brain development, due to loss of developmental apoptosis in the hindbrain and failure in neural tube closure, as well as increased apoptosis and degeneration of the forebrain. Deletion of either MKK4 or MKK7 leads to embryonic death, which in the case of MKK4 involves enhanced apoptosis and interference of liver development. Deletion of JNK3, which is expressed mainly in brain, heart and testis, leads to tissue-specific defects in stress-induced apoptosis, although developmental apoptosis is normal.

Stimuli that strongly activate the p38 MAP kinase pathway include hypoxia, hyperosmolarity and lipopolysaccharides (LPS or endotoxin), as well as cytokine and growth factor receptor activation. An important function of p38 MAP kinases is to promote inflammatory responses in diverse cell types, illustrated by their initial discovery as a target of drugs that blocked inflammatory cytokine synthesis in response to LPS. p38 MAP kinases also promote apoptosis, proliferation, differentiation and/or survival depending on cell type and conditions. As in ERK and JNK pathways, mechanisms behind these responses include regulation of gene transcription. For example, nuclear factors CCAAT/enhancer-binding protein-β (C/EBP-β) and peroxisome proliferator-activator receptor-γ (PPARγ) have been shown to be upregulated by p38 MAP kinases, and facilitate the role of this pathway in promoting fat cell differentiation.

Clinical relevance

MAPK pathways provide viable targets for pharmaceutical intervention of human disease. Perhaps the best validation of this concept is shown by inhibitors of p38 MAP kinases, which have been used to elucidate the role of this pathway in disease states such as pulmonary inflammatory diseases, arthritis, sepsis, viral infection, myocardial hypertrophy and ischemia. Selective inhibitors of p38 MAP kinase have shown promising therapeutic potential in suppressing inflammatory responses in animal models, thus inhibitors of the p38 and JNK stress-activated kinase pathways are an extre-

mely active area of pharmaceutical research. An important goal will be to understand which distinct cellular effects are regulated by p38 and JNK isoforms, and to fine tune drug effects by targeting inhibitors towards different isoforms.

Because ERK signaling promotes cell growth and transformation in most cell types, components in this pathway are important targets for treatment of proliferative diseases. Cancer models such as pancreatic or colon cancer are strongly correlated with oncogenic mutations in p21-Ras, suggesting that cancer cell growth may be retarded by inhibitors of this pathway. An inhibitor of MKK1/2 has recently been reported which causes significant reduction in colon carcinoma growth rate in animal models of tumor transplantation as well as cancer cell invasiveness.

In addition, growth regulatory and stress-activated MKKs have been shown to be cellular targets for inhibition by anthrax lethal toxin, the major cause of death in animals infected with *Bacillus anthracis*, and by YopJ, a virulence factor from the bubonic plague pathogen *Yersinia pestis*. Thus, therapies that protect MAP kinase pathway components from inhibition by bacterial toxins may eventually prove useful in conferring resistance to bacterial infection and agents of biological warfare.

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MAP Kinase Kinase

Synonyms

- MAPKK
- MKK
- → MEK

Definition

Mitogen activated protein kinase kinase.

MAPK

Synonyms

- → MAP kinase

Definition

Mitogen activated protein kinase.

MAP2K

Synonyms

- MAPKK
- MKK
- → MEK

Definition

Mitogen activated protein kinase kinase.

MAP3K

Synonyms

- MAPKKK
- → MEKK

Definition

Mitogen activated protein kinase kinase kinase.

MAPK Kinase Kinase

Synonyms

- MAPKKK
- MAP3K
- → MEKK

Definition

Mitogen activated protein kinase kinase kinase.

MAPKK

Synonyms

- MAP kinase kinase
- → MEK

Definition

Mitogen activated protein kinase kinase.

MAPKKK

Synonyms

- MAP3K
- → MEKK

Definition

Mitogen activated protein kinase kinase kinase.

Marker

Definition

- A DNA marker is an internal standard (usually DNA fragments obtained by digestion with DNA restriction enzymes) of known size that is used to determine DNA or RNA fragment size in an electrophoretic gel.
- A genetic marker is any allele of interest in an experiment.

MART-1

Definition

MART-1 stands for melanoma antigen recognized by T cells 1. The → HUGO nomenclature commitee symbol is MLANA (melan-A). Melan-1 is a protein of 118 aa and 13 kD, with an so far unidentified function. Its expression is restricted to melanoma and melanocytes, and the retina. MART-1 is used in as a vaccine for the immunotherapy of → melanoma.

MASH-2

Definition

Mammalian achaete scute homolog 2 (Mash-2) is a mouse → bHLH protein member of Achaete-scute family. The mouse mash-2 gene locus maps to chromosome 7 (69.30 cM).

Mast Cell Growth Factor Receptor

Definition

→ Kit/stem cell factor receptor.

Mastectomy

Definition

Mastectomy is the surgical removal of the breast.

Maternal Inheritance

Definition

Maternal inheritance describes the preferential survival in the progeny of genetic markers provided by one parent.

MATH1

Definition

Math1 (mouse atonal homolog 1 (*Drosophila*)), also known as Atoh1, is a → bHLH protein member of Atonal family (354 amino acids, 38 kD). The mouse atoh1 gene locus maps at chromosome 6 (29.69 cM). Math1 activates → E-box dependent transcription in collaboration with E47, but the activity is completely antagonized by the negative regulator of neurogenesis Hes-1. It may play a role in the differentiation of subsets of neural cells by activating E-box dependent transcription. Efficient DNA binding requires dimerization with another bHLH protein.

Matrix Metalloproteinase-9

Definition

The proteolytic activity of matrix metalloproteinase-9 is important for tumor invasion, tissue remodeling and → angiogenesis. Latent precursors have to be proteolytically cleaved to produce the active form. Matrix metalloproteinase-9 can exist in soluble or bound form (e.g. to → CD44), and their activity can be blocked by potent tissue inhibitors of MMPs (TIMP).

Matrix Metalloproteinases

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Synonyms

- matrix metalloproteinases (MMPs)
- matrixins

Definition

The matrix metalloproteinases (MMPs), or matrixins, are a family of zinc-dependent metalloendopeptidases that are widely expressed. They cleave a variety of extracellular substrates including, but not limited to, protein and proteoglycan components of the extracellular matrix (ECM). MMPs belong to the metzincin family of endopeptidases, characterized by the presence of three zinc-binding histidine residues within the active site (HexxHxxGxxH). MMPs are believed to catalyze localized hydrolysis of extracellular matrix proteins including collagens, fibronectin, laminins and proteoglycans, thereby modifying the integrity of the connective tissue.

Characteristics

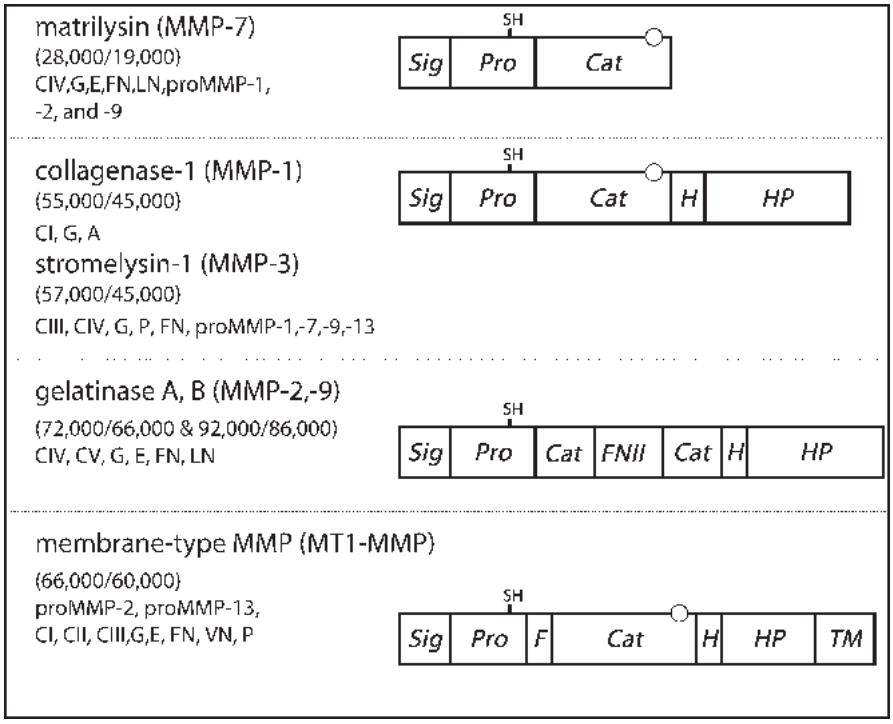
Domain structure

MMPs are synthesized as prepro-enzymes and, following removal of the signal peptide, are generally secreted in proenzyme or zymogen form. The latency of the zymogen is maintained by the presence of an unpaired cysteine residue in the propeptide domain (Fig. 1), contained within the conserved sequence PRCG(V/N)PD. This cysteine residue ligates the catalytically essential zinc atom in the enzyme active site, thereby preventing enzymatic activity. Exceptions exist including stromelysin 3 (MMP-11) and membrane-type MMPs (MT-MMPs), which contain a conserved RX(K/R)R sequence within the propeptide and can be activated intracellularly by proprotein processing enzymes

of the PACE family, such as furin. In addition to the catalytic zinc atom described above, an additional zinc and two calcium ions provide structural stability. Inserted into the active site of the gelatinases [MMP-2 (gelatinase A) and MMP-9 (gelatinase B)] are a series of three fibronectin type II repeats that function in substrate recognition. The catalytic domain is followed by a short connecting (hinge) region and, with the exception of MMP-7 (matrilysin), a hemopexin-like domain. The role of the hemopexin-like domain is the subject of current investigation, and recent studies have demonstrated that this region participates in cell surface association (MMP-2) and collagen recognition and cleavage (MMP-1). The MT-MMPs also contain an hydrophobic transmembrane sequence and a short (20 amino acids) cytoplasmic tail. Like the hemopexin domain, the cytoplasmic tail of MT-MMPs is also currently under active investigation to evaluate the potential for association with other cytoplasmic proteins that may regulate MT-MMP localization or function. The domain structures of the MMPs most widely expressed in tumor tissues are summarized in Fig. 1.

Zymogen activation

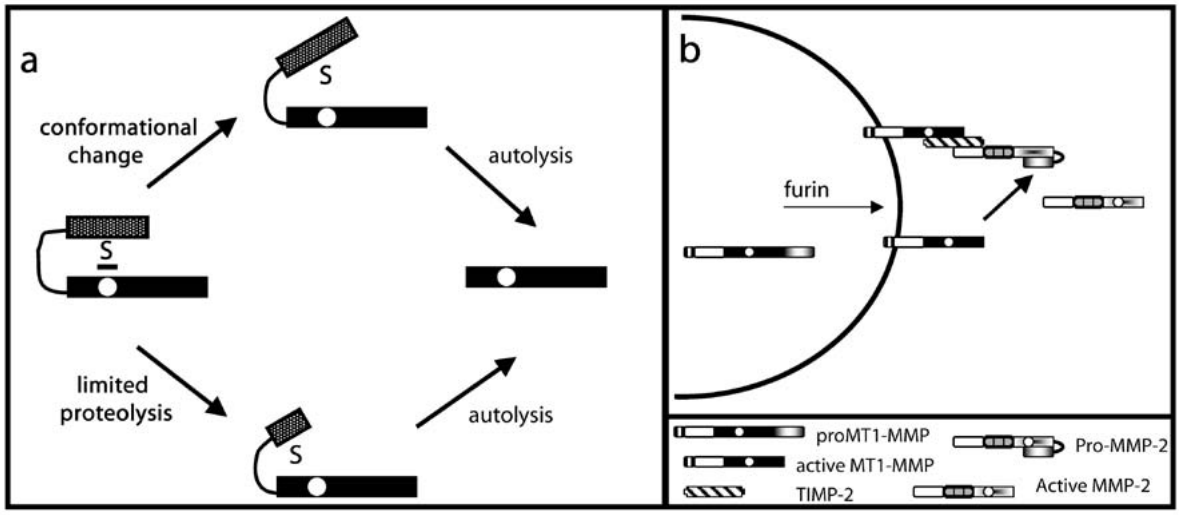
In addition to regulation of MMP gene expression by a number of growth factors and cytokines, the activity of MMPs is stringently regulated by post-translational mechanisms, predominantly zymogen activation and enzyme/inhibitor binding. Proteolytic activation of proMMP zymogens functions primarily via a



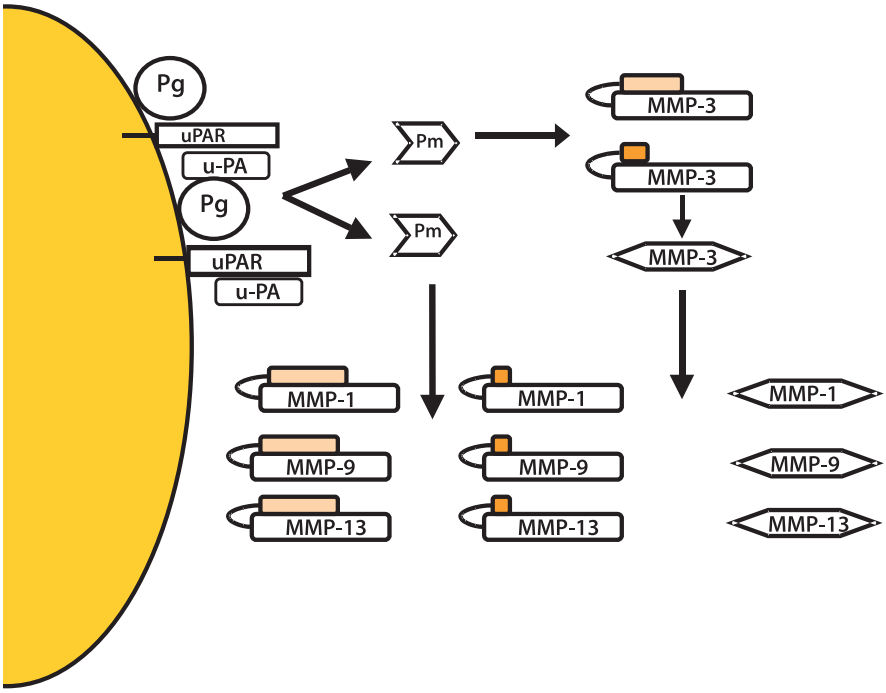
Matrix Metalloproteinases. Fig. 1 – Domain structure of selected tumor-associated MMPs. The common name and MMP number are listed, followed by the approximate molecular weight of the latent and active species, respectively. Reported substrates are summarized as follows: CI, CII, CIII, CIV, CV - collagen types I, II, III, IV, and V, respectively; G - gelatin; E - elastin; FN - fibronectin; LN - laminin-1; A - aggrecan; P - proteoglycan; VN- vitronectin. Structural/functional domains are denoted as follows: Sig - signal peptide; pro- propeptide domain containing unpaired Cys residue denoted by -SH; Cat - catalytic domain containing the zinc binding consensus sequence that chelates the catalytically essential zinc atom (circle); H - hinge region; HP - hemopexin-like domain; FNII - fibronectin type II-like repeats; F - furin cleavage site; TM - transmembrane and cytoplasmic domain.

cysteine switch mechanism (Fig. 2A) and involves cleavage of the propeptide, thus destabilizing the cysteine-zinc interaction and generating catalytically active enzyme. The initial cleavage in many MMP propeptides can be carried out by proteinases of other mechanistic classes, predominantly serine proteinases such as plasmin. Following the initial proteolytic event, the partially active enzymes often undergo further inter- or intra-molecular cleavages, resulting in complete removal of the prodomain and generation of fully active enzyme. Proteinase activity is frequently regulated by zymogen activation cascades, such that an initial event will generate an active proteinase that processes a downstream zymogen (Fig. 3). Many studies suggest that coupling of serine and MMP zymogen activation pathways may function to promote pericellular proteolysis during tumor invasion and metastasis.

proMMP-2 (gelatinase A) is an exception to serine proteinase activation of proMMPs. Detailed biochemical studies from a number of laboratories have demonstrated that proMMP-2 is activated on the surface of many neoplastic cells following formation of a trimeric complex containing the transmembrane proteinase MT1-MMP and a molecule of tissue inhibitor of metalloproteinases 2 (TIMP-2). The MT1-MMP/TIMP-2 complex forms a binding site for proMMP-2, which is then proteolytically processed by a second MT1-MMP molecule (Fig. 2B). This is an interesting example of a reaction in which a proteinase inhibitor (TIMP-2) is also required for zymogen activation. Activation of proMT1-MMP is believed to occur intracellularly, as the zymogen contains a dibasic recognition motif (Arg108-Arg-Lys-Arg) in the propeptide region that may be cleaved by proprotein processing



Matrix Metalloproteinases. Fig. 2 – Activation of proMMPs. (a) Cysteine switch mechanism for proMMP activation. The latency of MMP zymogens is maintained by an interaction between an unpaired cysteine residue in the propeptide region (S) and the catalytically essential zinc atom (circle). Following conformational perturbation via the action of agents such as SDS, the Cys-Zn^{2+} interaction is disrupted, leading to autolytic activation characterized by propeptide cleavage. Alternatively, limited proteolysis of the propeptide initiated by serine or metalloproteinases may result in partial propeptide processing and disruption of the Cys-Zn^{2+} interaction, followed by autolytic processing to generate the fully active specie. **(b) Cell surface activation of proMMP-2 by MT1-MMP.** ProMT1-MMP is processed intracellularly by proprotein convertases, such as furin, and active enzyme is inserted in the plasma membrane. A ternary 'activation complex' form, comprised of MT1-MMP/TIMP-2/proMMP-2. ProMMP-2 in the 'activation complex' is proteolytically processed by a neighboring MT1-MMP molecule, followed by additional autolytic activation and release of the fully active proteinase.



Matrix Metalloproteinases. Fig. 3 – Cell surface-initiated zymogen activation cascade. Interaction of the serine proteinase uPA with its cell surface glycosyl phosphatidyl inositol-anchored receptor (uPAR) initiates activation of cell-associated plasminogen (Pg), forming the broad-spectrum serine proteinase plasmin (Pm). Plasmin can initiate propeptide processing of MMP-1, -3, -9, or -13. This is followed by additional autolytic cleavages or MMP-3 processing events, leading to the generation of fully activated MMPs.

enzymes including the PACE family serine proteinase furin.

Inhibition

Aside from zymogen activation, a predominant mechanism for post-translational control of MMP enzymatic activity is via interaction with protein inhibitors. Tissue inhibitors of metalloproteinases (TIMPs) inhibit MMP activity via the formation of a tight, non-covalent enzyme/inhibitor complex in a 1:1 molar ratio. Four TIMPs (TIMPs 1-4) have been identified, although the function of TIMP-1 and TIMP-2 are the most well characterized. Recent studies have demonstrated that the amino terminal cysteine residue in TIMPs is required for inhibitory activity, functioning by coordinating the catalytically essential zinc ion. Additional contact sites have been described that may interfere with substrate binding or other proteinase functions, suggesting that the inhibitor presents an extended contact surface to the enzyme. In addition to TIMPs, MMPs are also inhibited by the non-specific plasma proteinase inhibitor α-2-macroglobulin. Substantial research efforts have also been directed toward generation of synthetic MMP inhibitors to prevent pathologic proteolysis prevalent in diseases such as cancer and arthritis. These compounds contain a zinc binding functionality such as an hydroxamic acid group coupled with a peptide or peptidomimetic sequence designed to target the inhibitor to the enzyme active site. Most synthetic inhibitors are broad spectrum, blocking the activity of a wide range of MMPs. However, a current alternative strategy in inhibitor design is the generation of more specific compounds that target only a specific subset of MMPs (for example, gelatinase inhibitors that do no alter collagenase function).

Substrate cleavage

Although it is widely believed that MMPs function *in vivo* to process extracellular matrix macromolecules, the precise substrates for the majority of MMPs remain unclear. *In vitro* experiments have demonstrated a wide range of substrates for MMPs including native and denatured collagens, adhesive glycoproteins such as fibronectin, laminins, and vitronectin, and proteoglycans. Substrate gel electrophoresis, or zymography, is a commonly utilized method to evaluate relative MMP activity levels in tissue extracts or tumor cell conditioned media. Although not quantitative, this method provides a rapid and sensitive initial evaluation of cellular MMP profiles. However, data obtained using zymography are frequently misinterpreted as

a) MMP zymogens attain proteolytic activity in the presence of sodium dodecyl sulfate without propeptide cleavage;
b) MMP/TIMP complexes are non-covalent and are thus dissociated during electrophoresis, leading to the potential for over-estimation of enzymatic activity.

Preliminary results obtained using zymography should be confirmed by other methods such as those based on solution interaction of the MMP and its potential target substrate such as collagen, gelatin or other matrix macromolecules. Cleavage may then be assessed by electrophoretic examination of reaction products or other methods. Alternatively, a number of quenched fluorescent peptide MMP substrates have been described that have broad utility for continuous kinetic monitoring of peptidase activity. These synthetic substrates have proven useful for comparative evaluation of synthetic MMP inhibitors. A more complex approach for elucidation of MMP substrates *in vivo* involves generation of mice genetically engineered for a specific MMP deficiency by targeted gene inactivation (MMP 'knockouts'). The majority of these animals display relatively mild phenotypes, with the exception of animals deficient in MT1-MMP expression. These mice display severe connective tissue abnormalities characterized by dwarfism, osteopenia and arthritis, suggesting that MT1-MMP is required for pericellular collagenolysis *in vivo*.

Control of MMP gene expression

MMP gene expression is tightly regulated and transcription of many MMP genes is inducible by a wide variety of effectors including hormones, growth factors, cytokines and tumor promoters. In addition to soluble mediators, recent studies have demonstrated changes in MMP expression or activity associated with physical factors such as alterations in cell/cell or cell/matrix contact, or changes in cell shape associated with conditions such as physical stress that induce cytoskeletal rearrangement.

Clinical relevance

MMPs have been implicated in a wide variety of pathologic processes including arthritis, cardiovascular disease, periodontal disease, emphysema and cancer. In tumor tissues, enhanced or *de novo* expression of MMPs is often correlated with disease progression. Conversely, experimental manipulation of TIMP levels has been shown to decrease tumor invasion and metastasis in many experimental models. MMP production in malignant tissues is not necessarily limited to neoplastic cells, as several studies have demonstrated that tumor cells can 'recruit' stromally produced enzymes to promote pericellular proteolysis. Furthermore, MMPs have also been implicated in tumor-associated angiogenesis, suggesting an additional mechanism whereby MMPs can contribute to tumor progression. Therapeutic administration of MMP inhibitors is currently under investigation as a potential strategy to prevent tumor invasion and metastasis, and several synthetic MMP inhibitors have demonstrated efficacy in preclinical models of human cancers including colon, breast, and lung carcinoma and melanoma. The observed inhibition of tumor growth may be due in part to inhibition of tumor angiogenesis. In human trials, a decrease in the rate of rise in cancer antigens was observed in patients with prostate, ovarian, colorectal and pancreatic cancer. However treatment

was associated with musculoskeletal pain and inflammation. Additional studies are necessary using MMP inhibitors alone or in combination with cytotoxic chemotherapies to assess the efficacy of these compounds in inhibition of tumor growth, metastasis and angiogenesis.

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Matrixins

Definition

Matrixins, or matrix → metalloproteinases, are a family of zinc-dependent proteinases used by cells to break down extracellular matrix.

Mature B-cell Tumors

Definition

In the scheme of Revised European-American Classification of Lymphoid Neoplasm (REAL), B-cell tumors are divided into those arising from immature cells and those representing more mature B-cell phenotype. The latter group consists of 11 subcategories, most of which are included in the more widely used clinical term, non-Hodgkins lymphoma (NHL). Immunoglobulin genes of the neoplastic cells are rearranged, and the cells express 'pan-B' markers as well as immunoglobulin molecules on their cell surface. Oncogene rearrangement, clinical features and response to treatment vary among the subcategories.

MAX

Definition

MAX (Myc associated protein x) associates to dimers with either → MYC or MAD, which bind to DNA and CACGTG. Whilst MAX/MYC dimers activate gene transcription, MAX/MAD dimers repress transcription. MAX is a nuclear protein of 160aa and is a member of the helix-loop-helix family of transcription factors. The gene maps to 14q23, and two protein forms exist resulting from differential splicing at N-terminus.

MAX Binding Protein

Definition

→ MNT.

MAX Interacting Protein

Definition

→ MNT.

MAZ

Definition

MAZ is a → MYC-associated zinc finger protein (also, purine-binding transcription factor) that is also known as ZF87, Pur-1 and Zif87. It is a

protein of 477 amino acids and 48kD. Like MYC, is ubiquitously expressed in many tissues. The protein binds to two sites, mda1 and mda2, within the MYC promoter thus regulating MYC expression. The gene maps to chromosomal position 16p11.2.

MCAM

Definition

MCAM, also known as MUC18, MelCAM, is a cell surface adhesion molecule associated with tumor progression, particularly of → melanoma; expression regulated by → AP-2.

MCH

Definition

MCH is short for mammalian Ced-3 (for cell death defective) homolog of the nematode → *Caenorhabditis elegans*. *Ced* genes function in the developmental cell death pathway (→ apoptosis) of the nematode. In dying cells the activity of *Ced-3* and *Ced-4* is necessary for cell death to occur. The *Ced* genes encode aspartate-specific cysteine proteases (ASCPs). A number of MCH genes have been identified in human cells, they are now referred to as *CASPASE* (*CASP*) genes. *MCH3* is synonym for *CASP7*, *MCH4* for *CASP10*, *MCH5* for *CASP8* and *MCH6* for *CASP9*. → Caspases.

MCM/P1 Proteins

Definition

These proteins are a family of 6 closely related minichromosome maintenance proteins (Mcm2-7) that form the RLF-M component of the replication licensing system.

MC1R

Definition

Melanocortin 1 receptor (MC1R) is an α-melanocyte stimulating hormone receptor of 317 aa and 34 kD. It is an integral membrane receptor for MSH-α, MSH-β and MSH-γ: activation of the receptor is coupled to → G-proteins, which activate adenylate cyclase. The gene maps to 16q24.3.

MDM2

Definition

Human homolog of mouse double minute 2 (MDM2); the protein was first identified as the product of the *mdm2* gene amplified in transformed murine cells. It is a → p53-binding nuclear protein that antagonizes and down-regulates p53 activity. The gene maps to 12q14-q15 and is amplified in certain human tumors, including sarcomas, glioblastomas and astrocytomas.

MDR1

Definition

MDR1 is the human multidrug resistance gene that encodes the → P-glycoprotein, which actively exports various toxic substances (including chemotherapeutic drugs) from cells.

Medulloblastoma

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Synonyms

- Infratentorial primitive neuroectodermal tumor (PNET)

Primitive neuroectodermal tumor of children occurring in the child's posterior fossa; see also → brain tumors.

Definition

Medulloblastomas are malignant embryonal tumors of the cerebellum, composed of densely packed small cells with round to oval nuclei and scanty cytoplasm with a high tendency to metastasize throughout the CNS. The current World Health Organisation (WHO) classification of 'Tumors of the Nervous System', groups medulloblastomas as Primitive Neuroectodermal Tumor (→ PNET) and recognizes four variants apart from the classic histological subtype:

- → desmoplastic medulloblastoma,
- → large cell medulloblastoma,
- → medullomyoblastoma,
- → melanotic medulloblastoma.

Characteristics

Clinical data

Medulloblastomas are the most common malignant → brain tumors occurring in childhood, affecting each year approximately six of one million children (<20 years) in the United States. Two age peaks at 3-4 and 8-9 years and a male predominance ranging from 1.4 to 2.1 have been reported. However, medulloblastomas have been described in neonates and in 1% of primary adult CNS tumors. Medulloblastomas are rarely observed beyond the 4th decade.

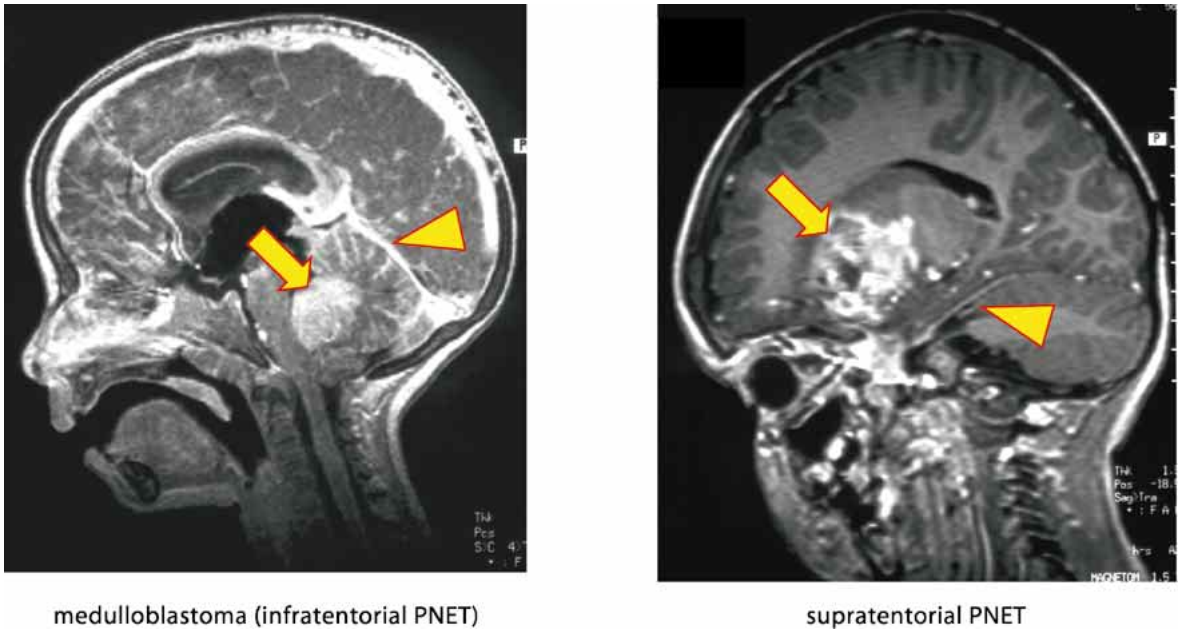
Medulloblastomas are most often found in a midline localization in the vermis of the cerebellum, projecting and obstructing the fourth ventricle. With increasing age tumors are more commonly found in the cerebellar hemispheres. Medulloblastomas have a high propensity to metastasize throughout the CNS; metastasis to bone, bone marrow, peritoneum, liver and lung have been reported mainly in children with ventriculo-peritoneal shunts. Clinical symptoms consist of truncal ataxia, gait disturbances and, caused by increased intracranial pressure, headache, lethargy and morning vomiting. On computed tomography (CT) and magnetic resonance imaging (MRI) medulloblastomas usually present as solid, homogeneously contrast-enhancing masses (Fig.). Diagnosis and staging are established by clinical examination, imaging studies using CT and/or MRI and lumbar puncture.

For treatment planning and prognostic evaluation, patients are broadly divided into two risk groups, based on the extent of surgical resection as assessed by postoperative imaging and the neurosurgeons report:

- Average risk patients are 7 years or older and have a localized tumor, which can be totally or near totally resected.
- Poor risk patients are three years or younger, show gross nodular seeding within the neuraxis, sometimes in addition extraneurally, and have a significant postoperative residual tumor.

Recently molecular markers have been used as assets in the prognostic evaluation of medulloblastoma patients. The negative association of → amplification of the *MYC* gene and the positive association of high levels of the Neurotrophin 3 [→ neurotrophin] receptor TrkC, are accepted indicators for the outcome, while the prognostic value of loss of heterozygosity (→ LOH) for 17p is not yet clear.

Current treatment protocols for medulloblastoma consist of radio- and chemotherapy, with the attempt to delay radiotherapy in children 3 years and younger to avoid damage to the developing CNS. In more recent high-dose chemotherapy regimen, autologous bone marrow transplantation or stem cell reinfusion are included.



Medulloblastoma. Fig. – Primitive neuroectodermal tumors (PNET) of the CNS. Axial T1-weighted MRI images of two children with PNETs. The left panel shows the MRI of a 2 year old boy with a classic medulloblastoma (infratentorial PNET) located in the midline of the cerebellum. The right panel is an MRI image of a supratentorial PNET originating in the right cerebral hemisphere of a 3 year old child. Arrowheads indicate the location of the tentorium of the cerebellum, while open arrows point to the tumors.

With current treatment strategies, 5-year survival rates of 74 to 83%, depending on the risk group, are achieved. However, the cure of a recurring medulloblastoma remains a rare event.

Aetiology

The origin of medulloblastomas is largely unknown. As shown immunohistochemically medulloblastoma cells predominantly express neuronal marker proteins (e.g. synaptophysin, neurofilaments). Occasionally, astrocytic differentiation (expression of GFAP) and, rarely, ependymal features are seen. Some evidence exists that the hypothetical progenitor cell, the 'medulloblast' (after Cushing, 1925), is derived from the → external granular layer (EGL) of the developing cerebellum. A multitude of factors have been implied in the tumorigenesis of medulloblastomas as they show expression differences between the tumors and normal cerebellum. Among many others are the transcription factor genes *PAX5*, *NEUROD3/neurogenin* and *ZIC*, the neuropeptides somatostatin and VIP as well as the growth factor receptor genes *HER2* and *HER4*.

Molecular genetics and biology

Several familial cancer syndromes are associated with medulloblastoma. 'Turcot's syndrome', also called 'brain tumor polyposis syndrome type II', is characterized by the simultaneous occurrence of adenomatous polyps or carcinomas of the colon. There, as well as in brain tumors, primarily glioblastomas and medulloblastomas, germline mutations of the *APC* gene are found. While for sporadic medulloblastomas no mutations in the *APC* gene have been reported, some of these tumors show activating mutations in the β-*catenin* gene, which is controlled by *APC* and eventually activates the *MYC* oncogene. Rarely, medulloblastomas have been seen in patients with the Li-Fraumeni syndrome or in neurofibromatosis. Sporadic medulloblastomas only rarely carry mutations of the genes *TP53* and *NF1* respec-

tively, causing these syndromes. In the Basal Cell Nevus (BCNS) or Gorlin's syndrome patients are affected by basal cell carcinomas, ovarian fibromas, keratocysts of the jaw, palmar and plantar pits, skeletal abnormalities and medulloblastomas, mainly of the desmoplastic variant. In such patients and in about 10% of the cases with sporadic medulloblastomas, mutations of the human homologue of the *drosophila melanogaster* segment polarity gene *PTCH1* in 9q22.3 have been described. Transgenic mice, heterozygous for *PTCH1*, develop tumors that exhibit intriguing similarities to medulloblastomas.

Reported cytogenetic abnormalities in medulloblastomas include isochromosomes 17q (up to 50%), trisomy 7, extra copies of 1q, monosomy 8 and 22 and deletions on 10q and 4q. Recent Comparative Genome Hybridization studies demonstrated loss of genetic material from chromosomes 10q, 11, 16q, 17p and 8p; gains were found for 17q and 7 and amplifications were detected in 5p15.3 and 11q22.3.

In molecular genetic studies, LOH for the short arm of chromosome 17 (17p) was demonstrated in up to 50% of sporadic medulloblastomas and the chromosomal breakpoint has been narrowed down to a region in 17p11.2. Loss of genetic material from chromosome 10q is in some medulloblastomas associated with homozygous deletions of the gene → *DMBT1*. More recent studies demonstrated an abundance of global DNA methylation in medulloblastomas. The combined incidence for amplifications of the genes → *MYC*, → *MYCN* and → *EGFR* in medulloblastomas is approximately 10%.

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Medullomyoblastoma

Definition

Medullomyoblastoma is a rare variant of medulloblastoma with foci of striated and nonstriated muscle fibres.

Meiosis

Definition

Meiosis occurs by two successive divisions (meiosis I and II) that reduce the starting number of 4n chromosomes to 1n in each of four product cells. These products may mature to germ cells (sperm or eggs).

MEK

Definition

MEK (or MKK, MAPKK) is short for mitogen activated protein kinase kinase/ extracellular signal-regulated kinase (ERK) family of protein Ser/Thr kinases. MEKs are the direkt activators of → MAP kinases and specific MEKs, such as the dual specifity Ser/Thr kinase MEK1/2, activate distinct MAP kinases, such → ERK 1/2.

MEKK

Definition

MEKK stands for MEK kinase, a protein capable of phosphorylating → MEK. → MAP kinase.

Melanocortin Receptor

Definition

The melanocortin receptor is a cell surface glycoprotein receptor for melanocortin (→ melanocyte stimulating hormone; MSH), a polypeptide with homology to other pituitary hormones. MSH is produced by the pituitary and also by keratinocytes in response to UV irradiation. A principle downstream target is tyrosinase, the rate-limiting enzyme in melanin production.

Melanocyte Stimulating Hormone

Definition

→ POMC.

Melanoma

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Definition

Melanoma is a tumor arising from melanocytes. Melanocytes are dendritic cells that migrate from the neuroectoderm to lodge at the base of the epidermis and in other epithelial sites including the eye, gastrointestinal tract and vagina. Melanoma can arise at any of these sites but most commonly arises in the epidermis (cutaneous melanoma, CM), where the function of melanocytes is to produce melanin, a protective skin pigment, in response to ultraviolet radiation. Ocular melanoma (OM) arises from melanocytes in the uveal stroma. Melanoma may or may not develop in a pre-existing → mole.

Characteristics

The incidence of cutaneous melanoma (CM) varies with racial distribution and geographic patterns of sun exposure. High incidence countries (>20 per 100,000) include Australia, Hawaii and New Zealand. Incidence rates are beginning to fall in younger age groups in some of these countries, attributed in part to intensive public health sun protection programs. Countries of intermediate incidence (5-15 per 100,000) include northern Europe and mainland USA. CM is the seventh most common cancer in US whites. In 1988-1992 the average age-standardized invasive CM incidence rates in the USA were 13.1 per 100,000 and 10.2 per 100,000 in white males and females, respectively. CM is very rare in US blacks (0.7 per 100,000 and 0.5 per 100,000 for males and females, respectively). The disease has increased dramatically during this century and continues to rise at a rate of approximately 1.8% per year in US whites, and by as much as 40 – 80% in the UK, France and Spain between the early 1980s and the early 1990s. A low incidence (<2/100,000) is found in Africa and South-East Asia. It was estimated that about 44,200 new CMs would be diagnosed in the USA in 1999 and that the disease would cause 7300 deaths. While mortality rates have begun to decrease in certain parts of the world, the burden of CM is likely to increase well into the next century due to the aging population, cohort effects in lifetime sun exposure and the possible effects of recent depletion of the ozone layer on ambient levels of ultraviolet (UV) light. Although the most common form of intra-ocular tumor, ocular melanoma (OM) incidence is less than 0.7/100,000.

Risk factors for the development of CM include the presence of multiple moles (nevi), family history of CM, history of a previous primary CM, fair skin that tans rather than burns,

freckling, red hair and blue eyes. Individuals with multiple atypical nevi (dysplastic nevi) are at particularly high risk. The fact that CM frequently develops *de novo*, even in those with multiple atypical nevi, suggests that these nevi are a marker of melanocytic stability, rather than being obligatory precursor lesions. It has been estimated that approximately 65% of all CM worldwide is due to sun exposure, and as much as 90% in the USA and Oceania (1). Epidemiological data best fit the hypothesis that CM is related to pattern as well as amount of sun exposure, with increasing intermittency of exposure being associated with increasing risk.

CM presents most commonly as a change in a mole, the majority of which are pigmented. Suspicion of malignant change is raised by the presence in a mole of asymmetry, border irregularity, distinguishing colour, including areas of red, blue or white and a diameter $>$ 5 mm. None of these features is invariable. Particular difficulty is presented by amelanotic primary CM. Approximately 15% of CM presenting to Sydney Melanoma Unit presents as metastatic disease with an occult primary, presumed in many cases to have regressed.

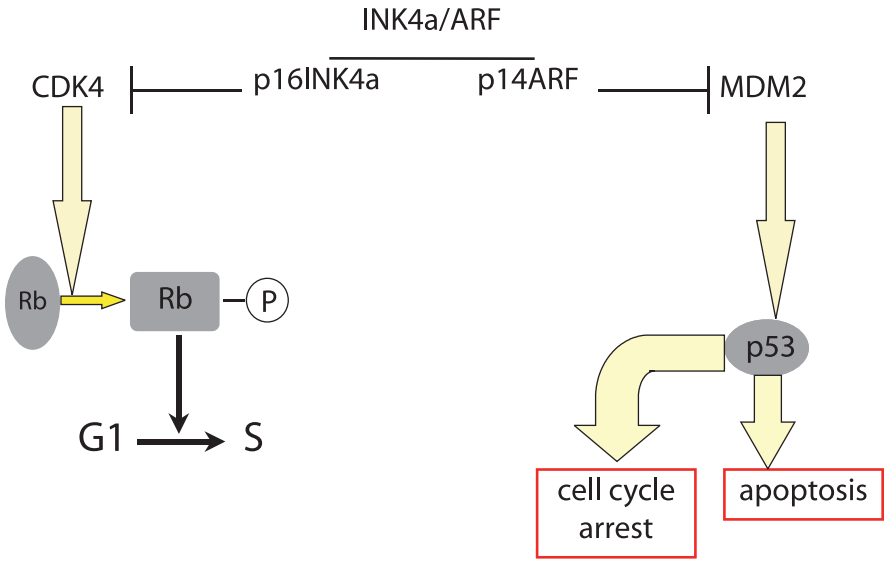
CM progresses through an *in situ* stage to radial growth phase and final vertical growth phase. Once cells penetrate the dermis, invasion of blood and lymphatic vessels leads to dissemination. The patterns of dissemination are highly varied and unpredictable. In certain cases, lymphatic spread dominates, and in selected cases removal of draining loco-regional lymphatics produces long-term survival. In other cases, hematological dissemination occurs early. The skin (Fig. 1), subcutis and lungs are particularly common sites for metastases, but in later stages nearly every organ and tissue can be involved. Cerebral metastases are present in over 80% of autopsy cases. OM metastasizes haematogenously, with a particular affinity for the liver, which may remain the sole site of indolent metastatic disease until the preterminal stage when lung, brain and distant lymph node sites are also often involved.

The prognosis of primary CM is linearly correlated with the thickness of the lesion (Breslow depth). More than 85% of patients with primary CM $<$ 1.5 mm in depth survive 5 years. Fewer than 10% of patients with hematogenous metastases survive two years.

Genetics

Approximately 5 – 10% of CM patients have a positive family history of the disease. In 20 – 40% of multiple ($\geq$3 case) families the cause is an inherited mutation in the CDKN2A gene, coding for the $p16^{INK4A}$ protein (2). Certain kindreds with CDKN2A mutations also show a propensity for pancreatic cancer. Other melanoma families have an increased incidence of breast cancer and ocular melanoma, although these have not been linked to the CDKN2A gene. CM-associated mutations in the CDKN2A gene prevent $p16^{INK4A}$ from binding to cyclin dependent kinase 4 (CDK4). The consequence is phosphorylation of the → retinoblastoma protein, release of the → E2F transcriptional regulator, and unrestricted passage of cells through the G1-S restriction point (Fig. 2). A second transcript derived from an alternative first exon of the CDKN2A gene encodes the protein $p14^{ARF}$, which stabilizes p53 levels through interaction with MDM2 (3) (Fig. 2). So far there is only indirect evidence for the involvement of $p14^{ARF}$ in the genesis of CM (4), although mutations that alter the structure of the protein have been demonstrated in hereditary CM families. Some of these

Melanoma. Fig. 1 – Multiple intracutaneous melanoma metastases.



Melanoma. Fig. 2 – Functions of the INK4a/ARF locus on chromosome 9p. Alternate splicing produces two proteins. p16INK4A inhibits CDK4, preventing phosphorylation of Rb, and restricting the cell cycle at the G1-S interface. p14ARF binds to MDM2, preventing degradation of p53, and permitting p53 to carry out its functions of cell cycle arrest or promotion of apoptosis.

mutations have been shown to alter the sub-cellular localization of the $p14^{ARF}$ protein. The location of other CM susceptibility genes is the subject of intense research by the International Melanoma Genetics Consortium, and genome screening has revealed several promising new loci. Potential modifier genes include those that determine skin colour including the → melanocortin receptor, MC1R (5). Data on the penetrance of CDKN2A mutations are still being generated, but it is clear that penetrance is higher at earlier ages in areas of high sun exposure, demonstrating a clear synergy between environment and genetic susceptibility in the induction of a human cancer. The use of genotyping to influence clinical management of those at high risk of CM is currently discouraged because of the lack of clarity concerning penetrance, the absence of functional data on many CDKN2A mutations and the demonstrated high risk of CM in non-gene carriers in hereditary CM families (2). The current best strategy is for all at high risk to be subject to the same high standards of sun protection and skin surveillance.

Twin studies have shown a strong genetic basis for mole number, although sun exposure clearly also has a strong influence. Neither mole count or presence of atypical nevi correlates well with genotype in CM families with CDKN2A mutations, suggesting that other genes may be involved. However, segregation studies suggest that CDKN2A, or another gene close to it, are important regulators of mole number (6).

Immunology

There continues to be keen interest in the potential for immunological strategies in the treatment of CM. This is based on the following observations:

1. Regression is common in primary CMs, and a predominantly CD4 T cell lymphocytic infiltration is commonly seen.
2. Well-documented instances of spontaneous regression have been reported in metastatic melanoma.
3. *In vitro* and animal evidence for a cell-mediated immune response to melanoma cell surface antigens.
4. There is an increased incidence of CM in immunosuppressed patients.

Immunotherapies which have shown some activity in metastatic disease in Phase II trials include the use of vaccination with melanoma cell-surface membranes or purified peptide components of common melanoma cell surface antigens. These antigens are derived either from differentiation antigens in melanoma such as tyrosinase, → MART-1, gp100, or from tumor specific gene products such as → MAGE, → BAGE and → GAGE proteins. The latter are expressed normally only in the testes but become expressed in melanoma and other tumors because of demethylation of the genes. The third group of antigens are specific to individual tumors and are usually due to mutations in the gene concerned, such as CDK4 or β-catenin. Recent advances in these strategies include the use of peptide-primed autologous dendritic cells as antigen-presenting cells, the use of improved adjuvants and the addition of cytokines such as GM-CSF, IL2 or → α-interferon. Cytokines may be administered systemically by subcutaneous infusion, or locally in the site of vaccination utilising autologous melanoma cells or tumor-infiltrating lymphocytes (TILs) transduced with a transgene constructs containing the cytokine gene. The latter approach constitutes the basis of a number of current gene therapy trials in metastatic melanoma.

Rational therapies

Surgery remains the mainstay of treatment for melanoma, and the screening and early detection of high risk groups remains a principle of melanoma control. Metastatic melanoma is relatively resistant to treatment with existing cytotoxic drugs and there is no evidence that any form of chemotherapy prolongs survival. The reason for this is probably the evolution within the tumor of robust and redundant mechanisms of inhibition of the apoptotic pathway. Partial responses to single agent chemotherapy occur in less than 25% of treated patients and complete responses in less than 5%. The single agent with the highest reproducible response rate is the alkylating agent dacarbazine (DTIC). These responses are usually short-lived (< 6 months).

Biochemical pathways of particular interest in the development of future rational and specific therapies for CM include receptor signaling pathways, cell-cycle regulation, angiogenesis, integrin expression and regulation of apoptosis. For example, the use of anti-sense molecules to bcl-2, which is over-expressed in 90% of melanomas, restores a high sensitivity to chemotherapy to melanoma cells (7). The promoter regions of the genes for tyrosinase and tyrosinase-related protein (TRP), relatively melanocyte-specific intermediates in the melanin synthetic pathway, offer potential targets for gene therapy approaches.

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Melanoma in Fish

Definition

→ Xiphophorus.

Melanotic Medulloblastoma

Definition

Melanotic medulloblastoma is a rare variant of medulloblastoma containing focal accumulations of melanin-containing epithelial cells next to typical medulloblastoma cells.

Melting

Definition

The melting of DNA refers to its denaturation.

Membrane Proteins

Definition

Membrane proteins have hydrophobic regions that allow part or all of the protein structure to reside within the membrane; the bonds involved in this association are usually noncovalent.

MEN 2

Definition

→ Multiple endocrine neoplasia type 2.

Menin

Definition

Menin is the protein encoded by the *MEN 1* gene; → multiple endocrine neoplasia type 1.

Meningioma

Definition

Meningioma is typically a benign tumor of the meninges; → brain tumors.

Merlin

Definition

Merlin is a protein encoded by the tumor suppressor gene eliciting → neurofibromatosis 2.

Mesenchymal Tumors

Definition

Mesenchymal tumors are tumors of connective tissue origin.

Mesenchyme

Definition

Mesenchyme is the meshwork of embryonic connective tissue that forms the connective tissues of the body.

Mesothelioma

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Synonyms

- malignant mesothelioma

Definition

Mesotheliomas are tumors derived from mesothelial cells that form the membranes surrounding the lungs, pericardium and peritoneum. Mesotheliomas are highly aggressive malignancies, with a median survival of 9 months from diagnosis. The incidence of mesothelioma is higher in men than in women (8:1) and these tumors usually occur during the 7th and 8th decades of life. Although mesothelioma is traditionally associated with occupational asbestos exposure, especially crocidolite asbestos, recent studies have linked this disease with a DNA tumor virus known as → Simian virus 40 (SV40). Rarely, mesotheliomas occur after radiation exposure and usage of Thorotrast. There are 2-3,000 cases of mesothelioma per year in the USA and more than 1,000 in England and Italy. Some countries, such as Finland, have a very low rate of mesothelioma, possibly because antophyllite, the form of asbestos used in Finland, is not oncogenic and/or because Finnish people have not been exposed to SV40-contaminated poliovaccines. The incidence of mesothelioma continues to increase in spite of measures adopted in the 1970s and 1980s to eliminate (Italy) or reduce (USA) the use of products containing asbestos.

Characteristics

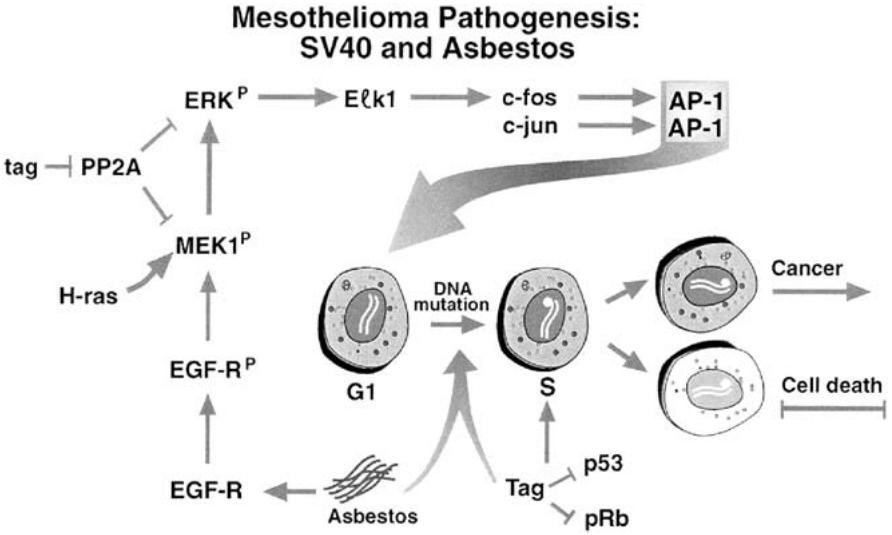
Pathogenesis

Asbestos. Epidemiological studies have clearly linked exposure to crocidolite → asbestos, a form of amphibole asbestos, to the development of mesothelioma. Other forms of amphibole asbestos, such as tremolite have also been linked to mesothelioma, but the risk is lower compared to crocidolite. Whether other forms of asbestos, such as antophyllite or chrysotile (the latter is also known as serpentine asbestos) cause mesothelioma is controversial. The mechanisms responsible for asbestos carcinogenicity are largely obscure. Asbestos does not transform human cells, including mesothelial cells *in vitro*; instead, when mesothelial cells in tissue culture are exposed to asbestos the cells die. Other cell types are much less sensitive to the toxic effects of asbestos. Asbestos has been shown to induce → AP-1, which may enhance cell division. When phagocytes ingest asbestos, these cells release oxygen radicals that can contribute to genetic damage. Finally, asbestos has been shown to impair the immune system, which could allow mesothelial cells transformed by SV40 to escape immunosurveillance. Up to 5% of asbestos miners develop mesothelioma. However, it is unknown how many mesotheliomas are associated with exposure above background in the general population. Asbestos is a ubiquitous carcinogen. Therefore, virtually everyone may have some levels of exposure. It is hypothesized that there is a threshold level of exposure above which the risk of developing mesothelioma increases. But the threshold is unknown and individual susceptibility and/or infection with SV40 may influence this threshold. It is generally accepted, however, that about 80% of mesotheliomas develop in conjunction with higher than background levels of exposure.

SV40. In animals, SV40 induces mesotheliomas at a much higher rate than does asbestos. 100% of hamsters injected in the pleural space develop and die of mesothelioma within 6 months, compared to approximately 20% of those injected with asbestos, which develop mesotheliomas after about 2 years. SV40 can transform human cells in tissue culture, and mesothelial cells are transformed at a rate that is much higher than in any other cell type tested. This effect appears to be related to the unusually high levels of the p53 tumor suppressor normally present in mesothelial cells. p53 binds and inhibits the SV40 oncoprotein, large T antigen (Tag), thereby limiting viral replication. Thus, in these cells infection is not lytic, as

observed in other human cell types. Instead, mesothelial cells are infected and SV40 establishes a 'parasitic' relationship within mesothelial cells, i.e., replicating without lysing the cells. SV40 has been detected in about 60% of human mesotheliomas, and Tag has been shown to bind and inhibit the tumor suppressors p53 and pRb. Microdissection, immunohistochemistry and *in situ* hybridization experiments have shown that SV40 is present in tumor cells and not in nearby stromal cells. Finally, *in vitro* experiments indicated that asbestos increases the carcinogenicity of SV40. Some of the molecular events thought to lead to malignant transformation of mesothelial cells are shown in the figure. These events are thought to be important during the initial steps of malignant transformation. However, during tumor formation and/or progression, additional genomic damage occurs which leads to some characteristic features of mesothelioma.

Chromosome changes. Experiments with rodent cells indicate that asbestos can interfere with normal chromosome segregation by interacting with the mitotic apparatus, which can lead to → aneuploidy. Furthermore, *in vitro* studies have shown that human mesothelial cells acquire extensive numerical and structural chromosomal abnormalities shortly after exposure to low concentrations of asbestos fibers.



Mesothelioma. Fig. – Working Model Depicting Possible Mechanisms Involved in the Pathogenesis of Mesothelioma. Arrows indicate stimulatory effect; crossed bars indicate inhibitory effect. Tag, SV40 large tumor-antigen; tag, SV40 small tumor antigen. Autophosphorylation of the EGF receptor, or activation of H-ras, etc., leads to phosphorylation of MEK1 kinase, which in turn phosphorylates ERK kinases (MAP kinases). Activation of these kinases leads to the activation of c-fos, c-jun and other members of the AP-1 family, which through their transcriptional activity stimulate cell division. The phosphatase PP2A downregulates AP-1 activity by dephosphorylating MAP kinases. Crocidolite induces both DNA damage and autophosphorylation of the EGF-receptor, which leads to increased levels of c-fos and c-jun expression, AP-1 activation and cell division. When Tag is expressed, it binds and inhibits p53 and pRb; it also contributes to the development of DNA alterations (Tag is known to be directly and indirectly - through p53 inactivation - mutagenic). When SV40 tag is also expressed, it binds and inactivates PP2A and contributes to cellular transformation. In this model, crocidolite may increase the amount of c-fos and c-jun and to some extent their activity, while tag further increases their activity. If dividing cells have developed DNA damage through the mutagenic effects of asbestos and/or Tag, these mutations may not be repaired, because of the Tag inactivation of the G1/S checkpoint mediated by p53 through p21. In this scenario, the cell may complete division and possibly continue to divide. The combined effects of asbestos and SV40 may overwhelm the cell cycle control mechanisms and result in unregulated cell division and, occasionally, in cellular transformation, which may lead to mesothelioma development.

SV40 can also cause genetic damage. SV40 Tag is known to be mutagenic and clastogenic as it can cause point mutations, chromosome rearrangements and aneuploidy. While many of the DNA alterations caused by asbestos or SV40 will either be of no significance or lead to cell death, a few cells could potentially develop perturbations of key cell cycle regulatory genes, leading to tumor formation and/or progression.

Karyotype studies and comparative genomic hybridization (CGH) analyses have revealed multiple chromosome alterations in most human mesotheliomas. Although a specific chromosomal change is not shared by all mesotheliomas, several prominent sites of chromosomal loss have been identified. Deletions of specific regions in the short (p) arms of chromosomes 1, 3 and 9 and long (q) arm of 6, 13 and 15 are repeatedly observed in these tumors. Loss of a copy of chromosome 22 is the single most consistent numerical change seen in mesotheliomas. Monosomy 4 and monosomy 14 are also common. These recurrent losses frequently occur in combination in a given tumor. Chromosomal gains appear to be less common than losses in this disease, although recurrent gains of 5p and 7 have been reported. It is not possible to determine a temporal sequence of these pathogenetic events due to the lack of data for early stages of this disease. The high frequency of genomic losses in mesothelioma is consistent with a recessive mechanism of oncogenesis. Loss and/or inactivation of tumor suppressor genes residing in recurrent sites of chromosomal deletion are thought to contribute to the development and progression of mesothelioma. As a prelude to the isolation of these genes, investigators have begun to molecularly map these regions by performing → loss of heterozygosity (LOH) analysis with polymorphic DNA markers. Such molecular studies have demonstrated high frequencies of allelic loss from 1p22, 3p21, 4q, 6q, 9p21, 13q13-14 and 15q11-15. To date, tumor suppressor gene loci within two of these regions have been shown to be frequently altered in mesotheliomas.

Tumor suppressor genes

A high frequency of mesothelioma cell lines exhibit homozygous deletion of the 9p21 region. The → *CDKN2A* locus, which encodes the alternative tumor suppressor gene products $p16^{INK4a}$ and $p14^{ARF}$, is located in this region. The $p16^{INK4a}$ protein is capable of binding to the cyclin dependent kinase CDK4, thereby inhibiting the catalytic activity of the CDK4/cyclin D enzymes. Therefore, loss/inactivation of $p16^{INK4a}$ leads to cell-cycle deregulation through the loss of a key inhibitor of G1/S progression. More than 80% of mesothelioma cell lines have homozygous deletions of one or more $p16^{INK4a}$ exons, and most of the remainder have greatly down-regulated expression of $p16^{INK4a}$ due to epigenetic mechanisms, i.e., promoter hypermethylation. Homozygous deletions of $p16^{INK4a}$ have been documented at a much lower frequency in mesothelioma tissues than in cell lines. This discrepancy may be an *in vitro* phenomenon resulting from the selective advantage provided by $p16^{INK4a}$ deletion during the culturing process. On the other hand, mesothelioma samples often contain a significant amount of contaminating normal stroma that can mask the existence of a homozygous deletion in the malignant cell population. Immunohistochemistry studies suggest that loss of $p16^{INK4a}$ expression is a universal finding in mesothelioma tissues. Fluorescence *in situ* hybridization analysis has revealed absence or reduced copy numbers of the $p16^{INK4a}$ gene in most mesothelioma specimens. Taken collectively, the available data suggest that loss of at least one copy of $p16^{INK4a}$ occurs in most of these tumors, with the remaining allele silenced by promoter hypermethylation. In a given tumor, all or a subset of cells may contain homozygous loss of $p16^{INK4a}$, with these cells presumably having a proliferative advantage when placed into long-term culture. Importantly, alteration of $p16^{INK4a}$ appears to play a critical role in mesothelial cell tumorigenesis, since re-expression of $p16^{INK4a}$ in mesothelioma cells has been shown to result in cell-cycle arrest and → apoptosis, as well as inhibition of tumor formation or diminished tumor size.

Homozygous deletion of $p16^{INK4a}$ also leads, in most cases, to the inactivation of $p14^{ARF}$ since these two genes share exons 2 and 3, although their reading frames differ. The product of the $p14^{ARF}$ tumor suppressor gene is required for activation of p53 in response to the action of oncogenes such as *RAS*. Since the product of the $p16^{INK4a}$ gene induces a G1 cell-cycle arrest by inhibiting the phosphorylation of → pRb, homozygous loss of $p14^{ARF}$ and $p16^{INK4a}$ would collectively affect both the p53- and pRb-dependent growth regulatory pathways, respectively. Recent experiments have demonstrated that adenovirus-mediated transfer of $p14^{ARF}$ in mesothelioma cell lines induces G1-phase arrest and apoptosis. Together, the available data suggest that alteration of either product of the *CDKN2A* locus, i.e., $p14^{ARF}$ or $p16^{INK4a}$, contributes to the pathogenesis of mesothelioma. The success of the experiments with $p14^{ARF}$ and $p16^{INK4a}$ adenoviral constructs have led investigators to propose that such → gene therapy based approaches may prove useful in the treatment of mesothelioma patients.

Extensive LOH analysis of chromosome 22 losses in mesothelioma has not been performed because an entire copy of chromosome 22 is lost in most cases. Although the → neurofibromatosis 2 tumor suppressor gene, *NF2*, predisposes affected individuals primarily to tumors of neuroectodermal origin, somatic mutations of *NF2* have occasionally been identified in seemingly unrelated malignancies. *NF2* somatic mutations predicting either interstitial in-frame deletions or truncation of the *NF2* gene product (merlin or schwannomin) have been reported in 40%-55% of mesothelioma cell lines. In many cases, it was possible to confirm the mutation in matched primary tumor DNA. In some samples that showed *NF2* gene transcript alterations, no genomic DNA mutations were detected, suggesting that aberrant splicing may constitute an additional mechanism for merlin inactivation. Western blot analyses revealed complete absence of merlin expression in cell lines that exhibited alterations of the *NF2* gene, suggesting that truncated forms of the protein are unstable. LOH analyses documented allelic losses at the *NF2* locus in more than 70% of mesothelioma cases. All cases exhibiting mutation and/or aberrant expression of *NF2* showed allelic losses, implying that inactivation of *NF2* in mesothelioma occurs via a 'two-hit' mechanism.

Crocidolite asbestos has been shown to induce the proto-oncogenes *c-fos* and *c-jun*, which encode transcription factors (→ AP-1) that activate various genes critical in the initiation of DNA synthesis. The persistent induction of these transcription activators following asbestos exposure may enhance cell division. Mesothelioma may begin with stem cell proliferation via such an epigenetic mechanism, followed by the progressive accumulation over several decades of spontaneously occurring mutations in tumor suppressor genes and asbestos-induced missegregation of chromosomes. Thus, such activation of proto-oncogenes and inactivation of tumor suppressor genes may cooperate in a multi-step process to regulate critical events intrinsic to the pathogenesis of mesothelioma.

Diagnosis

Chest pain and accumulation of fluid in the pleura or abdomen are often the first symptoms. Radiological tests, thoracoscopy or laparoscopy reveal that the patient has a tumor, but the final diagnosis relies on pathology. Because of their undifferentiated state, mesothelial cells can evolve along either an epithelial type or a fibroblastic type of differentiation. Thus, morphologically mesotheliomas are distinguished as epithelial and sarcomatous, the latter also known as fibrous or spindle cell mesothelioma. Sufficient sampling, however, will often reveal both components, thus the terminology of biphasic or 'mixed type' mesothelioma. The exact percentage of biphasic mesotheliomas is unclear because different pathologists use different criteria to consider a mesothelioma biphasic: some will call biphasic any tumor in which both components can be identified, others require the presence of a given percentage of the minor component. In any case, those mesotheliomas with a predominant spindle component behave like fibrous mesotheliomas, and those who have a predominant epithelial component have longer survivals. Histologi-

cally, epithelial mesotheliomas must be differentiated from carcinomas of the lung and breast, biphasic mesotheliomas from synovial sarcoma, and sarcomatous mesotheliomas, from other types of sarcomas. Electron microscopy (EM) reveals long branching microvilli in epithelial mesotheliomas and is very useful to distinguish these tumors from carcinomas. Immunohistochemistry also helps to distinguish mesotheliomas from carcinomas. Almost all epithelial mesotheliomas stain positive for calretinin, about 50% are positive for SV40 Tag, have membranous staining with HBME-1 and are negative for CEA, LeuM1, BerEp4, and B72.3. Carcinomas are positive for at least two among CEA, LeuM1, BerEp4, and B72.3, have cytoplasmic staining for HBME-1 and are usually negative for calretinin and SV40 Tag. Synovial sarcomas are often positive for BerEp4 and have a characteristic X;18 translocation. Sarcomatous mesotheliomas stain positive for cytokeratin, which distinguish them from other sarcomatous tumors of the pleura. All mesotheliomas are malignant. However, histologically malignant mesotheliomas have occasionally been associated with long survivals of years or even decades. Whether the latter should be called mesotheliomas is debatable. There are also some unrelated tumors called 'benign mesotheliomas', which are histologically different from mesotheliomas and are clinically benign. It is unfortunate that the term mesothelioma is often used to identify these lesions because it generates confusion and patient anxiety. Multicystic mesothelioma, also called multilocular peritoneal inclusion cyst, is a totally benign mesothelial lesion characteristically formed by multiple cysts arranged in grape-like clusters. Adenomatoid mesotheliomas are benign mesothelial lesions of the genital system. Mesothelioma of the atrioventricular node is neither a mesothelioma nor a tumor. This lesion represents congenital heterotopia of the endodermal sinus in the atrioventricular node. Well differentiated papillary mesothelioma is found more often in the abdominal cavity of young women. Histologically, it is formed by multiple papillary structures covered by cytologically benign mesothelial cells. The lesion is benign but there are very rare cases in which several years after diagnosis the patient developed a true mesothelioma. Localized mesothelioma, better referred to as localized fibrous tumor of the pleura, is thought to originate from the submesothelial cells. The tumor cells have a benign fibrous appearance and are characteristically negative for cytokeratin and positive for CD34. Occasionally, localized fibrous tumors of the pleura are malignant and are characterized by multiple recurrences after resection and are histologically and cytologically malignant.

Clinical approaches

To date, no therapy has been definitively shown to significantly influence the natural course of this tumor. Patients with early stage disease are good candidates for surgical resection. Surgery should be performed by surgeons experienced with this type of procedure to reduce the risk of operative mortality, which in experienced hands is about 2%. Either pleurocotomy or extrapleural pleurectomy has the potential to cure very early disease. However, mesotheliomas are usually diagnosed at an advanced stage when curative resection is not possible. Chemotherapy has been disappointing to date, although new drugs are presently being tested alone or in combination with conventional chemotherapeutic agents. The finding that a substantial percentage of mesotheliomas contains SV40 Tag has led to the development of immunotherapy and gene therapy approaches, aimed at eliminating Tag-positive mesothelial cells, which will be tested in future clinical trials.

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MET

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Definition

Met is a member of the → receptor tyrosine kinase family. Like other members of this protein family, Met possesses a highly glycosylated extracellular ligand binding region, a hydrophobic membrane spanning region, an intracellular region that contains a tyrosine kinase domain and a C-terminal multisubstrate binding site that mediates interactions with several signal transduction pathways upon receptor activation. Activation of receptor tyrosine kinases results from binding a protein growth factor. The growth factor that activates Met is hepatocyte growth factor/scatter factor, referred to as HGF/SF, as it was identified independently as both a growth factor for hepatocytes (HGF) and as a fibroblast-derived cell motility factor, scatter factor (SF). It was later discovered that HGF and SF were identical proteins and HGF/SF-Met signaling can induce different biological affects depending on the cell context. Since then, HGF/SF-Met signaling has been implicated in a variety of cellular responses including proliferation, motility, invasion, chemotaxis, and morphogenic differentiation, and regulates a diverse series of biological processes ranging from lumen formation to neuronal development to tumor cell invasion and metastasis.

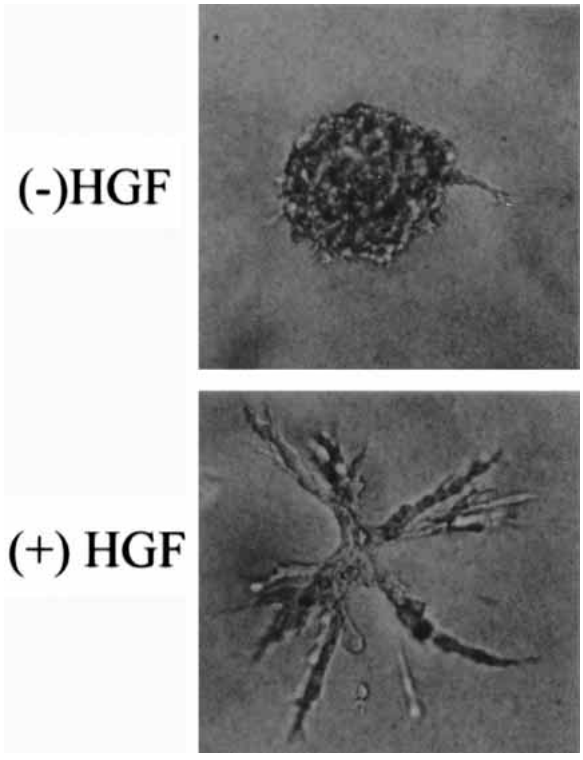
Characteristics

Met was identified in the early 1980s as an oncogene. A chromosome rearrangement resulted in the fusion of the N-terminal protein-protein dimerization motif of *tpr* (translocated promoter region) to the C-terminal tyrosine kinase domain of *met*. The resulting chimeric protein, Tpr-Met, has high constitutive tyrosine kinase activity and can potently transform cells *in vitro*. Isolation of the *tpr-met* cDNA led to the identification of the full length *met* receptor.

Human *met* spans over 120 kilobases of genomic sequence and consists of 21 exons, which when spliced together produce a primary mRNA transcript that encodes the complete receptor. Several *met* splice variants have been identified, but the physiologic role of these variants is not well understood. Mature, full-length, human Met is produced by proteolytic cleavage of a single 1408 amino acid, partially glycosylated precursor protein into an N-terminal α-chain, which is entirely extracellular, and a larger C-terminal β-chain. The β-chain consists largely of an extracellular portion, a membrane spanning segment and an intracellular region that contains the tyrosine kinase domain and the multisubstrate binding site. Similar to other receptor tyrosine kinases, such as the insulin receptor, the α- and β-chains of Met are joined via a disulfide linkage and further glycosylated during transport to the cell surface. While at the cell surface, Met clusters together and may interact with cell adhesion molecules, such as cadherin, to help regulate cell-cell interactions.

Met is normally expressed in the epithelial cells of many embryonic and adult organs (kidney, liver, lung, skin, stomach and placenta), while HGF/SF expression is usually restricted to the surrounding mesenchyme (fibroblast stroma and other mesenchymal cells). Several aspects of organogenesis, such as tissue growth and morphogenic differentiation, are regulated by interactions between the organ epithelia and the surrounding mesenchyme (1). Endocrine mediated signaling between HGF/SF and Met is believed play an important role in regulating normal epithelial-mesenchyme interactions. If HGF/SF-Met signaling is inhibited by gene dis-

ruption in mice, the mouse embryos die in early gestation with several defects in liver and placental organogenesis. Furthermore, while not directly observed in the mouse mutants (partly due to the early embryonic lethality and partly due to compensation by other factors), HGF/SF-Met signaling has been implicated in regulating other epithelial-mesenchyme interactions such as mammary gland duct formation, lung tubule formation and kidney development in organ culture models. In addition, several epithelial cell types undergo significant morphogenic differentiation to form branched tubule structures complete with interior lumens when grown in a 3-dimensional matrix and stimulated with HGF/SF (Fig. 1). These tubule structures are very similar to structures found *in vivo* and in order to form require complex interactions between cell proliferation, survival, movement, (requiring both motility and matrix degradation) and differentiation. While other growth factors such as epidermal growth factor (EGF) may induce proliferation and/or movement, HGF/SF is somewhat unique in its ability to induce all of the cellular responses required for branching morphogenesis.

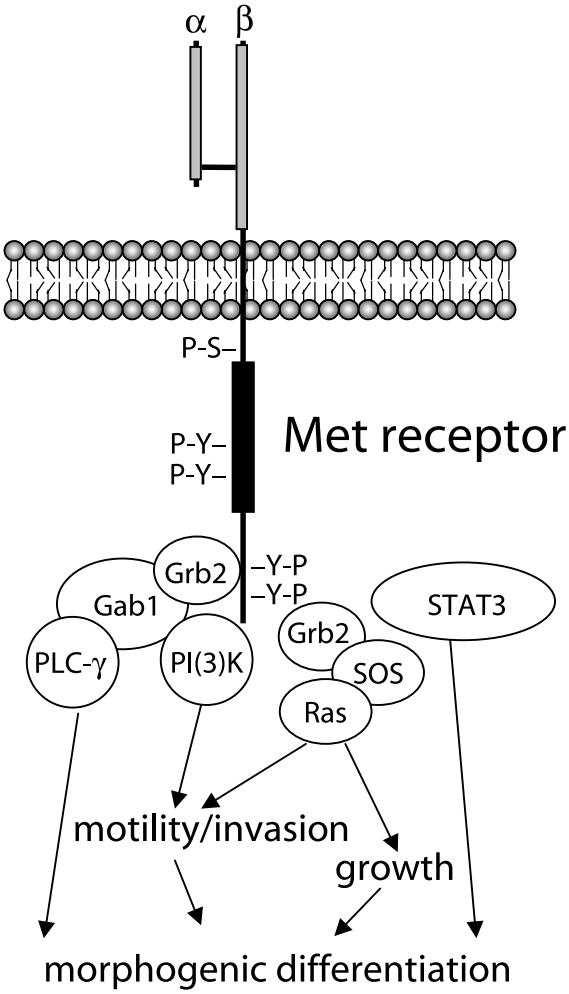


MET. Fig. 1 – Met induced branching morphogenesis of cells grown in a 3-dimensional matrix. Several epithelial cell lines (cell line C127 is shown) that express Met undergo significant changes in cellular morphology and 3-dimensional organization following stimulation with HGF/SF.

However, HGF/SF-Met signaling is not exclusively involved in regulating epithelial morphogenesis. Met is also expressed in skeletal muscle and in the developing nervous system, and HGF/SF-Met signaling is required for the migration of myogenic precursors in limb bud formation and has been implicated in neuronal development. HGF-Met signaling also stimulates the proliferation of hepatocytes, renal epithelial cells and vascular endothelial cells, and increases the motility of both epithelial and vascular endothelial cells and may play a significant role in wound repair and tissue regeneration. The ability of HGF/SF to induce increased growth, motility and capillary-like tubule formation in vascular endothelial cells *in vitro* and to promote blood vessel formation *in vivo* suggests that Met may stimulate angiogenic processes as well.

Cellular and molecular features

Normal Met activation by HGF/SF is believed to occur through receptor dimerization and induction of transphosphorylation of tyrosine residues, which are critical for growth factor mediated signal transduction (Fig. 2). Much work has been done to determine which of the phosphorylated tyrosine residues in the intracellular region of Met (there are 16 of them) are important for stimulating cellular responses. Phosphorylation of two tyrosines located within the activation loop of the tyrosine kinase domain (amino acid positions 1242 and 1243 in human Met) greatly enhances the intrinsic kinase activity of the receptor and are critical for potent receptor activation. Phosphorylation of two closely spaced tyrosines at the C-terminal of Met (amino acid position 1,367 and 1,374 in human Met) generates a multisubstrate docking site that several SH2-, PTB- and MBD-domain containing signal transduction and adaptor proteins can bind (2). Proteins that bind to this region include phosphotidyli-



MET. Fig. 2 – The Met receptor tyrosine kinase stimulates multiple signal transduction pathways. The Met receptor is a membrane spanning α/β heterodimeric protein that binds the HGF/SF growth factor. The intracellular region of Met contains a tyrosine kinase domain (dark box). Phosphorylation of tyrosine residues (-Y-P) within this domain, following growth factor binding and receptor dimerization, activates the tyrosine kinase domain while phosphorylation of a serine residue (-S-P) near the membrane spanning region can inhibit the kinase activity of this receptor. Phosphorylation of tyrosine residues in the C-terminal end of Met activates a multisubstrate binding site that mediates interactions with several signal transducing and adapter proteins to stimulate growth, motility, and morphogenic differentiation. However, cells that contain abnormally active Met as a result of gene mutation, receptor overexpression or constant HGF/SF stimulation, are frequently highly invasive *in vitro* and both, tumorigenic and metastatic *in vivo*.

nositol-3-OH kinase (PI(3)K), Grb2, Gab1, SHC and phospholipase C-γ (PLC-γ). Several assays that monitor HGF/SF-Met meditated cell proliferation, motility/scattering and branching morphogenesis have been instrumental in identifying the signaling pathways that are responsive to Met activation. However, detailed analysis of which protein pathway mediates each of these cellular responses has been complicated by prevalent signaling pathway cross-talk and a limited knowledge of specific downstream substrates. Generally, activation of the Ras-MAP kinase pathway via the Grb2/SOS complex is important for both cell proliferation and motility, while activation of PI(3)K, the Rac/Rho pathways and disassembly of cadherin/catenin complexes at the cell surface promotes loss of intercellular adhesion and gain of cell motility. Induction of epithelial morphogenesis likely requires additional factors including Gab1, PLC-γ and STAT3. Overexpression of Gab1 can stimulate both cell scattering and branching morphogenesis in the absence of HGF/SF, possibly by sustained recruitment/activation of PLC-γ. Activated Met also recruits and tyrosine phosphorylates the Stat-3 transcription factor. Subsequent nuclear translocation and site specific transcription of Stat-3 is also involved in epithelial tubule formation.

As several of the proteins activated by Met such as Ras have also been implicated in tumor generation and progression, down-regulation of the activated Met signal is important to prevent hyperactivation of these protein pathways and subsequent cellular transformation. One important means of down-regulating growth factor receptors is degradation of the active receptors. Endocytosis can clear activated growth factor receptors either transiently, if they are recycled back to the cell surface, or permanently if they are sorted to the lysosome and degraded. Alternatively, activated growth factor receptors can be ubiquitin-tagged and degraded by the proteosome. Upon ligand binding, Met is rapidly endocytosed and polyubiquitinated. The combination of endocytosis and ubiquitination ultimately leads to degradation of the receptor and down-regulation of Met signaling. Another way to attenuate Met signaling is by phosphorylating a serine residue located in the receptor

juxtamembrane region (serine 1003 in human Met). Phosphorylation of this serine by protein kinase C (PKC) inhibits the tyrosine kinase activity of Met and may also be used to downregulate Met signaling.

Clinical Relevance

While Met plays several important roles in normal growth and development, abnormal Met signaling likely contributes to generation and progression of human tumors (3). As previously mentioned, Met was originally isolated as the constitutively activated oncoprotein Tpr-Met. However, Met missense mutations have been implicated in the cause of hereditary and sporadic human papillary renal carcinoma (HPRC). The missense mutations are located in the tyrosine kinase domain of Met and produce a constitutively active receptor that promotes tumor formation and/or progression. In the absence of an activation mutation, Met and HGF/SF have now been identified in most solid tumors and may play a major role in invasion and metastsis. Met can also become constitutively activated by co-expression of HGF/SF in the same cell, forming an autocrine stimulatory loop. Numerous tumor types, including several different carcinomas (lung, breast and others), sarcomas (osteosarcoma, → Kaposi sarcoma and others) and tumors of the brain (gliomas), express Met and/or HGF/SF. In model systems, Met-HGF/SF-expressing cells are not only invasive *in vitro*, but also rapidly metastasize when injected into mice. Part of the mechanism behind the invasive phenotype of HGF/SF-Met expressing cells is upregulation of the urokinase proteolysis network. Induction of the proteolysis network following Met activation can degrade the surrounding extracellular basement membrane and facilitate invasion and metastasis.

Overexpression of Met is also observed in several gastric carcinoma cell lines as well as carcinomas of the lung, pancreas, thyroid, colon and stomach and is thought to participate in cell transformation and tumorigenicity. As HGF/SF has been found in tumor stroma and normal human serum it is possible that activation of Met in some of these cells may result from paracrine or endocrine stimulation. The paracrine model may be responsible for some breast cancers. Identification of HGF/SF and Met in breast cancer tumors is a strong negative prognostic indicator of reoccurrence and survival. The effects of HGF/SF in tumor progression may in part result from an increase in tumor angiogenesis. Invasive breast cancers that contain high levels of HGF/SF or brain tumors that are engineered to overexpress HGF/SF have increased microvessel density and increased levels of other vascular markers. Other effects, such as sustained proliferation and/or misregulated differentiation, may also contribute significantly to HGF/SF-Met mediated tumor growth/progression. As such, treatments that inhibit HGF/SF-Met signaling may be therapeutic in preventing the onset and progression of many cancer types and may also play significant roles in preventing cell metastasis and angiogenesis in these cancers.

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Metabolic Activation

Definition

Metabolic activation is the process by which a carcinogen is converted into a more reactive form that can bind to DNA.

Metabolic Detoxification

Definition

Metabolic detoxification is the process by which a carcinogen is converted to a form that is excreted without reacting with DNA; → detoxification.

Metabolic Polymorphisms

Definition

Metabolic polymorphisms are polymorphisms of genes encoding enzymes involved in the metabolism of carcinogens or anticarcinogens. These are predisposing gene variations with relatively low penetrance that may result in a moderate increase in the risk of specific cancers. The scientific interest in such genes (e.g. sequence alterations in genes encoding cytochrome p450's, null-polymorphisms for genes encoding glutathione S-transferases, or phenotypic alterations of the activities of N-acetyltransferases) is based on the possibility of identifying population subgroups that are at elevated risk of developing environmentally induced cancer.

Metabolic Polymorphisms and Cancer Susceptibility

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Definition

The genetic basis for inter-individual differences in cancer susceptibility.

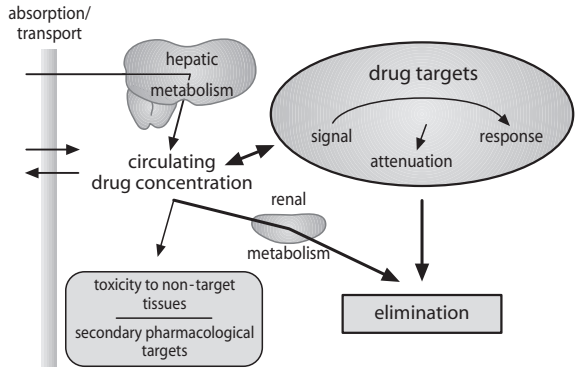
Characteristics

History

The term → pharmacogenetics has been used for approximately the last 40 years to describe genetic or inherited factors that determine individuality in response to drugs and other therapeutic agents. In studies starting in the mid-1950s, it became apparent that some individuals were hypersensitive to certain drug treatments. This sensitivity was also observed within other family members and was subsequently shown to be an inherited trait. Early epidemiological studies were focused on the drugs used in the treatment of malaria and tuberculosis. Inter-individual variation in the ability to metabolise these drugs was subsequently attributed to inherited differences in the enzymes glucose 6-phosphate dehydrogenase, which generated sensitivity to the anti-malarial drug primaquine, and in the N-acetyl transferases, which generated sensitivity to antitubercular drugs such as isoniazid. The term pharmacogenetics was first coined by Vogel in the late-1950s. In the 1960s and 1970s there was increased interest in this research area and many genetic variants in a range of genes involved in determining drug response were identified.

Important genetic factors

In order to elicit a therapeutic response, a drug first needs to enter the circulation, i.e. to cross the gastrointestinal (GI) tract. We now know that the entry of many drugs into the body is regulated by a multi-gene family of drug transporters that have the capacity to pump drugs in and out of cells. In addition, the GI tract also expresses enzymes that can metabolise and in-



Metabolic Polymorphisms and Cancer Susceptibility. Fig. – Factors which can limit the therapeutic effectiveness of drugs.

activate drugs. Variability in the level of expression or activity of these drug metabolising enzymes is therefore an important determinant of circulating drug concentrations. Once a drug enters the body it is transported to the liver, which is the main organ involved in drug → metabolism. Nearly all therapeutic agents are subject to hepatic metabolism before they are eliminated from the body. The rate of metabolism will determine the biological half-life of the drug. Hepatic drug metabolism is carried out by many multi-gene families of proteins. Of key importance are the cytochrome P450-dependent monooxygenases that convert lipophilic drugs into more water-soluble products predominantly through hydroxylation reactions. Many anti-cancer drugs are substrates for one or more of the P450 monooxygenases (Table 1). The products of these P450-catalysed reactions are further conjugated to co-factors such as glucuronic acid or sulphate, further increasing their water solubility and facilitating elimination in the bile or via the kidneys in the urine. The rate of uptake of drugs into liver cells and their rate of metabolism is therefore of central importance in determining therapeutic outcome. Finally, the capacity of a drug to exert its therapeutic effect is determined by its concentration at the target cell and the level of expression and activity of specific receptors and enzymes with which it interacts.

Pharmacogenetic variability resulting in altered drug response can involve any or all of these pathways, and allelic forms [→ alleles] of many drug metabolising enzymes, drug transporters and drug receptors are now known to exist within the population. In addition to individual genetic variability, for certain types of therapy genetic variability of the target cell will also be a key determinant of therapeutic efficacy. This is particularly the case in cancer chemotherapy where the genetics of the tumour cell is often a major additional determinant of the effectiveness of drug therapy. This is also of central importance in the chemotherapy of infectious agents and in the use of antibiotics, antiviral agents, etc. In these cases, drug resistance is well characterised and has been ascribed to altered patterns of gene expression in the target organism (6). Interestingly,

Metabolic Polymorphisms and Cancer Susceptibility. Table 1 – Anticancer P450 drugs.

6-aminochrysene	flutamide	retinoic acid
anastrozole	genistein	*all-trans*-retinoic acid
artemisinin	halomon	senecionine
BCNU	ifosfamide	tamoxifen
O_6-benzylguanine	irinotecan	tangeretin
bropirimine	irsogladine	tauromustine
coumarin	liarozole	taxol
cyclophosphamide	lomustine	teniposide
dexamethasone	lovastatin	tirapazamine
docetaxel	mofarotene	toremifene
ellipticine	17α-methyltestosterone	trofosfamide
(−)-epicatechin-3-gallate	nilutamide	vesnarinone
ethyl carbamate	onapristone	vinblastine
ethynylestradiol	oncostatin M	vincristine
etoposide	prednisolone	vindesine
flavone acetic acid	prednisone	vinorelbine

many of the factors which determine drug response in these organisms are the same as those that determine circulating drug levels in man, i.e. drug transporters and drug metabolising enzymes.

Relevance of pharmacogenetics

The importance of pharmacogenetics is well illustrated by studies of genetic variability in the cytochrome P450 system, in particular in cytochrome P450 CYP2D6. In the 1970s, clinical trials identified a group of people who were hypersensitive to certain types of drug including sparteine, an anti-hypertensive agent which is prescribed for the treatment of arrhythmia and debrisoquine. It was subsequently shown that this variability in response was inherited and that between 5 – 10% of the white population, 'poor metabolisers', do not express a functional form of CYP2D6. In later studies, the molecular basis of this polymorphism was elucidated and shown to be due to gene-inactivating changes in CYP2D6 DNA. This subsequently allowed the development of DNA-based tests to identify individuals with this metabolic defect. Cytochrome P450 CYP2D6 is responsible for the metabolism of up to 25% of therapeutic drugs, including a large number of the drugs used in the treatment of psychiatric illness (Table 2). This polymorphism has now been attributed to aberrant metabolism, and disposition of many of the drugs has been associated with adverse drug reactions induced by many of these agents.

In addition to gene inactivating alleles of CYP2D6, certain individuals contain multiple copies of this gene that generates an 'ultra-rapid metaboliser' phenotype. These individuals metabolise and eliminate drugs at a much faster rate than the rest of the population and as a result the desired therapeutic effect is not achieved. Therefore, pharmacogenetic variability can result in adverse drug reactions, exacerbated drug toxicity or lack of therapeutic effect.

Ethnic differences

The genes and proteins that determine therapeutic outcome following drug treatment evolved to protect us against the effects of toxic environmental chemicals. Part of the genetic variability in response to these agents may be explained by different populations needing a different spectrum of enzymes to cope with their particular environment and diet. This is exemplified by the large variability in the distribution of pharmacogenetic polymorphisms between different ethnic groups. For example, there is an allele of CYP2D6 that is present in 40% of the Chinese population but is not found in the white population. This allele generates a slower metaboliser phenotype and explains why a high percentage of the Chinese population cannot tolerate doses of drugs prescribed routinely in Europe and the Western world.

The future

The current aim of research in pharmacogenetics is to exploit the information from the Human Genome Project to identify novel variant forms of genes, to establish their functional significance and to apply these usefully in the clinical environment. The ability to identify rapidly and test for variant sequences of genes will allow pharmacogenetic testing to be generally applied in medical practice so that optimal drug doses can be used. This is particularly the case in the treatment of diseases such as cancer

Metabolic Polymorphisms and Cancer Susceptibility. Table 2 – CYP2D6 drugs used in the treatment of psychiatric, neurological and cardiovascular disease.

psychiatric disease	amitriptyline, clomipramine, clozapine, desipramine, fluvoxamine, fluoxetine, haloperidol, imipramine, levomepromazine, nortriptyline, olanzapine, paroxetine, perphenazine, thioridazine, tranylcypromine, zuclopenthixol
cardiovascular disease	alprenolol, amiodorine, flecainide, indoramine, mexiletine, nimodipine, oxprenolol, propranolol, timolol

where inappropriate drug dosing is often life threatening and the success or failure of treatment may depend on the genetics of the tumour cell. It is anticipated that with the identification of pharmacologically important genetic variations within the population, rapid tests will be available to hospitals that will allow clinicians to prescribe drugs at optimal dose regimens or to decide whether specific drug therapies will be effective for individual patients.

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Metabolism

Definition

Metabolism comprises the chemical reactions that occur in the cell; individuals can be.

- Extensive metaboliser: an individual with the ability to metabolise drugs at a rate that falls within the normal population distribution.
- Poor metaboliser: an individual with a genetically determined deficiency in their ability to metabolise certain drugs.
- Ultra-rapid metaboliser: an individual with increased expression of a particular gene. Drugs which are substrates for these genes are metabolised very rapidly, often requiring increased doses to achieve any therapeutic benefit; → metabolic polymorphisms and cancer susceptibility.

Metalloproteinases

Definition

Metalloproteinases are a family of extracellular enzymes that depend on Ca^{2+} or Sn^{2+}. They are involved in tumor → progression, where they degrade several components of the extracellular matrix during metastasis of tumor cells.

Metaplasia

Definition

Metaplasia is the abnormal transformation of a differentiated tissue of one kind into a differentiated tissue of another kind.

Metastasis

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Definition

Metastasis is the process by which tumor cells disseminate to distant sites to establish discontinuous secondary colonies. The metastatic cascade refers to the series of sequential steps by which tumor cells migrate from a tumor mass, enter a circulatory compartment (e.g., blood vessel or lymphatic), transport to the secondary site, arrest, exit the circulation and proliferate at the secondary site.

Characteristics

At the time that a cancer patient is first diagnosed with the disease, on average half will already have identifiable metastases. The initial clinical evaluation of a cancer patient is designed to search for evidence of these metastases. Each type of cancer has a distinct pattern of spread. For example prostatic carcinoma tends to metastasize to pelvic lymph nodes and to the bones. In contrast colon carcinoma tend to metastasize first to the colonic lymph nodes, and then to the liver and finally the lungs and brain. Breast cancer often will be found in the regional or nearby lymph nodes, lungs, liver, bone, brain, adrenal glands and ovaries. Thus the clinical evaluation for each cancer will be tailored to look for metastases at the most likely places for that particular form of cancer.

Some cancers are much more likely to have metastasized at the time of diagnosis than others. For example squamous cell carcinomas of the skin rarely metastasize, while small cell carcinomas of the lung almost always have spread by the time of diagnosis. As will be noted below, the presence of metastasis greatly alters a patient's treatment options and prognosis.

The metastatic tumor cells travel by two main routes to get to locations distant from the original tumor. In lymphatic spread, the tumor cells enter the lymphatic vessels and this route usually results in the tumor spreading to lymph nodes adjacent to the tumor. The pattern of the lymphatic tumor spread can be predicted by knowledge of the anatomy of the lymphatics at that site. Tumors also use the blood stream. This route results in the more distant spread to sites far from the original tumor and is called hematogenous metastasis.

When a tumor has metastasized to another site, one might ask how can you tell that it is a metastasis and not another primary cancer. In practice this can sometimes be a difficult distinction. The determination rests upon the fact that the metastases retain the characteristics of the original tumor and its tissue type and do not take on the characteristics of the new tissue or organ. For example a prostatic tumor metastasis may continue to make prostate specific antigen (PSA) and the presence of this marker will show that the tumor originated in the prostate. Sometimes to distinguish from a new primary in the same organ such as a second breast carcinoma in the same breast or a second liver tumor from a metastasis or hepatocellular carcinoma in the liver may not be easy with the clinical tools currently used.

Another feature of some metastatic cancers is that the metastases may not reveal themselves immediately, but can remain dormant for many years. This is a notable feature of breast cancer where it is not uncommon for metastases to remain dormant for five or ten years and the reappearance of metastases after as long as twenty years has been described. On the other hand, this feature of latency is unusual with other types of cancer such as many lung cancers.

Cellular and molecular aspects

To some extent the set of genes that must be expressed for metastasis to occur differs from those that are required merely for tumor growth. The ability to isolate cells that can or can not metastasize from the same tumor demonstrates that the metastatic phenotype can be separated from the ability to form tumors. Particular genes have been identified as being required for metastasis, but not necessary for primary tumor expression, and conversely the decreased expression of other genes is also necessary for metastasis. For example, a surface molecule called E-cadherin, which functions in normal tissues to form the bridges that hold the cells together, is often found to be absent in tumors that metastasize. The loss of expression of this gene could act simply to loosen the attachments between cells and this weakened aggregation allowing movement of the cells away from the primary tumor. In addition, there is more recent evidence that the interactions of the E-cadherin molecule on the surface with other molecules inside the cell leads to signaling that affects the transcription of genes. Therefore, the absence of E-cadherin not only changes the ability of the tumor cells to attach to its neighbors but may also drastically alter the expression of other genes affecting the

malignancy of the tumor and its ability to metastasize.

Proteases or enzymes that cleave other proteins also have been shown to affect the ability of cells to metastasize. Plasminogen activator, an enzyme that activates plasmin and can dissolve blood clots, is required for metastasis in some tumor cells. This enzyme too appears to have a dual function in metastasis, both enzymatically cleaving other proteases that are also required for metastasis and a signaling function in binding to a surface receptor, the plasminogen activator receptor. This receptor is more frequently expressed on metastatic tumor cells, while the plasminogen activator is often made by the host's own normal cells and then secreted where it binds to the tumor cell receptor.

Another class of proteinases, the matrix metalloproteinases, may also play a role in metastasis. These enzymes are often overexpressed in tumors. They have the capability to degrade the components of the extracellular matrix, the material between cells, and this function may allow the tumor cells to penetrate deep into tissues. There is evidence that two of this class of enzymes, MMP-2 and MMP-9, along with the enzyme MT1-MMP, which activates MMP-2, are also required for metastasis.

Thus one approach to the study of metastasis has been to determine which genes may be required for a cell to metastasize. Another approach has been to determine the various functions a cell will need to metastasize. Hence, the first step would be for the tumor cells to break away from the primary tumor and to enter the blood stream. Thus the tumor cells would be expected to lose their attachments to neighboring cells, for example through downregulation of E-cadherin, and gain motility. Perhaps less is known about the gain in motility, but in some cases secreted factors that are not dissimilar to growth factors can act as motility factors. Such proteins as epidermal growth factor (EGF) or autocrine motility factor enhances the ability of a tumor cell to move. Entrance into the blood stream appears to require proteases, but little is known about this step. Once in the blood stream the tumor cells need to survive. Resistance to apoptosis is critical to metastasis. The tumor cells then attach to the cells of the blood vessels or endothelia. While some literature has suggested that the cells then exit from the blood vessels in order to proliferate at distant sites, there is more recent evidence that the tumor cells begin to proliferate within the blood vessels. Lastly, tumor growth at the distant site results in metastasis.

Clinical relevance

The clinical relevance of metastasis cannot be overstated. Currently the most successful modes of therapy only affect the primary tumor. Surgery or surgery in concert with radiation are very effective at eradicating tumors if they have not spread beyond regional or adjacent lymph nodes. With only a few encouraging exceptions, once a tumor has metastasized to distant sites therapy is often ineffective. Thus, there are several important goals for future research. One is to develop a means of predicting whether a given tumor has developed metastases. As was noted above, many metastases can be dormant so that at the time of first diagnosis a patient may already have undetectable metastatic disease. A second important goal is to develop ways of treating these dormant metastases before they proliferate.

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Metastasis Suppressor Gene

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Definition

A metastasis suppressor gene blocks metastasis without affecting tumorigenicity. The suppressing activity could be the result of alterations at any of the steps in the metastatic cascade.

Characteristics

Although the definition of a metastasis suppressor gene would at first appear straightforward, it is important to emphasize the distinctions between tumorigenesis, tumor progression, invasion and metastasis. This section will briefly highlight the key aspects of those differences, but readers are encouraged to refer to individual entries for a more complete understanding of the terms.

Tumorigenesis refers to the ability of cells to proliferate continuously in the absence of persistent stimulation by triggering carcinogenic agent(s). Tumor progression is the evolution of already tumorigenic cells (populations) towards increasing malignancy. Invasion is the migration of tumor cells away from the primary tumor mass. This process can involve the breakdown of physical barriers (e.g., basement membranes) by secretion of proteinases (i.e., protein-degrading enzymes). Metastasis is the process by which a tumor cell(s) spreads to other sites in the body and establishes a secondary tumor colony. The process is complex and involves many steps (i.e., the metastatic cascade).

The genetics of metastasis can be conceptualized as consisting of two components; positive and negative regulators. The positive regulators (i.e., metastasis promoting genes) drive metastasis formation. These are genes, when expressed, enhance the ability of a cell to complete one or more steps in the metastatic cascade. An example is the MMPs (matrix metalloproteinases) that are involved in enzymatic breakdown of basement membrane matrices. It is important to note that most metastasis promoting genes are neither necessary (because of redundancy) nor sufficient (because of the multiple steps in the metastatic cascade) to confer metastatic competency upon cells. Two genes, → RAS and → MEK1, however, do confer both tumorigenic and metastatic potential upon NIH3T3 cells, which are murine cells established in culture and are often used for analyzing growth parameters of tumor cells.

In contrast, metastasis suppressor genes inhibit metastasis. Since metastasis requires cells to complete every step in the metastatic cascade, these genes can block any step(s) in the process. The first metastasis suppressor gene was discovered in 1984. Since that time several candidate genes have been identified, but only six have been shown to suppress metastasis *in vivo*, NME1, → KiSS1, KAI1, → E-cadherin, → BRMS1 and → MKK4.

Metastasis suppressor genes and metastasis promoting genes are analogous to tumor suppressor genes and oncogenes, but there are important distinctions. Tumor suppressor genes dominantly inhibit tumor formation when wild-type expression is restored in a neoplastic cell. By definition then, metastasis would also be suppressed (since the cells are non-tumorigenic). Metastasis suppressor genes, on the other hand, block only the ability to form metastases. Restoring expression of a metastasis-suppressor yields cells that are still tumorigenic, but are no longer metastatic.

How are metastasis suppressor genes identified?

Two general approaches have been used to identify metastasis-controlling genes. The first involves comparison of gene expression in poorly or nonmetastatic cells with matched metastasis-competent cells. The specific techniques employed are differential display and subtractive hybridization. The second takes advantage of clinical observations that identified nonrandom chromosomal changes that occur during tumor progression. This information lo-

calized the gene(s) from which cloning could commence. Based upon karyotypic patterns observed in human cancers, additional metastasis suppressor genes to those listed above are hypothesized to exist. However, the identities of the specific genes have yet to be determined.

Clinical Relevance

The existence of genes that block metastasis implies that the metastasis could be theoretically controlled by agents that regulate these genes or mimic their behavior. However, gene therapy is not yet possible. In the meantime, however, the differential expression of metastasis-associated genes is being used by pathologists to more accurately define the cancers.

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Metastatic

Definition

Metastatic refers to tumors that spread to more distant tissue *via* the lymphatic or blood stream; tumor cells growing in a new location.

Methylation

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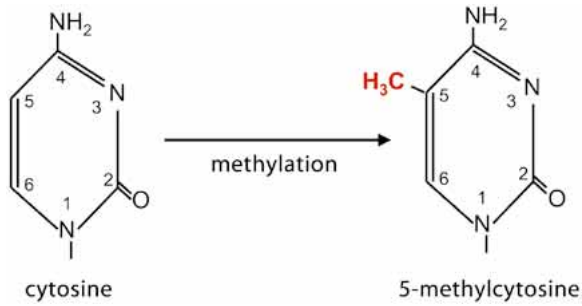
Definition

An epigenetic modification of DNA is the addition of a methyl (CH3) group to the 5′ position of a cytosine residue. The majority of methylation events in humans occur on cytosines that are located next to a guanine (5′-CpG-3′).

Characteristics

DNA methylation results from the addition of a methyl (CH_3) group to the 5′ position of a cytosine (Fig.). The addition of a methyl group is an → epigenetic modification to DNA that is maintained after cell division and does not change the DNA sequence. 5′-CpG-3′ dinucleotides are not uniformly distributed in the human genome. → CpG islands are short stretches of DNA, usually located in promoter regions of genes, with an unusually high GC content and a significantly higher frequency of 5′-CpG-3′ dinucleotides compared to the rest of the genome. It is well accepted that DNA methylation is involved in gene regulation. Inhibition of transcription factor binding by methylation of the target sequence was the first mechanism identified. This mechanism is limited to a subset of transcription factors that contain 5′-CpG-3′ dinucleotides in their recognition sequence. A second mechanism is the binding of methyl-CpG-binding protein complexes on proteins (MeCP1 and MeCP2) to methylated DNA. Transcription can then be repressed by either the inhibition of transcription factor binding or the recruitment of histone deacetylases (HDACs). HDACs mediate the deacetylation of lysine residues in the N-terminal tails of histones and thus cause an increase in compaction of the chromatin that makes the DNA less accessible for the transcriptional machinery.



Methylation. Fig. – Cytosine and 5-methylcytosine.

The establishment of normal DNA methylation patterns is a tightly regulated process of significant importance in many developmentally regulated pathways.

- Early embryonic development in mice is characterized by a genome wide demethylation in the early cell divisions (8-cell stage) to the blastocyst stage. The methylation pattern is re-established during implantation by a wave of *de novo* methylation.
- → X-chromosome inactivation is a developmentally regulated process in which DNA methylation plays an active role. In females most genes on one of the X-chromosomes are silenced. This mechanism assures the same expression levels in male and female cells. Dense methylation of only the inactive X-chromosome is correlated with transcriptional silencing of the genes located on this X-chromosome.
- While most genes are expressed from both the maternal and the paternal alleles, a small number of imprinted genes are expressed in a parent-of-origin dependent manner.

The underlying regulatory mechanisms of genomic → imprinting are unclear but allele-specific methylation in CpG islands associated with imprinted genes seems to be involved. Since methylation patterns are established in selected sequences of the genome, a sequence specificity for methyltransferases has been postulated. A first indication how this could be achieved came from a recent report describing a protein complex of DNMT1 with → RB, → E2F1 and HDAC1. This complex has the ability to target specifically those genes involved in growth regulation that contain E2F1 binding sites.

Cellular and molecular aspects

Several steps are required for the establishment of DNA methylation patterns. The methylation of an unmethylated sequence requires an enzyme with *de novo* methylase activity. This enzyme recognizes a potential target sequence and methylates both DNA strands. During replication of the genome a new DNA strand is synthesized that is unmethylated creating a hemimethylated state. A different enzyme that can detect and methylate the hemimethylated DNA is required for the maintenance of the methylation pattern (DNA maintenance methyltransferase). Since certain developmental processes require the erasure of the methylation pattern, an enzyme with demethylating activity was postulated. However, several rounds of DNA replication without maintaining the original methylation pattern could also accomplish demethylation. Recently, two methyltransferases, Dnmt3a and Dnmt3b, were shown to have *de novo* methyltransferase activity *in vivo* (4). The DNA maintenance methyltransferase, Dnmt1, was identified several years ago and fulfills the expected criteria. This enzyme targets hemimethylated DNA, is ubiquitously expressed in somatic tissue and interacts with the replication machinery at the replication fork. Furthermore, recent data suggests that DNMT1 can establish a repressive transcription complex that includes HDAC2, DMAP1 and the transcriptional co-repressor TSG101 (5).

Clinical relevance: Aberrant DNA methylation in cancer

Hypomethylation.

Both extensive aberrant hypo- and hypermethylation have been described for several human cancers. Global hypomethylation in human cancers was one of the earliest changes described to be associated with tumor progression. For example, some reports describe the activation of the → *MYC* oncogene in correlation with decreased methylation of CpG dinucleotides in the third exon of the gene. The underlying mechanisms however are unclear. In addition, there is convincing evidence that links hypomethylation with chromosomal breakage. Patients with → ICF syndrome are characterized by instability of the pericentromeric heterochromatin and decondensation in chromosomes 1, 9 and 16, resulting in multibranched chromosomes. The chromosomes involved show hypomethylation of satellite DNA, and recently several groups reported mutations in the *de novo* methyltransferase DNMT3B in ICF patients, consistent with the idea of a defect in the methylation pathway.

Hypermethylation.

Silencing of tumor suppressor genes either by mutations of both alleles, homozygous deletion or deletion of one allele (LOH) combined with mutation of the remaining allele, are well-documented mechanisms. More recently, homozygous DNA hypermethylation of promoter sequences has been identified as an additional mechanism of inactivation of tumor suppressor genes (1). The development of a methylation sensitive PCR (MSP; → PCR) allowed the rapid identification of methylated promoter sequences in various tumor samples (3). The list of genes that become hypermethylated in human cancers is growing rapidly and includes genes involved in growth/ development, repair and apoptosis. Hypermethylation of CpG islands located in promoter region of genes is correlated with the transcriptional silencing of the adjacent genes. Using → Restriction Landmark Genome Scanning (RLGS) for genome wide assessment of methylation patterns in CpG islands it was shown that up to 10% of the total 45,000 CpG islands in a tumor genome could be methylated (2). The methylation patterns are not random, suggesting a selective pressure for either the methylation of certain susceptible CpG islands or the selection of cells with a certain methylation pattern and a growth promoting transcriptional profile. In addition, by comparing different tumor types it was shown that some targets of methylation are shared between tumor types and others are specific for one tumor type.

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5-Methyl-cytosine

Definition

A cytosine modified by the addition of a methyl group through → methylation is called 5-methyl-cytosine. In vertebrate genomes, 5-methyl-cytosines are found mainly in the 5′ position of cytosine residues within CpG [→ CpG islands] dinucleotides.

Methylome

Definition

The term methylome describes the complete set of DNA methylation modification of a cell. Alterations in the methylome may be linked to aging and cancer.

MGF

Definition

STAT5A.

MHC

Definition

Major histocompatibility complex
→ HLA class I.

MIC2

Synonyms

- → CD99

Definition

Antigen identified by monoclonal antibodies 12E7, F21 and O13. MIC2 is a membrane protein of 185 and 18 kD that is involved in T-cell adhesion processes. MIC2 is localized on X and Y chromosomes and is therefore presumably the only human → pseudoautosomal gene; it maps toXp22.32 and Yp11.3.

Microarray (cDNA) Technology

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Synonyms

- cDNA chips
- parallel gene expression analysis

Definition

cDNA microarray technology is one of several developing functional genomics approaches to comparatively analyse genome-wide patterns of mRNA expression. Parallel gene expression profiling of cancer genomes with cDNA microarrays has revolutionized may aspects of cancer research. Gene expression 'fingerprinting' has been used for tumor classification, prediction of therapeutic response and resistance, and for elucidating genetic pathways associated with particular cancer phenotypes.

Characteristics

cDNA microarray technology entails the development and standardization of various hardware, analytical software, statistical methodology, biological resources and biochemical methodologies. The diagram in the Fig. demonstrates the various stages of cDNA microarray analysis.

- *cDNA microarray construction.* Construction of very high density cDNA microarrays of specific and distinct DNA hybridization 'targets', each one representing a single gene, are spotted in an arrayed format on a poly-L-lysine coated glass microscope slide. The printing process involves the sequential transfer of individual purified PCR amplified fragments (200bp to 2 kb) from a 96 well microtiter tray to an exact predefined location on glass slides. The 'arrayer' encompasses a robotic arm that moves (x,y,z axis) the 'quill pen probes' into position to either pick up or to spot down DNA (SDS solution), a manifold to hold the slides, a wash station to rinse the 'quill pens' when a different DNA is to be picked up, a place to house the microtiter plates, an air flow cabinet to keep everything in and a computer which orchestrates the operation of the components. Multiple glass slides, each containing thousands of spots of DNA, can be synthesized and used in subsequent experiments.
- *Hybridization with labeled cDNA.* For each experiment, complex 'probes' are made that consist of a pool of fluorescently labeled cDNAs. This step begins with the extraction and preparation of mRNAs from two populations of cells. Then each of the two mRNA is reverse transcribed separately with the incorporation of different fluorescently tagged nucleotides, producing two populations of differentially labeled cDNA probes. Typically Cy3 and Cy5 dyes (which emit light

at distinct wavelengths after laser excitation) are used to differentially label cDNA pools from different sources. The two complex labeled probes are combined and then simultaneously hybridized to the cDNA targets on the microarray.

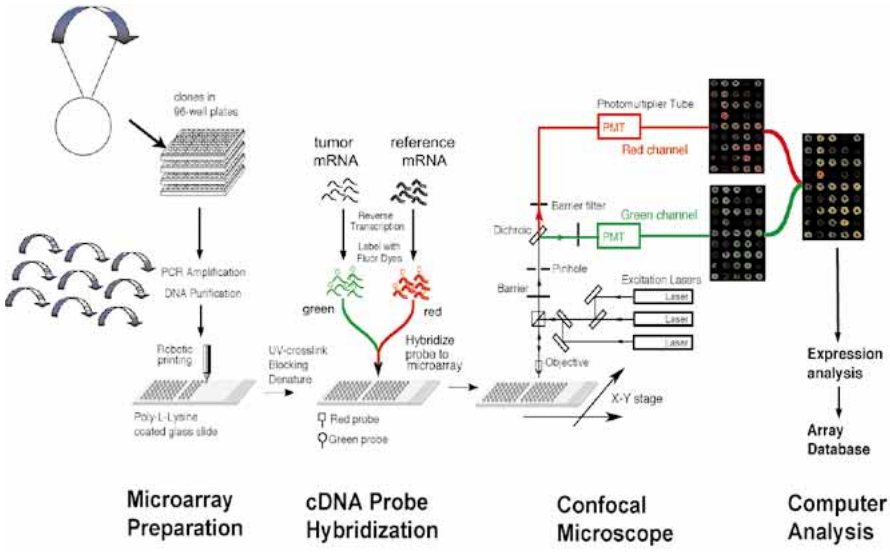
- *Scanning the cDNA microarray using a 'reader'.* To quantitatively measure the fluorescence of the hybridized probes, the cDNA microarray is scanned by a 'reader'. This device is a computer controlled inverted scanning fluorescent confocal microscope which detects the emitted fluorescence of the dyes after excitation with a double laser illumination system (a 532nm, 100mw NdYag laser is used for Cy3 and a 633nm, 35mw HeNe laser for Cy5). Digital images of fluorescence intensity data is generated by the 'readers' confocal laser scanning microscope.
- *Image Analysis and generation of cDNA microarray data.* Analysis of the image data generated by the 'reader' is done with a software for array target segmentation, target detection, background intensity extraction, target intensity extraction, ratio calculation and ratio normalization. Typically, cDNA microarray data is transferred to a relational database so they are available for analysis by query based data mining.
- *Analysis of large scale cDNA microarray data.* Management and analysis of the large volume of data that results from multiple cDNA microarray experiments represents a formidable challenge. Fortunately, new and effective bioinformatic methods have been developed to effectively analyze large scale expression data in ways conducive to extracting biologically relevant conclusions. Bioinformatic approaches using statistical tools can compare gene expression profiles from multiple experiments. One bioinformatic approach involves clustering, whereby the genes with similar expression profiles across multiple experiments are statistically clustered together. In some cases, the samples can also be clustered based on how similar the global gene expression patterns are. There are a wide variety of statistical methods to cluster microarray data. The most commonly used is unsupervised, hierarchical clustering that, unlike supervised clustering, is not based on fitting the data to a model. Plotting of hierarchical gene clustering results is aided by color representations of the relative gene expression ratios and branching dendograms. Other visualization methods, such as multi-dimensional scaling (MDS), have been developed to visually illustrate (by distance in a three-dimensional graph) the degree of correlation amongst multiple samples based on global scale gene expression profiles. In other words, MDS can cluster samples together in 3-D space based on the similarity of their gene expression profiles. More detailed information on cDNA microarray technology and related links is available in http://www.nhgri.nih.gov/DIR/LCG/15K/HTML/.

Application

DNA microarrays facilitate highly parallel analysis of gene expressionAnalysis of cDNA microarray data using statistical clustering has revealed groups of genes that have very similar gene expression profiles associated with a phenotype or a response to a physiological condition. Also, genes sometimes cluster together tightly across a large number of conditions revealing co-regulated genes. Furthermore, investigation of genes that cluster together often reveals that they have related structures and functions. As a consequence, clues about the function and regulation of unknown genes can be hypothesized based on other genes that cluster with it. An added advantage is that cellular phenotypes such as migration of cancer cells can be correlated with specific clusters and gene expression fingerprints.

Clinical relevance

Gene clusters from cDNA microarray data analysis have been used as cancer bio-markers for diagnosis, progression and prognosis. For example, the use of global gene expression profiles has successfully identified a gene cluster that distinguishes various classifications of malignancies. This type of classification by gene expression fingerprinting is useful in identify-



Microarray (cDNA) Technology. Fig. – Schema of microarray technology [adapted from Duggan et. al., Nature Genetics (1999) (1 Suppl):10-4].

ing sub-types of hematological malignancies that were histo-pathologically not distinguishable but had different clinical outcomes. These profiles can also identify genes that are associated with therapy response and resistance. For example, a gene expression program was identified in prostate cancer that is associated with androgen ablation therapy and resistance to hormonal therapy. As a consequence, such genes can be used to predict individual responses to therapy. Furthermore, identification of genes that mediate therapy response also permit rational drug design to target these genes and gene products.

cDNA microarray analysis of a small set of cancers can identify hundreds of gene expression alterations. Conventional molecular pathology techniques are relatively slow, thus, creating a 'bottle-neck effect' in the validation and translation of such alterations to a large population of clinical specimens. To alleviate this problem, tissue microarray technology has been developed for parallel high-throughput molecular pathology (immunohistochemistry, mRNA *in situ*, and fluorescence *in situ* hybridization) on hundreds of clinical tissue sections on a single glass microscope slide. The prevalence of candidate gene expression alterations can therefore be assayed in hundreds to thousands of clinical samples in a single experiment. These two types of microarray technology are highly complementary, allowing for rapid identification and translation of genes associated with cancer.

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Microcell-mediated Chromosome Transfer

Definition

Microcell-mediated chromosome transfer is a technique by which individual chromosomes can be introduced into a recipient cell. The transferred chromosomes are typically tagged with a selectable marker to facilitate experimental manipulations.

Microcirculation

Definition

Microcirculation is a term describing structure and function of blood (and lymph) vessels with diameters $\leq$ 250 μm (microvessels).

Micrometastasis

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Definition

Micrometastases were originally defined by pathologists as small occult metastases ($<$ 0.2 cm in greatest dimension). With the recent development of more sensitive diagnostic tools, such as immunocytochemistry and polymerase chain reaction (PCR), the term has been used more liberally in the literature. It now includes isolated disseminated tumor cells present in the peripheral blood or in a secondary organ (in particular bone marrow) or in a lymph node, classified as 'tumor-free' by conventional histopathological analysis. Considering the different biology of true micrometastases and isolated disseminated tumor cells, both types of findings should be distinguished.

Characteristics

Detection techniques

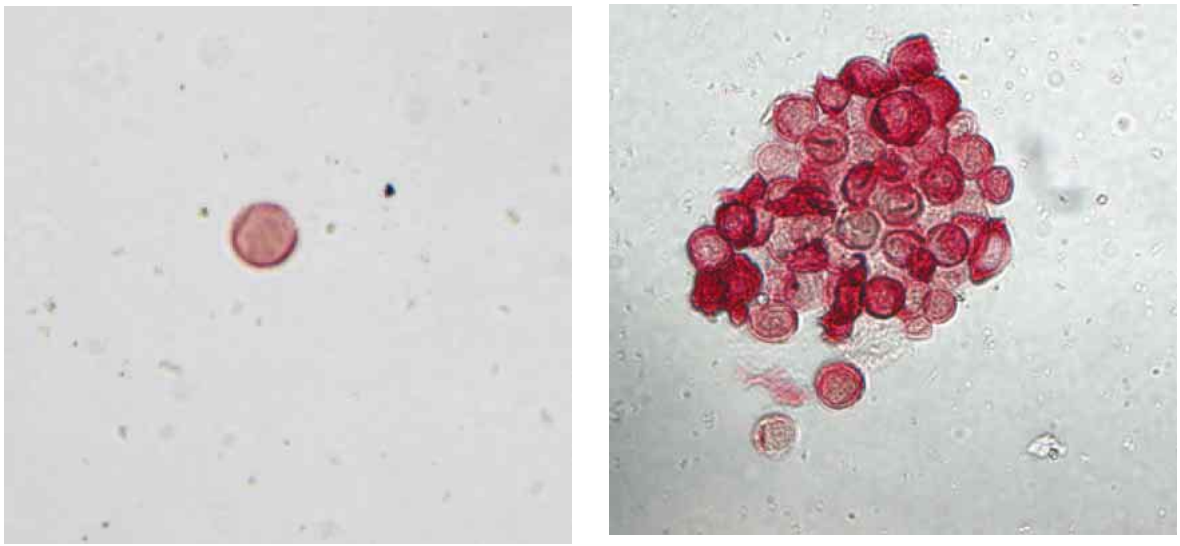
The identification of individual disseminated tumour cells in cytological blood or bone marrow preparations or lymph node sections on the basis of cytomorphological criteria is extremely difficult and not very practical because of the lack of sensitivity.

Immunocytochemistry.

Cytokeratins (CK), as integral components of the cytoskeleton of epithelial cells, are stably expressed characteristics of tumour cells that can be clearly identified in individual carcinoma cells by means of specific monoclonal antibodies (Fig.). Mucin-like tumour-associated cell membrane proteins are less well suited for analysis on account of their expression on haematopoietic cells. Immunohistochemical examinations of bone marrow biopsies have shown that CK-positive tumour cells are mostly situated in interstitial tissue outside the sinusoidal vessels, which indicates that extravasation, one of the last processes in the metastasis cascade, has been successfully completed. Although ectopic or illegitimate mRNA expression of cytokeratins in mesenchymal cells cannot be ruled out, numerous negative findings in patients without identifiable malignant processes show that ectopic expression of cytokeratin proteins in bone marrow is very rarely identifiable by immunocytochemistry. The time-consuming microscopic screening of large amounts of cytological samples could be facilitated in future through automated analysis of stained preparations using an image-analysis system (i.e., ACISR, Chromavision). As an alternative, density gradients and antibody-charged magnetic particles can be used to enrich tumor cells over several logarithmic units. However, the reproducibility of these enrichment techniques need to be confirmed on clinical samples.

Molecular techniques.

In the last few years molecular detection procedures have been increasingly used to identify disseminated tumour cells in organs remote



Micrometastasis. Fig. – Isolated disseminated carcinoma cells (left) and micrometastasis (right) on a bone marrow cytospin stained with anti-cytokeratin antibody A45-B/B3.

from the primary tumour. In principle, cDNA of disseminated tumour cells can be amplified millions of times as a result of polymerase chain reaction (PCR), so that even the smallest quantities of such tumour cells can be detected. For this to happen, however, the tumour cell must have specific changes in its genome or mRNA expression pattern that distinguishes it from the surrounding haematopoietic cells. As solid tumours are notable for their extreme genetic heterogeneity, the detection of tumour-specific genomic changes at the level of a single cell is highly complex. Each individual primary tumour must be genotyped so that the corresponding patient-specific genetic lesion can be identified and the appropriate PCR probes (primers) selected. This method is currently beyond the means of routine clinical diagnosis. The detection of tumour-specific expressed mRNA species, on the other hand, appears to present fewer obstacles to more widespread use of the PCR method. The cell mRNA is transcribed into cDNA by means of reverse transcriptase (RT) and the cDNA amplified in subsequent PCR reaction. Although this method offers great potential for future clinical use, the specificity of tumour cell identification is currently the greatest barrier.

Characteristics (cellular and molecular)

Immunocytochemical double staining methods have been developed for more precise characterization of disseminated tumour cells. In view of the malignant potential of CK-positive cells, a number of tumour-associated characteristics have been identified. This includes the frequent over-expression of the erbB2 oncogen and deficient expression of MHC class I molecules, which as restrictive elements help T lymphocyte mediated tumour cell recognition. The malignant nature of CK-positive cells in the bone marrow has been further confirmed through genomic analysis. Extensive cell culture experiments have shown that cells disseminating into bone marrow have a time-limited proliferative potential at the time of primary diagnosis of the tumour. It may therefore be assumed that at the primary stage these cells do not proliferate autonomously, but are in a latent state known as ‘dormancy’. This assumption is also corroborated by double staining studies, demonstrating that the fraction of disseminated tumour cells in bone marrow with proliferation marker expression (Ki-67, p120) is small. This could explain the relative resistance of micrometastatic tumour cells to chemotherapy and confirms the appropriateness of therapy strategies independent of the proliferation potential.

Clinical relevance

Bone marrow.

Several studies have confirmed the result of the cytokeratin assay as a prognostic factor unaffected by conventional risk factors (Table). The observed correlation to the total relapse rate is of particular interest, as clinically manifested skeletal metastases are rare in colon carcinoma. It would therefore appear that the presence of epithelial cells in bone marrow is more likely to be an indicator of early systemic tumour cell dissemination, the growth in bone marrow or other organs being determined by the milieu in question. In this context it is interesting that the repeat identification over a two-year period of tumour cells in the bone marrow of patients with operable gastric carcinoma have greater prognostic value than the primary identification of disseminated cells when the primary tumour was resected. Although the prognostic relevance of the immunocytochemical identification procedure has been confirmed in prospective clinical studies by various working groups (Table), doubts as to the value of the method have been expressed. A closer analysis of these reports shows that the techniques used differ considerably in terms of reproducibility. This might also explain the different detection rates that have been published, ranging from 4% to 45%, for example for mammary carcinoma. It is therefore necessary to define the critical variables in the immunocytochemical method and to introduce standardization so as to allow a reproducible and more precise determination of the residual cancer cell count.

Peripheral Blood.

Circulating tumour cells can also be identified in peripheral blood. Repeat blood sampling is superior to sequential aspiration of bone marrow as a monitoring procedure. Recent examinations of patients with prostate and colorectal carcinoma and melanomas whose periph-

Micrometastasis. Table – Immunocytochemical studies of the prognostic relevance of disseminated tumor cells in bone marrow.
[a] prognostic value as independent parameter confirmed through multivariate analysis
EMA,epithelial membrane antigen; CK=, cytokeratin; TAG12, tumor-associated glycoprotein 12; DFS, disease-free survival; OS, overall survival; NSCLC, non-small-cell lung cancer.

type of tumor	marker, proteins	detection rate	prognostic value	reference
mammary carcinoma	CK	199/552 (36%)	DFS, OS[a]	Braun et al., 2000
	EMA	89/350 (25%)	DFS, OS	Mansi et al, 1999
	EMA, TAG12, CK	38/100 (38%)	DFS, OS[a]	Harbeck et al., 1994
	CK	18/49 (37%)	DFS[a]	Cote et al., 1991
	TAG12	315/727 (43%)	DFS, OS[a]	Diel et al., 1996
colorectal carcinoma	CK-18	28/88 (32%)	DFS[a]	Lindemann et al., 1992
gastric carcinoma	CK-18	34/97 (35%)	DFS	Schlimok et al., 1991
	CK-18	47/78 (60%)	DFS	Heiss et al., 1995
	CK-18	95/180 (53%)	DFS[a]	Jauch et al., 1996
esophagus carcinoma	CK	37/90 (41%)	DFS, OS	Thorban et al., 1996
bronchial carcinoma (NSCLC)	CK	17/43 (40%)	DFS	Cote et al., 1995
	CK-18	83/139 (60%)	DFS[a], OS	Pantel et al., 1996
	CK-18	15/39 (39%)	DFS	Ohgami et al., 1997

eral blood was examined perioperatively by molecular methods have shown that a temporary intraoperative dissemination of tumour cells in the bed of the vessels can occur. Whether these cells reach and survive in secondary organs and form manifest metastases there is as yet unknown.

Lymphogenous dissemination.

Early haematogenous tumour cell dissemination is the commonest method of metastasis, but a second means, namely lymphogenous dissemination, is also of great clinical importance. The clinical importance of this type of dissemination was recently confirmed in a similar manner for carcinoma of the oesophagus. An important advance in the evaluation of regional lymph nodes has been the development of a more limited dissection, the sentinel lymph node dissection. This is based on the identification, with dyes or radioactivity, of the specific lymph node that drains the tumor and the removal of this lymph node for analysis. This approach has been extensively evaluated in patients with melanoma and breast cancer. Detection of occult tumor cells may be an important adjunct to the use of limited lymph node dissection for staging and for therapy.

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Microphthalmia

Definition

Microphthalmia, also known as MITF, is a basic helix-loop-helix/leucine-zipper transcription factor. It was named after a mouse mutant gene, where the corresponding gene is impaired, leading to albinism and small eyes. MITF exists in several isoforms, one of which regulates pigment cell-specific gene expression.

Microsatellite

Definition

A microsatellite is a locus specific DNA sequence and are present throughout the genome in high copy number. They are short nucleotide sequences (1-6 base pairs) that are tandemly repeated several times and flanked by unique sequences.

Microsatellite Instability

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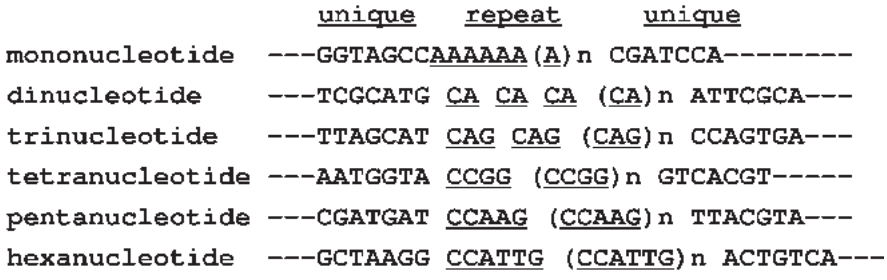
Definition

Microsatellite instability is the accumulation of novel microsatellite alleles in genomes (in bacteria as well as human) that have defective mismatch repair. Microsatellites are locus-specific DNA repeated sequences with high abundance throughout the genome. They consist of short repetitive DNA sequences of 1- 6 nucleotides in length that are tandemly repeated 10-60 times and flanked by unique sequences (Fig. 1).

Characteristics

Multiple different sized alleles accumulate within a tumour due to defective mismatch repair genes, and this phenomenon is referred to as microsatellite instability. Tumours having such instability are said to have a mutator phenotype. Most microsatellites are usually located in non-coding parts of the genome and mutations in these are selectively neutral. Occasionally the microsatellites are found in expressed



Microsatellite Instability. Fig. 1 – Microsatellites consist of short nucleotide units, tandemly repeated and flanked by unique sequences. The different types of one to six base pairs per unit are illustrated. The last unit within each type is in parenthesis followed by 'n', indicating additional units.

regions of a functionally critical gene and mutations may result in a non-functional or mutated protein with impact on tumourigenesis.

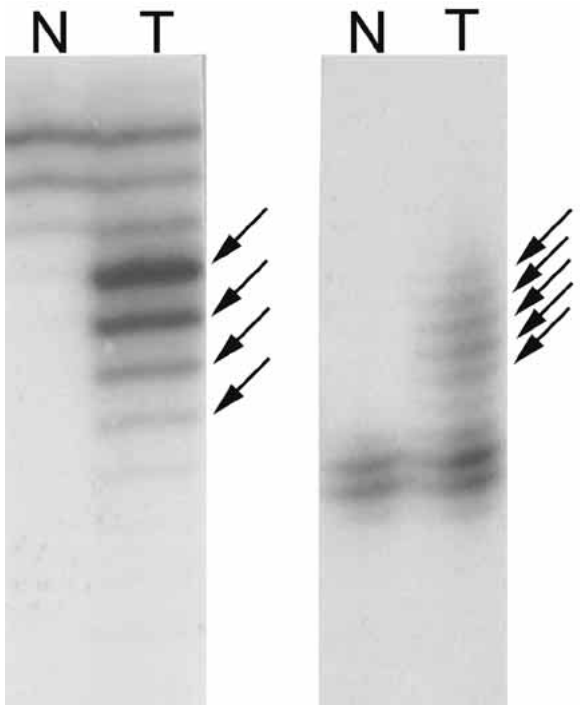
The high abundance and their polymorphic nature make microsatellites a valuable tool for a variety of applications. Microsatellites were used to identify the chromosomal map position of genes that in a mutated condition predispose to hereditary colorectal nonpolyposis syndrome (HNPCC). Several studies of tumours from HNPCC patients revealed the characteristic pattern of novel alleles at simple repetitive sequences. About 90% of colorectal carcinomas from HNPCC patients and a subgroup (15%) among sporadic cases have microsatellite instability. Other tumour types occurring in the HNPCC syndrome and a subgroup among sporadic cases of the same type also show microsatellite instability. This includes endometrial, ovarian and gastric cancers. Sporadic tumours of types not characteristic to the HNPCC tumour spectrum generally show microsatellite instability at lower frequencies, and the pattern is characterised by one or only a few new alleles, in contrast to the ladder of alleles often observed in the HNPCC tumour types (Fig. 2).

The HNPCC tumours and the sporadic cases of the same types share clinicopathological characteristics. In comparison with microsatellite stable colorectal carcinomas, the ones that show microsatellite instability are more often located in the proximal colon, are characterised by high lymphocyte infiltration, mucinous and undifferentiated growth pattern, exhibit diploid DNA content and normal genotype/phenotype of the tumour suppressor gene TP53.

Cellular & Molecular Regulation

The genome-wide microsatellite instability pattern seen in human tumours resembles a mutational fingerprint found in the bacterium *Escherichia coli*, which is caused by failure of the mismatch repair system, mutHLS, in this organism. Several human homologues to the prokaryotic genes have been identified, and germline mutations in some of these components predispose to the → HNPCC syndrome. Mutations in hMSH2 and hMLH1 account for 60% of the identified familial mutations. hMSH6 germline mutations are found in HNPCC with increased occurrence of endometrial cancer. Germline mutations are found in hPMS1 and hPMS2 but seem to occur rarely.

A shared requirement between mismatch repair genes and tumour suppressor gene is the need for homozygous inactivation at the cellular level. In addition to the germline mutation in a HNPCC patient, a somatic mutation of the second allele is necessary for tumour development. Mutations of both alleles of a mismatch repair gene have been reported in tumour studies, as well as mutation of one allele and inactivation of the other by hypermethylation. Biochemical analyses revealed that on the cellular level a single mismatch repair gene mutation



Microsatellite Instability. Fig. 2 – Microsatellite instability in a colon carcinoma. Constitutional alleles are shown in lanes with normal DNA of the patient (N). Instability, a ladder of novel alleles, is seen in the patient's tumor DNA (T). Instability is shown at two microsatellite loci. At one locus (left) all novel alleles are of smaller size than normal alleles, whereas at the other locus (right) all new alleles are larger. (It should be noted that at individual loci one or more novel fragments, of smaller, larger and/ or intermediate size, may be seen in microsatellite unstable tumors.)

(heterozygous condition) as opposed to homozygous inactivation does not severely affect mismatch repair function. Comparable interpretation can be drawn from studies with mice.

Mismatched nucleotides arise in DNA by:

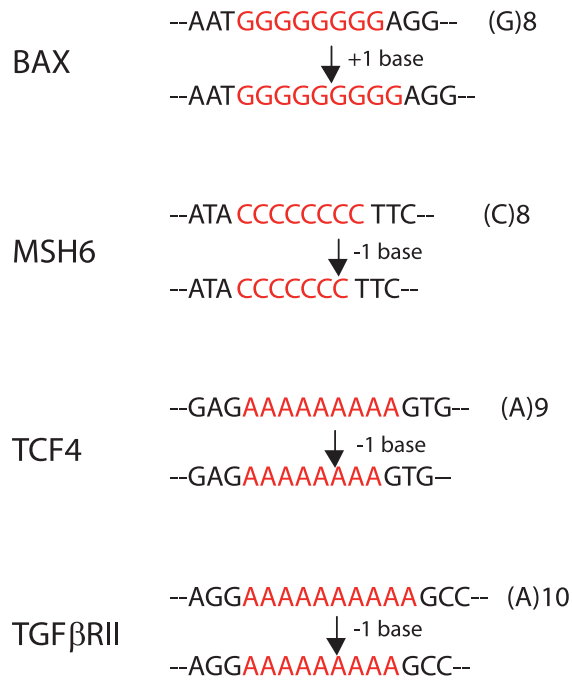
- polymerase misincorporation during replication,
- physical damage to existing nucleotides,
- as the result of forming heteroduplex intermediates during genetic recombination.

DNA polymerases are enzymes, which make mistakes especially in DNA with tandemly repeated sequences. Normally these errors are corrected by the mismatch repair system. Defects in one or more of these components causes insertion of inappropriate bases or the making of loops that are stabilised, and the following rounds of replication creates alleles of new sizes (Fig. 2).

The genome-wide microsatellite instability phenotype does not itself provide tumour cells with a proliferative advantage, let alone the ability to transform them malignantly. Nor do mutations in defective mutator genes, known to cause the phenotypic instability, necessarily provide a neoplastic-growth advantage. Instead, the genotypic and/or phenotypic instability increases the likelihood of additional mutations in other mutator genes and in more directly cancer-relevant genes. Mutations in mononucleotide repeat stretches in coding regions of different genes have been found in tumours with microsatellite instability. Insertions or deletions in a polymononucleotide sequence may cause frameshift mutations that lead to a shortened protein with no or abnormal function. Several target genes for defect mismatch repair have been identified, including TGF-β II receptor, important in transforming growth factor signal transduction, BAX, encoding a protein related to → apoptosis, E2F4, a cell-cycle regulator and TCF-4, a downstream transcription factor in the APC (adenomatous polyposis coli)-β-catenin pathway (Fig. 3).

Clinical Relevance

Several clinicopathological variables and the family history of HNPCC related cancers decide if further analyses should be performed to identify the disease-associated susceptibility gene mutation in a family. First the tumour samples of affected persons should be analysed for microsatellite instability and for altered expression of the mismatch repair proteins, hMSH2 and hMLH1. These analyses can be performed on serial sections of archival samples. The criteria for diagnosis of microsatellite instability have been standardised and suggest the analyses of 5 markers, including two mononucleo-



Microsatellite Instability. Fig. 3 – Genes revealing frameshift mutations as the result of defect mismatch repair. Examples of genes with mononucleotide repeat sequences within their coding regions prone for replication errors. If a gain (+) or loss (-) of one base(s) is not repaired, this will cause a frameshift mutation leading to a new coding sequence for the protein (each amino acid is read in base-triplets). *BAX*: encodes a protein that accelerate apoptosis, *TCF-4*: T cell factor 4, a downstream transcription factor in the APC (adenomatous polyposis coli) – β-catenin pathway, *TGF-β RII*: transforming growth factor β receptor II, *MSH6*: encodes a component of the mismatch repair system.

tide repeats (BAT25 and BAT26) and three dinucleotide repeats (D2S123, D5S356 and D17S250). If two (40%) or more markers show instability, the tumour is classified as MSI-H (high microsatellite instability). Immunohistochemical analyses of the hMSH2 and hMLH1 proteins add diagnostic information, since loss of expression of either one of them correlates highly with MSI-H and thus specifies which one is most likely to be mutated in the germline. Finally, constitutional DNA from family members should be submitted for germline mutation analyses of the relevant mismatch repair gene(s).

Microsatellite instability has been found in some studies to be associated with prolonged survival in colorectal cancer patients. This and other molecular markers may be used to describe the genetic profile of the patient's tumour and it remains to be seen how important such information will be in the choice of treatment and accurate prognostic evaluation.

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Microtubules

Definition

Microtubles are hollow, cylindrical polymers assembled from heterodimers of α- and β-tubulin. Microtubles also contain various other proteins called 'microtuble associated proteins' or MAPs. Interphase microtubles grow from the perinuclear microtuble organising centre (MTOC). During mitosis these microtubles are depolymerised and spindle microtubles are formed from the free subunits. Spindle microtubles are the tracks for chromosome movement, using microtuble-associated motor proteins.

MIN

Definition

→ Mom1.

Minimal Residual Disease

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Synonyms

- minimal residual disease (MRD)
- residual disease

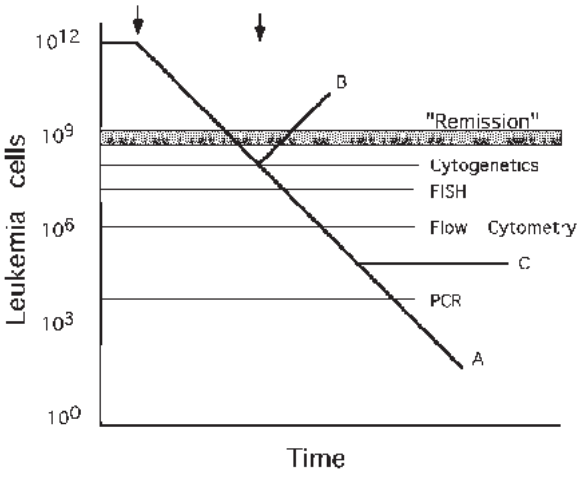
Definition

Minimal residual disease is the presence of disease detected in a patient who, by conventional clinical and pathologic measurements, is in remission.

Characteristics

At diagnosis a patient with cancer may have billions of malignant cells. After chemotherapy

many patients achieve 'remission,' meaning that their disease cannot be detected by conventional clinical, radiological and pathological examinations. Nonetheless, the major cause of failure in cancer therapy is the recurrence of disease, usually after the induction of a 'remission.' The problem of defining remission and relapse is illustrated in the Fig. In this essay leukemia, a malignancy of the white blood cells, will be used as a disease example. At diagnosis leukemia patients may have a disease burden up to 10^{12} leukemic cells. Thus, even if chemotherapy kills 99.9% of the leukemia, up to 10^9 cancer cells may remain, despite the microscopic appearance of remission. The study of minimal residual disease (MRD) aims to redefine the concept of remission and differentiate patients with leukemia destined for relapse (1B) from those with a stable or declining (1A) leukemia load. Once identified, patients at a high risk of relapse could undergo additional or alternative therapy while the disease burden is several orders of magnitude less than at frank hematological relapse.



Minimal Residual Disease. Fig. – The detection of MRD. At diagnosis, patients may have up to 10^{12} leukemia cells. Treatment (arrows) reduces the load of leukemia below the threshold of microscopic detection ('remission'). Additional treatment further decreases the burden of leukemia. The sensitivity thresholds of other detection methods are shown as horizontal, labeled lines. Studies of MRD attempt to distinguish patients with decreasing disease (A) from those with disease in remission, yet bound towards relapse (B). Finally, there is the poorly understood phenomenon of 'dormancy', in which patients have steady, although very low, disease burdens, persisting for years (C).

The limitation of the conventional definition of remission is apparent, since many leukemia patients who attain remission nevertheless relapse. Other methods to define residual disease include 'classic' metaphase and molecular → cytogenetics, cell cytometry studies and molecular genetic studies such as the polymerase chain reaction [→ PCR] (PCR) detection of specific genetic targets. Each method takes advantage of differences in the characteristics of the tumor cell compared to the normal cell, and each has relative advantages, disadvantages and sensitivity of detecting MRD. Cytogenetics can detect approximately one leukemia cell in 10-100 normal cells (denoted as 10^{-1} sensitivity). Limitations of cytogenetics include a small sampling size (since typically only 20-50 cell metaphases can be examined) and the examination of only cells that grow and divide in culture. To increase sensitivity and facilitate genetic screening in non-dividing cells, fluorescence *in situ* hybridization (FISH) using chromosome-specific or locus-specific probes has been developed to identify genetic targets in both metaphase chromosomes and interphase nuclei. FISH is most useful in detecting simple losses or gains of chromosomes and chromosomal translocations. The sensitivity of FISH is between 10^{-1}-10^{-2} and detection of numerical losses or gains of chromosomes is generally more sensitive than for detecting translocations.

Normal cells display a variety of proteins on their outside surfaces, known as cell surface antigens. The expression of cell surface antigens can distinguish malignant from normal cells. Cells labeled with fluorescent antibodies can be detected and quantified by a fluorescence-activated cell sorter (FACS). While truly tumor-specific antigens are rare, studies have shown that malignant cells often express cell surface antigens in subtly different patterns than normal cells. By using combinations of multiple antibodies, flow cytometric assays can use the patterns of aberrant expression to 'fingerprint' the malignant clone. The sensitivity of flow cytometry is 10^{-1}-10^{-3}. The advan-

tage of flow cytometry is that it is rapid, with results within hours of sample collection. However, to fully optimize the sensitivity of detecting the leukemic clone, combinations of several antibodies are needed to define the aberrant antigen expression, requiring considerable technical expertise.

The most sensitive approach to detect MRD involves nucleic acid amplification using PCR. The power of the technique is the exponential amplification of the target genetic sequence. Each round of the PCR produces an expondential amplification from the initial starting copy number, following the equation $N=No\ (1+E)^C$ (where N = PCR product, No = initial gene target number, E = efficiency of the PCR reaction, and C = number of PCR cycles). Thus, 30 cycles of amplification yields over a million-fold amplification of the target. However, PCR must have a specific genetic lesion as the 'fingerprint' of the malignancy in order for PCR-driven reactions to have the desired sensitivity and specificity.

Cellular and Molecular Regulation

Gene translocations occur when a piece of chromosome is transferred to a piece of another chromosome. These chromosome translocations are the most straightforward leukemia-specific markers for the detection of MRD, since they should occur only in the cancer but not in normal cells. The prototypical translocation is the t(9;22) in → chronic myeloid leukemia (CML), which causes the reciprocal translocation of the ABL gene on chromosome 9 to the BCR gene on chromosome 22. The juxtaposition of BCR-ABL forces the expression of the chimeric → BCR-ABL mRNA, which can be specifically amplified by reverse transcription (RT)-PCR. Other examples of leukemia-specific translocations include the t(15:17), t(8;21) and inv.(16) found in approximately 30%, collectively, of acute myeloid leukemia (AML) cases, and the t(14;18) rearrangement detected in follicular cell non-Hodgkin lymphoma [→ Hodgkin disease]. However, genetic research of leukemia has discovered a rapidly expanding list of disease-specific translocations, and now genetically characterized translocations can be detected and amplified in 40 – 50% of AML and → acute lymphoblastic leukemia (ALL).

Another type of leukemia-specific 'fingerprints' takes advantage of the rearrangement of the immunoglobulin heavy chain and T-cell receptor genes that normally occur in B- and T-cell lymphoid development. During the development of a B-cell, for example, immunoglobulin heavy chain variable, junctional and diversity genes are merged from their germline configuration to produce a shortened IgH V-D-J rearrangement. Each B-cell has a unique IgH V-D-J sequence, which determines the unique antibody that it produces. When a B-lineage cell transformed into a malignant clone, all leukemic cells will have the same IgH V-D-J rearrangement. This V-D-J gene rearrangement can serve as the leukemia-specific 'fingerprint' of the leukemia. PCR methods can be used to track the presence or absence of this clonal marker after therapy.

Clinical Relevance

The study of minimal residual disease (MRD) aims to understand the biology and clinical significance of leukemia that persists in patients who are in complete pathologic remission. Treatment of cancer during the level of disease burden below the threshold of detection by conventional pathology defines conventional 'remission' (Fig.). Further therapy may drive disease levels further downward (1A). Alternately, the cancer may acquire → drug resistance and begin to grow (1B). Lastly, some patients remain with MRD for years without relapse (1C). The mechanism of the → dormancy is unknown.

The detection of MRD in 'remission' patients most consistently has been associated with subsequent relapse in childhood ALL, t(15:17) AML, and CML following marrow transplantation. In these situations the presence of MRD often heralds the relapse of the leukemia; the risk of relapse in patients with MRD is generally greater than 5-fold the risk in patients without MRD. Studies are ongoing to test if the treatment of MRD will prevent frank relapse.

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Minisatellite

Definition

A minisatellite is a tandemly repeated DNA sequences in units of approximately 10 to 100 base pairs; microsatellites [→ microsatellite instability].

Minor Histocompatibility Antigens

Definition

Minor histocompatibility antigens are protein or carbohydrate structures, polymorphic between individuals of the same or different species. They can be recognized directly by recipient T lymphocytes, leading to less severe and rapid rejection reactions.

Mismatch Repair

Definition

Mismatch repair is a molecular process by which erroneously incorporated and not matching bases are removed from DNA double strands and are replaced by the correct base. MMR is under control of evolutionary conserved → mismatch repair genes. Germ-line mutations can impair the function of mismatch repair genes and is associated with hereditary cancer susceptibility (→ Lynch syndrome). Mutations in mismatch repair genes often manifest themselves as → microsatellite instability. Mismatch repair is a DNA repair system that is highly conserved from bacteria to humans. It occurs by excision of a DNA sequence from one strand, containing an incorrect paired nucleotide, followed by resynthesis of a new strand. The components of the mismatch repair system recognise normal nucleotides that are either mispaired or unpaired, and repair this by excision of a DNA sequence from one strand, containing an incorrect paired nucleotide, followed by resynthesis of a new strand.

Mismatch Repair Genes

Definition

Mismatch repair genes encode proteins that are involved in post-replication mismatch repair, eliminating from DNA newly incorporated not-matching bases. The principle groups include the *hMSH1-3* genes, *hMLH1* and *PMS1* to *2* (see below). These genes are highly conserved among living organisms and were ori-

Mismatch Repair Genes. Table

gene	homolog	chromosome
hMSH2	*mutS* (*E. coli*)	2p22-21
hMSH3	*mutS* (*E. coli*)	5q11-12
hMSH4	*mutS* (*E. coli*)	1p31
hMSH5	*mutS* (*E. coli*)	?
hMSH6	*mutS* (*E. coli*)	2p16
hMLH1	*mutL* (*E. coli*)	3p21
hPMS1	*postmeiotic segregation increased 1* (*S. cerevisiae*)	2q31
hPMS2	*postmeiotic segregation increased 2* (*S. cerevisiae*)	7p22

ginally identified in bacteria and yeast. Germline mutations of mismatch repair genes are responsible for hereditary nonpolyposis colorectal cancer (HNPCC), also →Lynch syndrome. HNPCC is an autosomal, dominantly inherited disease associated with marked increase in cancer susceptibility. It is characterized by a familial prediposition to early onset colorectal carcinoma and of extra-colonic cancers of the gastrointestinal, urological and female reproductive tracts. HNPCC is reportedly the most common form of inherited colorectal cancer, accounting for about 1% or more of all colorectal cancer cases. Mutation in two of the known mismatch repair genes, *hMSH1* and *hMSH2*, accounts for over 90% of mutations found in HNPCC families. Cancers in HNPCC originate within benign neoplastic polyps termed adenomas. Mutations of mismatch repair genes result in impairment of mismatch repair, with the manifestation of →microsatellite instability.

Mismatch Repair in Genome Stability

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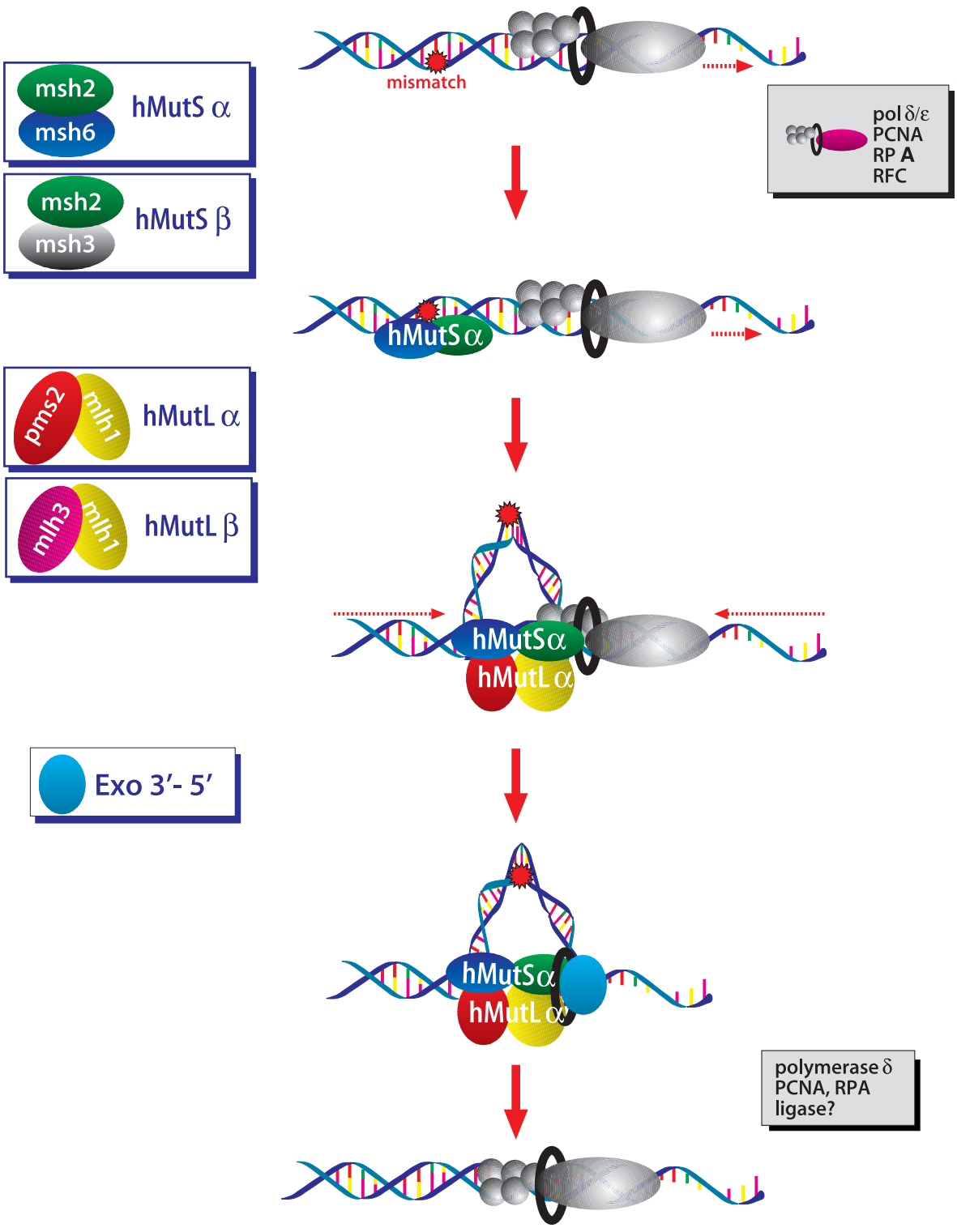
Definition

Mismatch repair acts after replication to correct mismatches which have escaped proofreading by the replication apparatus. Thus mismatch repair corrects unmodified DNA bases in non-Watson-Crick pairings or small distortions generated by the incorrect alignment of two normal DNA strands. Structural abnormalities such as these can also be generated during recombination between DNA molecules which are not perfectly homologous.

Characteristics

Several proteins participate in the 'long-patch' human mismatch repair (Fig. 1). The first step in the pathway is the recognition of DNA mismatches. Human cells contain at least three proteins that participate in mismatch recognition, hMSH2, hMSH3 and hMSH6, although none is a significant mismatch recognition factor on its own. Instead, binding to DNA mismatches is performed by the heterodimers hMutSα and hMutSβ, both containing the hMSH2 protein. In the hMutSα complex the partner of hMSH2 is hMSH6, while in the second heterodimer hMSH2 forms a complex with hMSH3. Biochemical evidence obtained with purified human proteins indicate that single base mispairs and loops of one base are preferentially recognized by MutSα, while larger loops of 3-4 unpaired bases are bound by the hMutSβ complex (Fig. 1). Some overlapping of the two complexes in the recognition of 1 and two-base loops seems to indicate some degree of redundancy in correction. ATP plays a major role in controlling the relative movement of the recognition complex and the mismatch which is pushed into a looped structure. Following the primary recognition step, the next step involves a third heterodimer, hMutLα, which comprises the hMLH1 and hPMS2 proteins. Recent evidence indicate that hMLH1 can also form another complex having as a partner the product of the *hMLH3* gene (hMutLβ). The precise biochemical function of these complexes remains undefined. Furthermore it is not clear yet whether there are selective interactions of these two complexes with MutSα and MutSβ to repair specific types of mispairs or whether there is some redundancy of mismatch repair functions. Repair of the mismatch requires an exonucleolytic activity to degrade the strand containing the mismatch, following strand displacement by a DNA helicase. 3′ or 5′ exonucleases are then needed to effect removal in one or other direction. The resynthesis step most likely requires polymerase δ, one of the replicative polymerases. The processivity of this polymerase is increased by an accessory protein, the proliferative cell nuclear antigen (PCNA). PCNA has been shown to interact directly with the hMutLα complex at a step preceding polymerization suggesting a dual role of this protein in mismatch repair. The human single stranded binding protein, replication protein A (RPA), which is also required for DNA replication, is



Mismatch Repair in Genome Stability. Fig. 1

also required for mismatch repair probably at the stage of repair synthesis. Finally it is not known which of the several ligases catalyzes the ligation of the newly synthesized DNA stretch to complete repair.

In the *in vitro* assays usually employed to analyze mismatch repair, a single strand nick provides the strand discrimination signal to identify the strand to be corrected. It is not known, however, whether human cells *in vivo* effectively use the same signal. It is possible that the transient presence of Okazaki fragments identifies the newly synthesized strand to be acted on by mismatch repair.

Biological consequences

Loss of mismatch repair leads to a mutator phenotype, affecting both, coding and non-coding regions such as microsatellites [→ microsatellite instability]. Thus inactivation of this pathway in human cell lines and in knock-out mice is associated with large increases in spontaneous mutation rates at functional genes (even by two or three order of magnitude). Molecular analysis of spontaneous mutations occurring in mismatch repair-defective cells shows that both frameshifts and base substitutions are significantly increased in the absence of a functional mismatch repair.

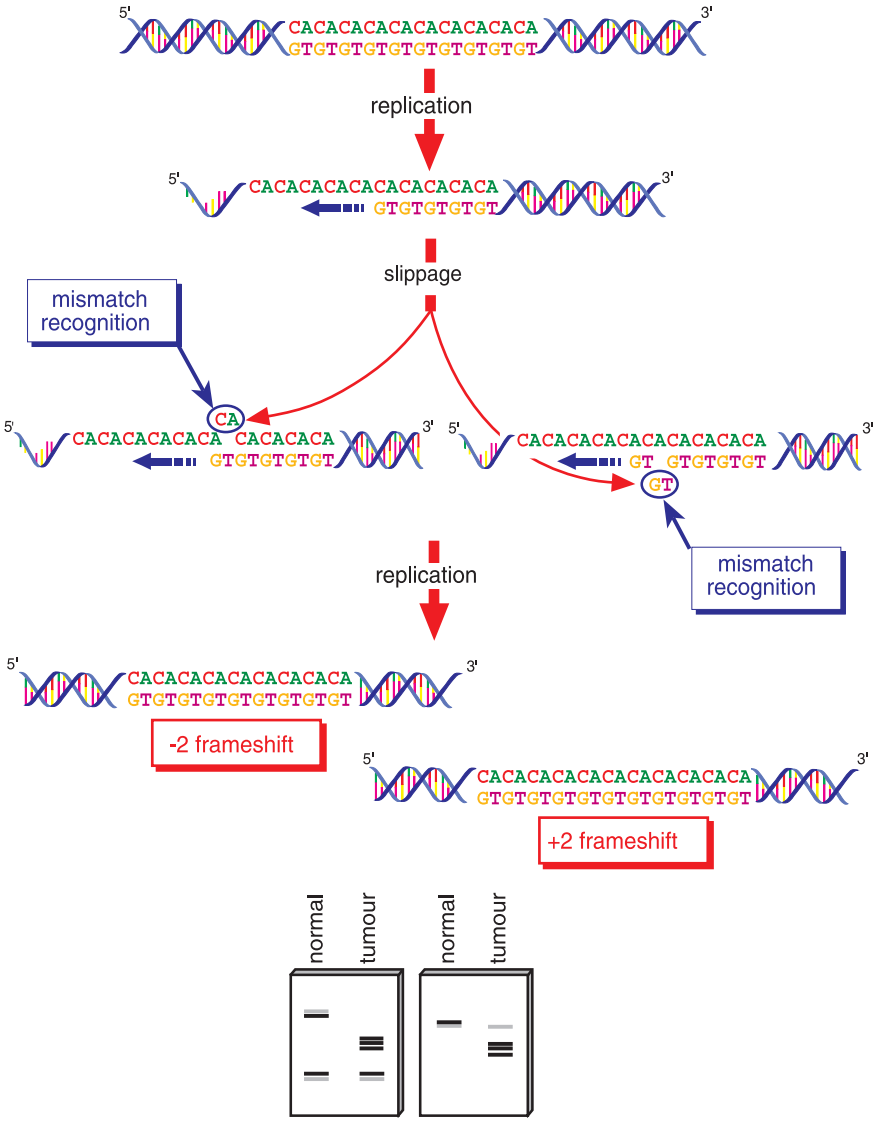
Regions containing microsatellite sequences are particularly prone to undergo slippage events between template and daughter strands during DNA replication (Fig. 2). Transient dissociation of the strands followed by realignement out of phase of the repeats generates extra-helical loops of one or more repeats. These structures are frameshift intermediates which leads to loss or gain of one repeat unit. Minus or plus frameshifts derive therefore from loops formed on the template or daughter strand, respectively. Mismatch repair is the main pathway for the correction of these slip-mispaired intermediates. Thus loss of mismatch repair ultimately results in a profound instability of microsatellite sequences and alterations in the number of mono- or di-nucleotide repeats reflects an accumulation of frameshift mutations. An example is shown in Fig. 1. The phenotype of tumors with defects in mismatch repair is defined as Replication Error positive (RER^+) or with microsatellite instability (MSI).

Loss of mismatch repair is also invariably associated with acquisition of a high level of resistance to methylating agents (tolerant phenotype). hMutSα can indeed recognize some modified DNA bases such as the methylated base O^6-methylguanine as well as the base analog 6-thioguanine and cisplatin adducts. It has been proposed that mismatch repair transforms these DNA modifications into killing events through 'abortive' repair attempts leading to the formation of double-strand breaks or triggering of a G2 checkpoint response which results in cell death. Thus loss of mismatch repair results in the modification of the sensitivity to therapeutic DNA damaging agents.

Clinical relevance

Mutations in genes encoding mismatch repair proteins underlie the predisposition to colorectal tumors in the familial syndrome Hereditary Non Polyposis Colon Cancer (→ HNPCC). The inherited germline mutation in at least one of four mismatch repair genes (*hMSH2*, *hMLH1*, *hMSH6*, *hPMS2*) and the subsequent functional inactivation of the second allele predisposes to colon cancer at an early age. It is proposed that the mutator phenotype, following loss of mismatch repair, facilitate the occurrence of mutations in genes controlling proliferation and/or apoptosis thus leading to an increased cancer risk. The majority of the mutations in the HNPCC families occur in the *hMSH2* and *hMLH1* genes. A peculiar characteristic of the *hMLH1* gene is that this gene is often silenced through methylation of its promoter. Some HNPCC families with unusual tumor spectra have mutations in the *hMSH6* gene.

At variance with the classical 'tumor suppressor' pathway that usually display gross genome instability (large chromosomal changes and aneuploidy), mismatch repair defective tumors are usually pseudo-diploid. It has been proposed that the multiple genetic changes needed for malignancy can be obtained in two alternative and mutually exclusive ways: in a minority of cases instability at gene level because of mismatch repair deficiency and



Mismatch Repair in Genome Stability. Fig. 2

gross chromosomal changes in the majority of cases. Indeed colorectal cancer occurring in HNPCC patients show a specific pattern of genetic changes compared to sporadic tumors with chromosomal instability. Runs of mononucleotide repeats in coding regions of genes become a preferential target of mutagenic events as a consequence of mismatch repair loss. For example tumors with microsatellite instability preferentially accumulate frameshift mutations in genes involved in the control of proliferation and death. Examples of frameshifts that are likely to confer direct or indirect proliferative advantages and which are particularly prevalent in mismatch repair defective tumors include transforming growth factor β receptor (*TGFβRII*), insulin-like growth factor type II (*IGF-II*), *β2-microglobulin*, *Bax* and *caspase 9* as well as the mismatch repair genes *hMSH6* and *hMSH3*.

Microsatellite instability is also quite common in some apparently sporadic tumors suggesting that loss of mismatch repair can be a relatively common event in human cancer. In particular 20% of sporadic colorectal tumors and, to a varying degree, tumors of several

Mismatch Repair in Genome Stability. Table

gene	homologue	chromosome
hMSH2	mutS - *E. coli*	2p22-21
hMSH3	mutS - *E. coli*	5q11-12
hMSH4	mutS - *E. coli*	1p31
hMSH5	mutS - *E. coli*	?
hMSH6	mutS - *E. coli*	2p16
hMLH1	mutS - *E. coli*	3p21
hPMS1	postmeiotic segregation increased 1; *S. cerevisiae*	2q31
hPMS2	postmeiotic segregation increased 2; *S. cerevisiae*	7p22

other organs (gastrointestinal tract, bladder, endometrium and ovary) display the mutator phenotype characteristic of mismatch repair defects.

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Mitogen-activated Protein Kinase (MAPK)

Definition

Mitogen-activated protein kinases (synonym: → MAP kinases; MAPKs) are important signal transduction enzymes and unique to eukaryotes. They belong to a family of serine /threonine kinases and are involved in diverse physiological functions such as → stress response, cell proliferation, → apoptosis, cellular differentiation, cell motility, and immune-responses, by phosphorylating specific target molecules, such as transcription factors or other kinases.

MAP kinases can be localized in different cellular compartments, such as the cytoplasm and the nucleus. Their activity is complex and involves three-tiered cascades composed of a MAPK, a MAPK kinase (also: MKK or → MEK) and a MAPKK kinase (also: MAPKKK or → MEKK).

MAPKs may activate specific effector kinases (MAPK-activated protein kinases, MAPKAPKs), they are inactivated by MAPK phosphatases.

In mammals, four distinctly regulated groups of MAPKs can be distinguished. These are the extracellular signal regulated kinases (→ ERK 1/2), the Jun amino-terminal kinases (→ JNK 1/2/3), the p38 proteins and ERK5, which are activated by specific MAPKKs. Each MAPKK, however, can be activated by more than one MAPKKK, increasing the complexity and diversity of MAPK signaling.

With few exceptions (mostly transcription factors) the MAPK substrates are not known to date.

Mitomycin C

Definition

Mitomycin C is an antibiotic chemotherapeutic agent that inhibits DNA synthesis and can reduce the risk of recurrence and progression.

Mitotic Catastrophe

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Synonyms

- mitotic cell death

Definition

Mitotic catastrophe is a form of cell death induced by radiation, chemotherapeutic drugs or hyperthermia. Mitotic catastrophe results from aberrant mitosis, which is followed by partial or complete fragmentation of interphase nuclei. Mitotic catastrophe is distinguishable from → apoptosis by morphologic criteria and by the insensitivity of this process to specific inhibitors of apoptosis (such as → Bcl-2).

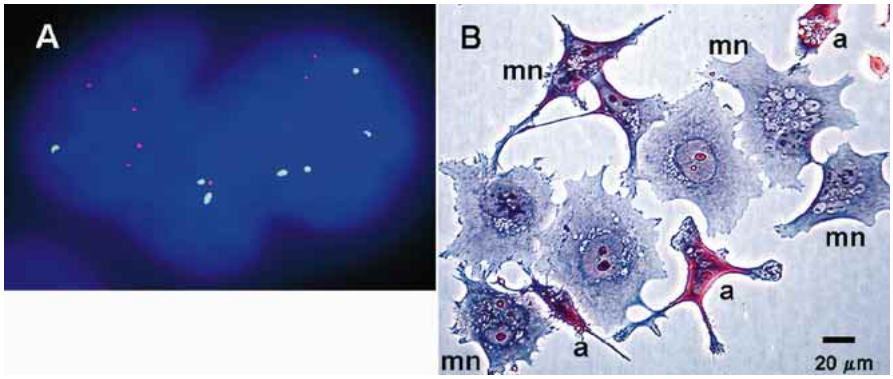
Characteristics

Interphase cells that suffer lethal damage from radiation or cytotoxic drugs may die before reaching mitosis. This rapid cell death usually occurs through the process of apoptosis. Alternatively, damaged cells may proceed into aberrant mitosis, which most often terminates at the stage of abnormal metaphase or, in some cases, abnormal anaphase or late prophase. This aberrant mitosis does not produce proper chromosome segregation and cell division, but results in the formation of large non-viable cells with two or more micronuclei that are completely or partially separated from each other. In the case of partial separation, the cell displays a large lobulated nucleus. The micronuclei arise through the formation of multiple nuclear envelopes around chromosome clusters at the end of abnormal mitosis. Chromosomes are randomly distributed among the micronuclei of a multilobulated nucleus of a drug-treated tumor cell (Fig. A). The described process is called mitotic cell death or mitotic catastrophe.

Micronucleated (mn) cells, indicative of mitotic catastrophe, can be easily distinguished by their morphology from apoptotic (a) cells (Fig. B). While apoptotic cells may have fragmented nuclei, they are characterized by shrunken cytoplasm and condensed chromatin, whereas micronucleated cells are large and contain uncondensed chromosomes. Cells that undergo mitotic catastrophe do not usually show DNA ladder formation or DNA breaks that are detectable by → TUNEL staining in apoptotic cells. Mitotic catastrophe and apoptosis often arise in the same population of cells treated with a cytotoxic agent. Furthermore, the onset of mitotic catastrophe may be followed in the same cells by the activation of the apoptotic program. For this reason, mitotic catastrophe has been sometimes described as an early stage of apoptosis. However, the importance of mitotic catastrophe as an independent determinant of radiation- or drug-induced cell death has been demonstrated by the suppression of apoptosis by ectopic overexpression of apoptosis-inhibiting proteins, such as Bcl-2 or → P-glycoprotein (the latter inhibits apoptosis through a presently unknown mechanism that is distinct from its function as a multidrug transporter). In the presence of these inhibitors, the number of apoptotic cells induced by drugs or radiation is greatly diminished, but the number of cells undergoing mitotic catastrophe (as well as terminal growth arrest in the form of → accelerated senescence) is correspondingly increased. As a result, inhibition of apoptosis was found in several studies to have little or no effect on the survival and regrowth of treated cells. In other words, cells that have initiated the process of mitotic catastrophe may be dying through apoptosis but not because of it. This functional distinction underscores the importance of mitotic catastrophe in cellular response to anticancer agents.

Cellular and molecular aspects

Some insights into the mechanism of mitotic catastrophe come from the studies where this response was induced in mammalian cells or in fission yeast by genetic manipulations. In particular, mitotic catastrophe was observed in cells that were forced into mitosis through untimely activation of the mitosis-initiating



Mitotic Catastrophe. Fig. – Morphology and chromosome distribution in micronucleated cells arising after mitotic catastrophe. A Random distribution of chromosomes among the micronuclei of a partially fragmented nucleus of human HT1080 fibrosarcoma cells treated with 20 nMol doxorubicin for 3 days. Fluoresecence *in situ* hybridization was carried out with probes specific for chromosomes 18 (green) and 21 (red); nuclei were stained with DAPI [reproduced with permission from Chang et al. (1999), Cancer Res 59:3761 – 3767]. **B** Micronucleated (mn) and apoptotic (a) cells in a population of HCT116 colon carcinoma cells treated with 50 nMol doxorubicin for 4 days [reproduced with permission from Chang et al. (1999), Oncogene 18:4808 – 4818].

complex, comprised of Cyclin B and Cdc2 kinase. This complex is regulated by a number of proteins that control the G2/M checkpoint [→ G_2/M transition] of the cell-cycle. Ectopic coexpression of cyclin B1 and Cdc2, cyclin A and Cdc2, and cyclin B1 and Cdc25C (an enzyme that dephosphorylates and activates Cdc2) was shown to induce premature mitosis and subsequent mitotic catastrophe in mammalian cells. The extent of mitotic catastrophe has been further increased through the use of a Cdc2 mutant, which is resistant to inhibitory phosphorylation. Mitotic catastrophe was also shown to result from mutations or inactivation of other genes that act in mitosis, such as RCC1 involved in chromosome condensation, the nuclear-mitotic apparatus (NuMA) protein and the centrosome-associated protein → survivin. Survivin is best known as an inhibitor of apoptosis, and the loss or displacement of survivin during abnormal mitosis may potentially account for the link between mitotic catastrophe and apoptosis.

Induction of mitotic catastrophe by heat or ionizing radiation has been associated with increased cellular levels of cyclin B1 and Cdc2 kinase activity, suggesting that cellular damage may induce premature mitosis. The entry of damaged cells into mitosis is delayed by growth arrest at the G2/M checkpoint. Agents that abrogate this checkpoint, such as caffeine, okadaic acid or staurosporine, were shown to promote mitotic death in irradiated or drug-treated cells. Mitotic catastrophe is also enhanced by genetic inactivation of G2/M checkpoint control genes, such as the cyclin-dependent kinase inhibitor → p21 (Waf1/Cip1/Sdi1) or 14-3-3-σ, a protein that maintains cytoplasmic sequestration of cyclin B1 and Cdc2. Damage-induced p21 induction is mediated largely (but not exclusively) by p53 protein, and p53 was found in several studies to be a negative regulator of mitotic catastrophe (in contrast to the usually positive effect of p53 on apoptosis). While the key role of the G2/M checkpoint in preventing mitotic catastrophe is well documented, the events resulting in the aberrant mitosis of damaged cells remain to be elucidated.

Clinical relevance

Mitotic catastrophe has been characterized as the main form of cell death induced by ionizing radiation and is recognized as a prominent response to different anticancer drugs, such as doxorubicin, etoposide, taxol, cisplatin or bleomycin. This response has been documented in many cell lines derived from different tumor

types, including apoptosis-resistant lines. As a general trend, mitotic catastrophe is more frequently induced by lower doses and apoptosis by higher doses of cytotoxins. Mitotic abnormalities and micronucleation have been observed both *in vitro* and *in vivo*; in fact, micronuclei formation has long been used as a common indicator of genotoxic damage.

The importance of mitotic catastrophe as a major response of tumor cells to chemotherapy and radiation has been obscured over the past decade by the emphasis on apoptotic cell death and the view that mitotic catastrophe is merely an early stage of apoptosis. However, the interest in this topic has been recently revived by the demonstration of the importance of the G2/M checkpoint as a determinant of cytotoxicity of anticancer agents and by the demonstration that the inhibition of apoptosis increases the fraction of cells that die by mitotic catastrophe. Mitotic catastrophe is potentiated by p53 deficiency and a weakened G2/M checkpoint. These are distinguishing characteristics of many tumors, suggesting that tumor cells may be more susceptible to mitotic catastrophe than normal cells. The elucidation of the molecular determinants of mitotic catastrophe may help to exploit this preferential mode of tumor cell death in a therapeutic setting.

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Mitotic Recombination

Definition

Mitotic recombination is the crossing over between two homologous double stranded DNA molecules leading to physical exchange of DNA and genetic information. This phenomenon, which is known to occur frequently during meiosis, is relatively rare during the mitotic cycle. One of the consequences of mitotic recombination is '→ loss of heterozygosity' (LOH), which may play an important role in the carcinogenic process by facilitating the loss of → tumor suppressor genes.

Mitoxantrone

Definition

→ Anthracycline.

MKI67

Definition

MKI67 is a protein of somatic cells, identified by monoclonal antibody → Ki-67. The gene maps to chromosome 10q25-qter. The protein (3256 amino acids, 358 kd) is thought to be required for maintaining cell proliferation. Its subcellular localization is nuclear; in G1 phase the protein is in the perinucleolar region, during mitosis it is present on all chromosomes. In non-dividing cells the protein cannot be detected. Analysis of cells with Ki-67 antibody is therefore a widely employed diagnostic tool to determine the proportion of dividing cells within a tumor.

MKK

Synonyms

- MAPKK
- → MEK

Definition

Mitogen-activated protein kinase kinase.

MLH1

Definition

MutL (*E.coli*) homolog 1 (MLH1), also known as → COCA2, is a protein (756 aa, 84 kD) involved in mismatch repair of DNA. It forms heterodimers with → PMS2. The gene maps to 3p21.3. Mutations are associated with familial hereditary nonpolyposis colon cancer (→ HNPCC) (→ Lynch syndrome); colon cancer, nonpolyposis type 2.

MLL

Definition

The MLL gene (also referred to as ALL1 or HRX), maps to 11q23, a region involved in recurring chromosomal translocations. The MLL gene is rearranged with several partners, from different chromosomes, in both myeloid and lymphoid acute leukemias.

MMAC1

Definition

→ PTEN.

MMP

Definition

Matrix → metalloproteinase (MMP) is family of zinc-dependent proteinases used by cells to break down extracellular matrix. MMPs are essential for the degradation of the extracellular matrix during cellular invasion; many MMPs are Ets responsive [→ ETS transcription factors]. Matrix metalloproteinases are the most extensively studied → AP-1 target genes in tissue culture cells and in the animal; → matrix metalloproteinases.

MMP-7

Definition

Metalloproteinase-7, or matrilysin, is a member of the matrix metalloproteinase family believed to have a critical role in tumour invasion. It is often expressed in gastrointestinal cancers. The gene maps to 11q21.

MMR

Definition

→ Mismatch repair.

MNT

Definition

MNT (Myc antagonist), also known as → MAX binding protein, encodes a nuclear basic helix-loop-helix protein of 582 aa and 62 kD. It binds DNA as a heterodimer with MAX at the → E-box CACGTG, and represses transcription by antagonizing MYC. The *MNT* gene maps to 17p13.3.

Modifier Loci in Cancer

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Definition

Cancer modifier loci are genetic loci characterized by allele-specific effects on cancer development, e.g., stimulation or inhibition of tumor development and/or progression. They are involved in fine tuning the tumor phenotype. The term is most often used to define loci characterized by an inhibitory effect on tumorigenesis.

Characteristics

Evidence for the existence of cancer modifier loci has come from studies in rodent models, characterized for their genetic susceptibility to spontaneous and induced tumorigenesis. Crosses between cancer-susceptible and -resistant strains have resulted in F_1 hybrids either susceptible or resistant to tumor formation, depending on which of the two traits (susceptibility/resistance) is dominant in the cross.

Gastric and intestinal cancer

Tumors of the alimentary tract are rare in untreated stocks of laboratory rats and mice. However, treatment with chemical carcinogens renders ACI and Buffalo rats susceptible and resistant, respectively, to gastric tumorigenesis (1). In F_1 hybrids, the genetic resistance of the Buffalo parent is dominant and associated with a differential proliferative response of gastric mucosa to carcinogen insult (2). The → *Min* mouse spontaneously develops intestinal tumors, due to a mutation in the *Apc* gene, but the → *Mom1* locus (*Pla2g2a*), which maps to mouse chromosome 4, decreases the multiplicity and size of Min-induced intestinal tumors (3; 4) (Fig.).

Hepatocellular cancer

The high susceptible C3H strain carries multiple, unlinked hepatocarcinogen sensitivity (*Hcs*) loci (5). The trait affects tumor growth and is dominant in some crosses. However, when C3H and BALB/c strains are crossed, the F_1 mice develop hepatocellular tumors that are >20-fold smaller than those of the C3H/He parent (5). Thus, the BALB/c strain must carry dominant tumor resistance loci that affect liver tumor growth (promotion/progression). Two hepatocarcinogen-resistance (*Hcr*) loci, derived from DBA/2 mice, have been mapped (6). The BN rat strain also carries *Hcr* loci that greatly decrease hepatocellular tumor growth in (BNxF344)F_1 hybrids (7) (Fig.).

Lung cancer

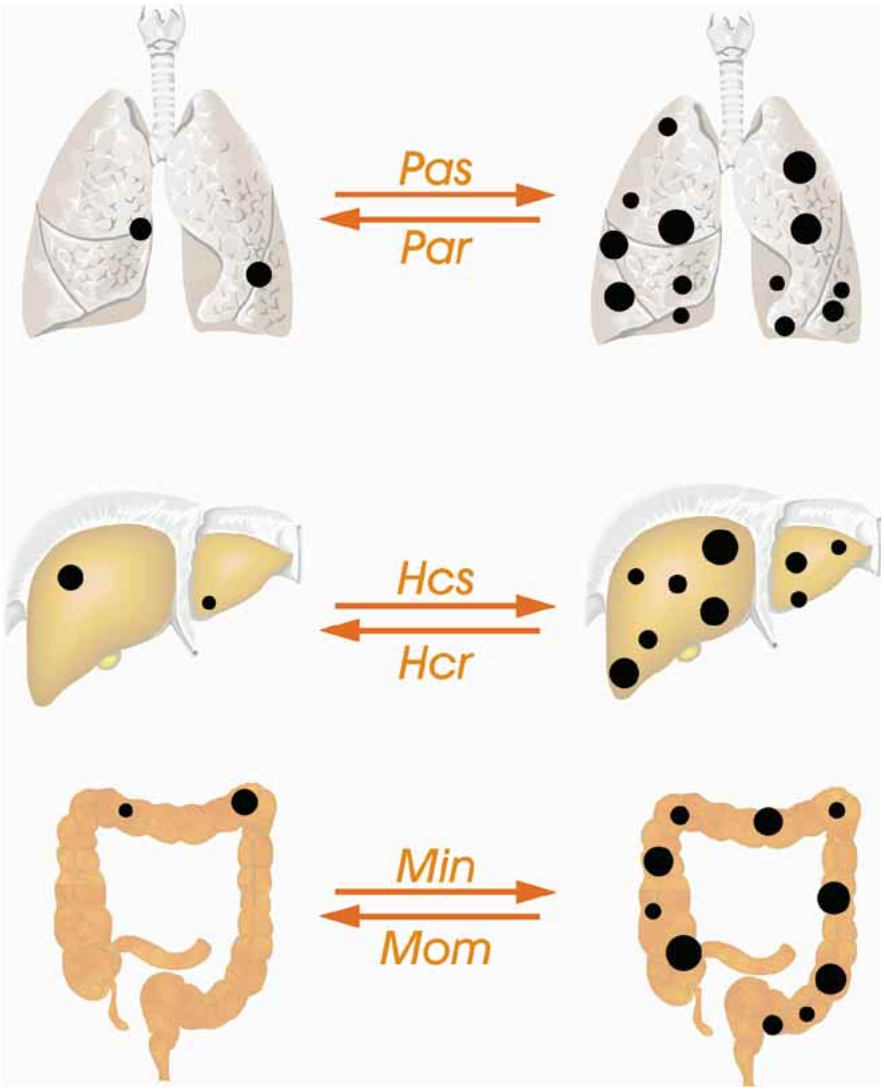
Inbred mouse strains are characterized by high, intermediate and low susceptibilities to lung tumorigenesis, with the *Pas1* locus on chromosome 6 playing a major role in the inherited predisposition (8; 9). Pulmonary adenoma resistance (*Par1-4*) loci are carried by the *M. spretus*, SM/J and BALB/c strains and are not linked to the *Pas1* locus (Fig.). *Par* loci inhibit in a dominant way the genetic susceptibility to lung tumorigenesis provided by the *Pas1* susceptibility allele (9).

B-Lymphoma

SL/Kh mouse strain displays a high incidence of spontaneous pre-B lymphomas, but tumor development is inhibited in F_1 hybrids after crossing with low-lymphoma strains. Two lymphoma-resistance loci carried by the MSM/Ms strain (derived from *Mus molossinus*) have been mapped (10).

Mammary tumors

The COP rat is resistant to mammary tumor induction and carries a dominant mammary carcinoma suppressor locus (*Mcs1*) (11). The frequencies of N-methyl-N-nitrosourea-induced *Ha-ras* mutations are the same in susceptible



Modifier Loci in Cancer. Fig. – Cancer resistance genes act as modifiers of genes that cause inherited predisposition to cancer, partially suppressing their effects on tumor development and growth.

and resistant rat strains (12), suggesting that the identified resistance *Mcs1* locus does not operate at the initiation step but instead during tumor progression.

Melanoma

Inbred mouse strains do not spontaneously develop melanomas. Transgenic mice with inherited predisposition to nevi and melanoma development have been constructed by using different mutated or over-expressed genes. F_1 crosses between melanoma-prone metallothionein/ret transgenic mice and BALB/c mice show delayed and reduced melanoma development, despite the same level of transgene expression as in the transgenic parent (13). Three loci (*Melm1-3*), inhibiting melanoma development, have been mapped (14).

Plasmacytomagenesis

BALB/c mice are highly susceptible to the induction of plasmacytomas, whereas DBA/2 mice carry two dominant plasmacytomagenesis resistance genes (15).

Renal cancer

The Eker rat carries a *Tsc2* germline mutation that confers a strong predisposition for renal carcinoma development. Crosses with the BN strain still develop renal tumors, but of much smaller size than in the Eker parent. Thus, BN-derived resistance gene(s) exists that inhibits renal tumor growth (16).

Squamous cell skin cancer

Three loci responsible for dominant resistance to skin cancer have been found using inter-specific crosses with the M. spretus strain (17). A mouse outbred line, carcinogenesis resistant (Car-R) has been phenotypically selected for high genetic resistance to two-stage skin tumorigenisis (18).

Identification of cancer modifier genes provides a step towards understanding the biochemical mechanisms of inherited resistance/susceptibility to cancer. Eventually, it may become possible to devise new chemoprevention and therapeutic strategies for cancer based on the biochemical effects of such genes. An important consideration is that an organism's natural tumor resistance gene product would not, in principle, be expected to cause the adverse side-effects often associated with the cancer therapeutic drugs now available.

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Modular Domains

Definition

Many proteins are composed of more than one structural unit or domain; some of these structurally defined units can be incorporated in many different proteins as modules, performing the same of different function (modular domains).

Mole

Definition

A mole, or → naevus, is caused by the clonal proliferation of melanocytes in the skin. The precise location of the naevus cells determines a histological classification into dermal, junctional or compound nevi. The majority of moles are acquired. Moles are more common in sun-exposed areas of the body and the number and size typically increase during puberty (→ melanoma). Atypical naevi may be distinguished by their asymmetry, border (indistinct irregular margins), colour (presence of unevenness of pigmentation, red-brown colour) and diameter $\geq$ 5 mm. Multiple naevi of atypical appearance are called dysplastic naevi (DN), the atypical mole syndrome (AMS) or familial atypical multiple mole-melanoma (FAMMM) syndrome. Typical naevi may be found on non-sun-exposed areas, the genitalia and the iris.

Molecular Cancer Epidemiology

Definition

Molecular cancer epidemiology is a novel research approach in which advanced laboratory methods are used in combination with analytic epidemiology to identify specific exogenous and/or host factors that play a role in human cancer causation at the biochemical and molecular level.

MOM1

Definition

MOM1 stands for '*modifier of Min*'. Mutations of the human *adenomatous polyposis coli* (*APC*) gene are prominent genetic alterations in both sporadic and familial cases of colorectal cancer. *Min* (*multiple intestinal neoplasia*) mice carry a dominant mutation in their *Apc* gene that predisposes them to develop multiple intestinal polyps. Genetic analysis lead to the identification of the murine *Mom1* locus. The latter is responsible for some of the genetic variability in a number of polyps, which are found in inbred mouse strains that carry the *Min* mutation. The secretory type II phospholipase gene (*Pla2s*, synonym *Pla2g2a*) has been demonstrated to be a candidate for *Mom1*. In comparison to mice without the *Pla2s* mutation, the double mutation of *Pla2s* in mice that carry the *Min* mutation appears to account for their increased number of polyps.

In humans, *PLA2S (PLA2G2A)* maps to 1p35, a region that is frequently altered in sporadic colorectal cancers. In this case, however, a → modifier locus role is not established. The protein (144 aa, 16 kD) is thought to be involved in the regulation of phospholipid metabolism of biomembranes. The expression is enhanced in various cell types by inflammatory cytokines as well as by liposaccharides. Expression is elevated in numerous inflammatory diseases including sepsis, Crohn disease and pancreatitis.

Monolayer

Definition

A monolayer describes the growth of eukaryotic cells in culture as a layer only one cell deep.

Monomeric GTPases

Definition

Monomeric GTPases are a large superfamily of GTP-binding proteins that function in various cellular processes, such as growth control (→ Ras family members), organization of the cytoskeleton (→ Rho family proteins) or intracellular transport between different compartments of the cell (Rab, ARF, Sar1, or Ran family members). They function as molecular switches by cycling between a GDP- and a GTP-bound state, in an analogous way to the heterotrimeric → G-proteins.

Monosomy

Definition

Monosomy is the presence of only one of the two homologous chromosomes in the cell.

Morphogenesis

Definition

Morphogenesis is the development of body shape and organization during the embryonic development of an organism. It refers to a process in which cells organize into well defined three-dimensional structures that resemble the tissue of origin. For example, → scatter factor has been shown to induce kidney epithelial cells (MDCK) to organize into renal tubular-like structures, breast epithelial cells to organize into mammary duct-like structures, lung epithelium to organize into alveolar-like structures, and microvessel endothelial cells to organize into capillary-like tubes. Apparently, scatter factor, acting through the c-→ Met receptor, activates a cell type-specific program of differentiation.

Morphogenic Differentiation

Definition

Morphogenic differentiation refers to the drastic changes in shape and three-dimensional organization of undifferentiated precursor cells in developing tissues and organs.

MORT-1

Definition

→ FADD.

Mosaic Animals

Definition

Mosaic animals have a heterozygous genetic constitution that contains homozygous mutant clones. Most mosaic animals are generated by the induction of → mitotic recombination.

Mosaic Screen

Definition

A mosaic screen is a type of genetic screen that relies on inducing homozygous mutant clones in otherwise heterozygous animals. Currently, it is only feasible in the *Drosophila* model system, where the yeast → FLP/FRT system has been adapted to allow efficient and reliable generation of mutant clones by inducible → mitotic recombination.

Mosaicism, somatic

Definition

Somatic mosaicism is a situation where the body of an individual consists of cell lines of different genetic make-up. This situation appears to be the rule rather than the exception, if one considers the following: the probability for a specific gene to become mutated is, per cell generation, in the order of 10^{-6} to 10^{-8}/cell. In humans, the number of gene copies per (diploid) cell is estimated at 160,000. Given that an adult human has in the order of 10^{13} cells, it is clear that we are all bound to be mosaics of genetically different cell lines. When specifically considering a disease gene for a recessive disease, one can distinguish forward mosaicism and reverse mosaicism. This can be exemplified by the situation of → Fanconi anemia (FA):

- 'forward' (inactivating) mutation in the wild type copy of a FANC+/FANC- carrier cell will produce a full-fledged FANC-/FANC- cell
- a reverse (correcting) mutation in a defective FA gene copy in a FA patient will produce a heterozygous carrier cell, which is phenotypically normal. Considering that mutations occur at random, correcting mutations are evidently much more rare than inactivating mutations.

Motility

Definition

Motility is cellular migration that can be either random or directed. Cell movements can result in cell translation from one place to another, or in rearrangements of cell shape with no cell translation.

MRD

Definition

→ Minimal residual disease.

MRE11

Definition

Mre11 partners with → Rad50 and Nbs1 [→ Nijmegen breakage syndrome], having nuclease activity on both single- and double-stranded DNA. It is involved in a number of roles in DNA maintenance; → repair of DNA.

MRF

Definition

Muscle related factor (MRF) is a → bHLH protein family involved in myogenesis, comprising MyoD, Myogenin, Myf5, Myf6, Mist1 and Mrf4.

MRI

Definition

Magnetic resonance imaging (MRI) is a computerized assisted imaging technique that uses magnetic fields to scan the human body for masses and diseases.

mRNA

Definition

Messenger RNA (mRNA) is the transcription product made from a gene as a template. It is the type of RNA that transports the genetic information coded in DNA, residing in the nucleus, to the cytoplasm where it is translated into protein.

MRP

Definition

→ Multidrug resistance-associated protein.

MSH

Definition

→ POMC.

MSH-R

Definition

Melanocyte stimulating hormone receptor; → MC1R.

MSIN3

Definition

Mammalian Sin3 (mSin3) is a transcriptional co-repressor that links DNA binding proteins to → histone deacetylases.

MSP

Definition

→ Macrophage-stimulating protein.

MST1

Definition

Macrophage stimulating 1; → macrophage-stimulating protein.

MST1R

Definition

→ Macrophage stimulating 1 receptor.

MTS1

Definition

→ CDKN2A.

Mucin

Definition

Mucin, also known as PUM (peanut-reactive urinary mucin) or mucin 1, is a transmembrane protein (1255 aa, 122 kD) that interacts directly or indirectly with the actin cytoskeleton. It is aberrantly expressed in human epithelial tumors, such as → breast cancer. The gene maps to chromosome band 1q21.

Muclin

Definition

Muclin, an alternative name for → CRP-ductin, was identified as the major sulfated protein of the pancreas. It accumulates in mucous plugs of mice that resemble the features of cystic fibrosis.

Mucosal Immune Defense

Definition

Mucosal immune defense is immune defense at epithelial surfaces (except for the skin) that are in permanent contact with the environment (e.g. oral, alveolar and gastrointestinal epithelium).

Multidrug Resistance

Definition

Multidrug resistance is cross-resistance to multiple cytotoxic drugs with different mechanisms of action; → P-glycoprotein.

Multidrug-resistance Protein

Definition

Multidrug resistance protein (MRP), also called P-glycoprotein, is a member of the ATP-binding cassette superfamily of transporter proteins. It is responsible for the efflux of hydrophobic xenobiotics [→ xenobiotic] from cells, many of which are not metabolised. Substrates for the transporter include *Vinca* alkaloids, anthracyclines, epipodophyllotoxins and antibiotics. MRP is located in the cell membrane of some normal and tumor cells. It exports specific chemotherapeutic agents from the cell, resulting in low cellular concentrations of drug and lack of antitumor effect; → detoxication.

Multidrug Resistance-associated Protein

Definition

Multidrug resistance-associated protein is a member of the ATP-binding cassette superfamily of transporter proteins. It is responsible for the efflux of anionic conjugates of lipophilic compounds from the cell. Many of the substrates are products of phase 2 drug metabolism; → detoxification.

Multidrug Transporter

Definition

→ P-glycoprotein.

Multi-modal Therapy

Definition

Multi-modal therapy is a treatment involving two or more approaches, including surgery, chemotherapy or radiotherapy.

Multiple Endocrine Neoplasia Type 1

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Synonyms

- MEN 1

Characteristics

Clinical Manifestations

Multiple endocrine neoplasia type 1 or MEN 1, is transmitted as an autosomal dominant trait which is classically characterised by a triad of neoplasia affecting the parathyroid glands (90 – 97% of patients), enteropancreatic endocrine tissues (30 – 80%) and the anterior pituitary gland (15 – 50%). It has a near complete penetrance and an equal sex distribution with a prevalence of 0.02-0.2/1000. The clinical manifestations of MEN 1 are primarily those of endocrine gland hyperfunction. In addtion to the three types of endocrine tumors mentioned above, MEN 1 patients may develop tumors in a range of endocrine and non-endocrine tissues: adrenocortical neoplasia, lipomas, foregut

and midgut carcinoids, thyroid neoplasms, skin tumors, spinal ependymoma, renal angiomyolipoma and leiomyoma of the esophagus.

Genetics

The *MEN 1* gene fulfills the paradigm of tumor suppressor gene. MEN 1 patients inherit one mutated gene copy and later in life, acquire a second mutation which is usually in the form of chromosomal deletion or loss of heterozygosity (LOH), causing complete elimination of its tumor suppressive activity and setting off tumor development. In accordance with this theory, the second hit mutation was first found in two MEN 1-related endocrine pancreatic tumors in chromosome 11 which was confirmed to be the *MEN 1* locus by linkage analysis on MEN 1 families. The search for the *MEN 1* gene went through the full process of positional cloning involving the development of:

- a comprehensive physical map,
- new genetic markers for narrowing the interval,
- a transcript map, covering the *MEN 1* region.

From the latter, a number of genes were selected for mutation analysis until the *MEN 1* gene was finally identified.

The *MEN 1* gene and it homologues

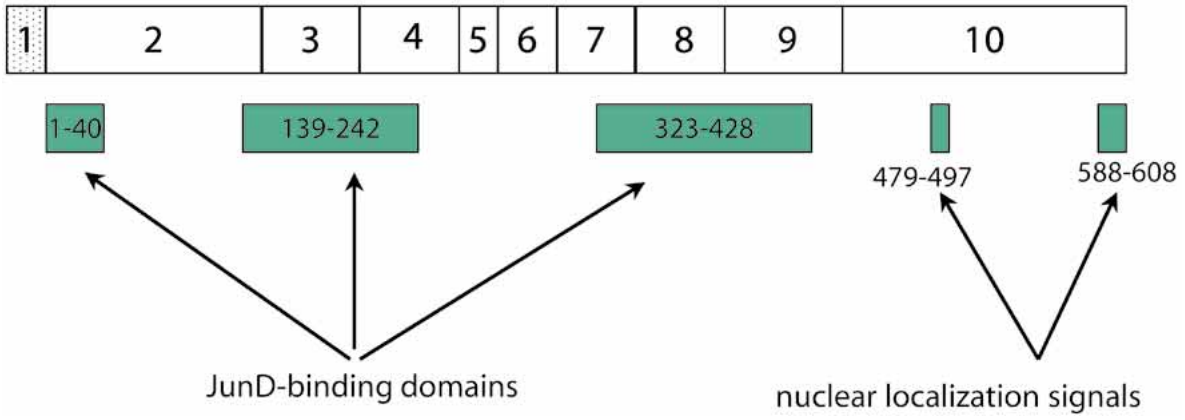
The *MEN 1* gene contains ten exons (only exons two to nine are transcribed) which encode a 610-amino acid protein product. So far, two transcripts have been identified, most likely as the result of alternative splicing: a 2.9 kb transcript expressed in all tissues and a 4.2 kb transcript in pancreas and thymus. The *MEN 1* murine homologue, *Men 1*, has been characterized which is located in the mouse synteny region of chromosome 19 and showed 97% homology compared to *MEN 1*. Like its human counterpart, it has ten exons and two transcripts (3.2 and 2.8 kb), a result of alternative splicing in intron 1. During mouse embryogenesis, the *Men 1* activity is already detected from day 7. It is generally expressed in all tissues although by day 17 it is most prominent in thymus, skeletal muscles, and CNS system. In adult tissues, it is also generally expressed although most prominent in testis (perinuclear spermatogonia), cerebral cortex (nerve cell nuclei), and thymus. Its universal expression pattern, especially in tissues, not affected by multiple endocrine neoplasia type 1, suggests its universal functional role, both in development and housekeeping. To date, the *MEN1* homologues of rat, zebrafish and *Drosophila melanogaster* have also been identified.

Menin and its Interacting protein(s)

The MEN 1 protein, menin, has no homology to any proteins known to date. By immunofluorescent staining, subcellular fractionation and Western Blotting, it was found to be predominantly nuclear. Two nuclear localization signals (NLS) or regions involved in nuclear localization were identified in the C-terminus of menin, one as a stretch of 19 amino acids (amino acid position 479 to 497), the other 20 amino acids long (position 588 to 608). To date, one interacting protein of putative importance has been identified, the AP-1 transcription factor JunD. The AP-1 transcription factors have basic leucine zipper (bZip) domains that pair to bind DNA as a Y-shaped heterodimer. Menin has been found to specifically bind to JunD via its N-terminal region but not to the other members of the Jun or → Fos families of transcription factors. MEN 1 mutations that interrupt this binding activity, increase JunD activated transcription. Based effect on the binding that MEN1 mutations have, when at locatised elsewhere, three binding domains have been identified. They are located at amino acid positions 1-40, 139-242 and 323-428.

MEN 1 Mutations

Mutation analysis of MEN 1 patients have identified over 250 different mutations which spread over the translated exons two to nine. Approximately 80% of these mutations result in truncated proteins and are likely to eliminate protein function, and further support *MEN 1* in its role as a tumor suppressor gene. Several mutations, such as 357del4 have been found more



Multiple Endocrine Neoplasia Type 1. Fig. – Diagram of the gene showing coding regions for three JunD-binding domains and two nuclear localization signals.

frequently in patients of different ethnic origin, representing mutational 'warm spots'.

Studies were undertaken in two large families with familial isolated hyperparathyroidism (FIHP) that is considered to be a milder form of MEN 1. Two missense mutations, located in close proximity to each other, have been identified in the fourth exon. One of the mutation affected seven, the other 14 family members. Interestingly, these two mutations, E255K and Q260P, are outside the sites for nuclear localization signals and JunD binding (Fig.) suggesting a correlation of functionally 'milder' mutations with a milder form of disease.

It is known that a number of tumor suppressor genes also demonstrate loss-of-function mutation in the sporadic counterparts of familial cancer. Somatic *MEN 1* mutations have also been identified in a number of sporadic tumors including parathyroid, gastrinoma, insulinoma and bronchial carcinoids.

Mitogenic factor and chromosomal instability in MEN 1

One unresolved issue in the study of MEN 1 is the reported mitogenic factor found in MEN 1 patients. Plasma of MEN 1 patients has mitogenic activity on cultured bovine parathyroid cells. This mitogenic factor, estimated to have a molecular weight of 50, 000 to 55, 000 kD, appears to be a fibroblast growth factor-like factor that might be secreted by the pituitary tumors. It is possible that this putative mitogenic factor interacts with the protein product of the MEN 1 gene, similar to mechanisms occurring in the von Hippel-Lindau disease (VHL). In the latter, the vascular endothelial growth factor (VEGF) is suppressed by the *VHL* gene.

The other problem is chromosome instability in MEN. Increased frequency of chromosome breakage, numerical and structural abnormalities and more recently premature centrosome division, have been reported in MEN 1 patients. Whether the MEN1 gene has a role in chromosome instability or DNA repair needs yet to be determined.

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Multiple Endocrine Neoplasia Type 2

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Synonyms

- multiple endocrine neoplasia type 2 (MEN 2)
- MEN II
- MEA II
- Sipple syndrome

Subtypes of MEN 2 are: MEN 2A, MEN 2B, FMTC (familial medullary thyroid carcinoma).

Definition

MEN 2 is a syndrome of inherited susceptibility to tumours of the thyroid 'C' cells, the adrenal medulla and the parathyroid glands; associated in some subtypes with developmental abnormality. It is caused by dominantly inherited mutations in the → ret receptor tyrosine kinase, which result in activation of the kinase in the absence of the specific ligand.

Characteristics

The principal tumour in MEN 2 is the medullary thyroid carcinoma (MTC), which arises from the 'C' cells of the thyroid (→ thyroid carcinogenesis). The normal function of 'C' cells is to produce the hormone calcitonin; but the manifestations of MTC are those of local and invasive cancer rather than of hormone overproduction. MTC may first be manifest in early childhood (in the MEN 2B subtype, as early as the first year of life), but the age of appearance of symptoms is variable and only 70% of individuals who inherit the susceptibility gene will have clinically significant disease by the age of 70.

Involvement of the adrenal medulla and of the parathyroids are less frequent, affecting roughly 50% and 10 – 30% of individuals. Their occurrence varies both within and between families. The adrenal tumours are usually (but not always) benign, and cause their effects through overproduction of adrenaline and related substances, leading to high blood pressure. The parathyroid glands may be affected either by a diffuse enlargement (hyperplasia) or by benign tumours (adenoma): these are commonly silent but may have clinical effects through overproduction of parathyroid hormone leading to elevated blood calcium levels. Some individuals with MEN 2 have associated developmental abnormalities that are characteristic of the clinical subtype (Table). The tissue most commonly involved is the → autonomic nervous system of the gastro-intestinal tract that can lead to altered gut motility and constipation or diarrhoea.

Cellular and molecular regulation

Most of the tissues involved in MEN 2 are derived in development from the neural ectoderm (the exception is the parathyroid tissue, which arises from the embryonic pharyngeal wall close to the point where migrating neural ectoderm cells pass through to enter the thyroid and form the thyroid C cells). The ret receptor tyrosine kinase gene is expressed in these neuroectodermal cells and in the pharyngeal wall during development. The normal function of ret is still not known in detail. In general, such receptors receive specific signals at the surface of cells by binding of a ligand, a specific signalling molecule. In the case of the Ret protein, two molecules are involved in binding to the receptor; a ligand and a co-receptor. Several ligands and co-receptors for Ret have been described, which probably act in a variety of different pairwise combinations in different circumstances. The binding activates the receptor, which signals to the interior of the cell and modifies gene expression and thus cellular behaviour. In this way, development of the RET-expressing cells is controlled. The effect of the MEN 2-associated mutations is to cause the receptor to be active and to signal even when the specific ligand is absent. This presumably perturbs normal development in a way that is not yet understood. This leads to abnormal growth and ultimately tumour formation. The disordered growth is manifest as multiple areas of

Multiple Endocrine Neoplasia Type 2. Table – Clinical subtypes of MEN 2 and tissues involved.

	MEN 2A	MEN 2B	FMTC
thyroid C cells	tumours	tumours	tumours
adrenal medulla	tumours	tumours	not involved
parathyroid	hyperplasia or tumour	not involved	not involved
gastrointestinal autonomic plexus	absence or incomplete development in a few cases	characteristic overgrowth with impaired function	not involved
other	–	skeletal abnormalities; overgrowth of nerve tissue in lips and conjunctiva leads to abnormal facial appearance	–
comments	most common form	less common; earlier onset; impaired reproductive success; high proportion of isolated cases which represent 'new' families.	less common; later onset; generally less aggressive

hyperplasia of the thyroid C cells and of the adrenal medulla in individuals with a MEN 2 mutation. Disturbances of signalling are presumed to cause the developmental abnormalities.

The MEN 2 associated mutations in *RET* result in substitution of one of a limited set of amino acids in the protein, thereby altering the properties of the protein and leading to its activation. The mutations fall into 3 main groups (Fig.). The different groups of mutants tend to be associated with different subtypes of MEN 2. The mechanism for this correlation between specific mutations and disease is not known, but may relate to:

- the strength of activation of the receptor by different mutations;
- the stimulation of signalling down different pathways from the receptor.

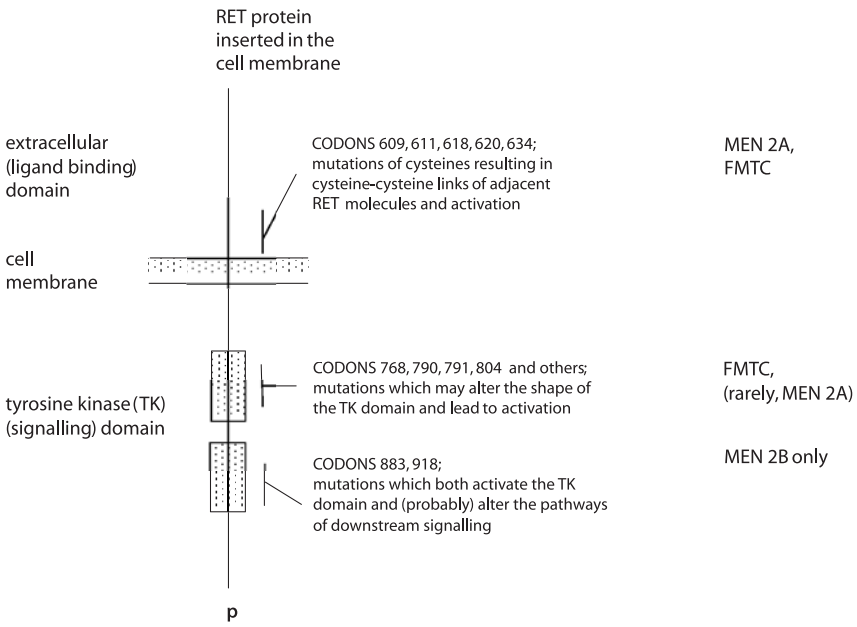
The failure of development of the gastrointestinal autonomic system in some individuals with MEN 2A is confined to those with mutations in codons 609, 618 and 620 and may reflect impaired signalling from these receptors that is insufficient to maintain the viability of the developing nerve cells in the embryonic gut.

Clinical aspects

MEN 2 is rare, with an estimated 20-25 new cases per year in the UK (population 55 million). However, it is important because early recognition and surgery is effective in preventing both deaths and unpleasant symptoms.

The production of calcitonin by thyroid C cells, and adrenaline and parathyroid hormones by adrenal and parathyroid, provide the basis of sensitive biochemical tests for the detection of tumours at an early stage. Detection of hyperplasia of the thyroid C cells before invasive tumour is possible with sensitive tests in which stimuli such as intravenous pentagastrin or calcium are used to provoke calcitonin release from the C cells, providing a measure of increased cell number.

As with other inherited cancers, the same types of tumour that occur in MEN 2 also occur, and more commonly, in a non-inherited form. The first step whenever a new patient presents with one of the component tumours is therefore to determine whether this is 'sporadic' - non-inherited - disease with no implications for further tumours in that individual or in family members or whether it is inherited, in which case both these possibilities must be in-



Multiple Endocrine Neoplasia Type 2. Fig. – MEN 2 associated mutations in the Ret protein.

vestigated further. The presence of a family history of disease is strong evidence for inheritance, with young age at diagnosis or pathology findings of multiple hyperplasias (see above) being suggestive. Before the MEN 2 gene was identified, biochemical testing was offered to families at risk. Now, testing a blood sample for the presence of an MEN 2-associated inherited mutation is definitive if a mutation is found and is the first investigation of choice. The presence of a mutation confirms heritable disease. Absence of a mutation is strong evidence against inherited disease, but not absolutely conclusive because not all the mutations are yet known. If a mutation is found, other family members can be offered testing. Those that test positive can be offered prophylactic surgery, with or without preliminary biochemical testing as clinical circumstances suggest. In general, thyroidectomy is a straightforward and acceptable operation that is recommended in childhood to those who have an MEN 2 mutation. Adrenalectomy carries higher morbidity and is only recommended when there is evidence of developing adrenal disease.

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Multiple Myeloma

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Definition

Multiple myeloma is a malignant plasma cell disorder characterized by accumulation of malignant plasma cells within the bone marrow and the secretion of a monoclonal protein, usually IgG or IgA.

Characteristics

Multiple myeloma is responsible of 1% of cancer-related deaths, is more frequent in males and in US black population. Its incidence grows with age, with a median age of 65 years. An unknown percentage (maybe as high as 50%) of cases develops in patients displaying a monoclonal gammopathy of undetermined significance (MGUS), a condition characterized by the presence in the serum of monoclonal IgG or IgA, without any clinical symptoms.

Malignant plasma cells accumulate in the bone marrow. The major clinical manifestation is bone destruction, with frequent bone fractures. Other frequent abnormalities at diagnosis are anemia, bacterial infections and renal insufficiency. Malignant plasma cells are characterized by a typical immunophenotype: CD38+, CD138+, CD56+, CD19-. Even though a high serum monoclonal Ig concentration is found in most patients, and in contrast to the normal counterpart, these cells have lost most of their secretion capabilities. This tumor is also characterized by high levels of interleukin-6, which promotes both survival and growth of plasma cells. At least 90% of patients display chromosomal abnormalities, the most frequent being chromosome 14q32 rearrangements.

Treatment

Treatment is based on chemotherapeutic agents, especially melphalan and steroids. The classical MP (melphalan-prednisone) regimen is still the treatment of choice in older patients (after 65 years). Recently, intensive therapy regimens followed by autologous bone marrow transplantation has been demonstrated to produce superior results (essentially longer survival) in younger patients (before 60 years). Biphosphonates have shown efficacy in preventing bone complications. More recently, thalidomide has been demonstrated to be highly effective, even in relapsing patients.The main poor prognostic factors are high serum β2-microglobulin and C-reactive protein levels. More recently, monosomy 13 has been identified as a strong independent poor prognostic factor. Despite recent improvements in therapy, multiple myeloma remains a fatal disease, except in the rare patients treated by allogeneic transplant. The median survival is 3 to 4 years, but may vary from 1 year in patients with high β2-microglobulin level and monosomy 13, to more than 10 years in patients with low β2-microglobulin level and normal chromosomes 13.

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Multistep Development

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Definition

Multistep development is the stepwise development of malignant tumors by successive changes in multiple characteristics.

Characteristics

Tumor development can be regarded as a Darwinian process, based on variation and selection. In the neoplastic microevolution, 'survival of the fittest' means the successive emergence of increasingly emancipated subclones that are less and less restrained by the multifactorial growth controls of the organism.

History

Epidemiological studies have shown that the incidence of cancer increases exponentially with age. The analysis of age incidence curves indicated that between 3 to 7 mutations are required for full development of cancer. This is consistent with the modern molecular analysis. Carcinomas and sarcomas appear to require a larger number of mutations than leukemias.

Participating genes

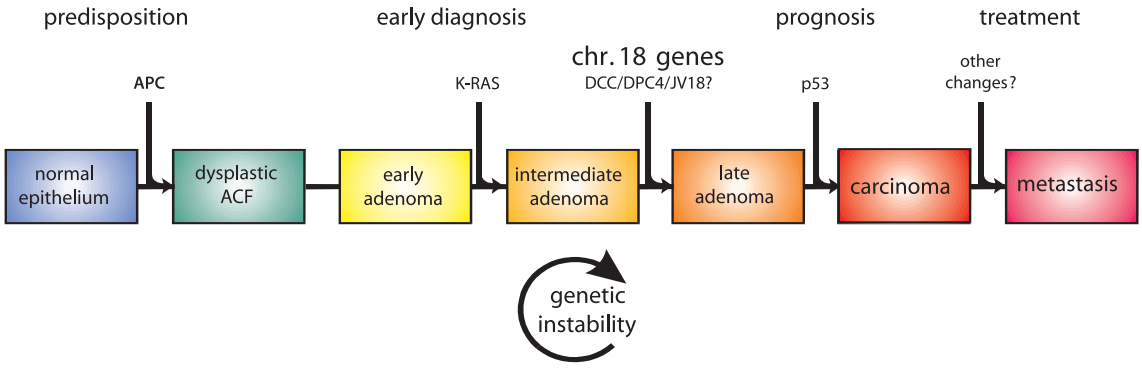
The four major known categories are:

- oncogenes that drive the cell towards an S-phase when activated;
- suppressor genes that can arrest the growth of illegitimately driven cells and contribute to tumor development when inactivated or deleted;
- genes that raise the apoptotic threshold when activated or genes required for the apoptotic process that contribute by their loss;
- DNA repair genes whose dysfunction may create genetic instability.

Less well known genes may contribute to → immortalization, → angiogenesis, → metastasis, hormone insensitivity and escape from immune rejection.

Minimum requirements for tumor development

Genetic analysis of tumor development in many different tissues has created a scenario where transformation of a normal cell into a



Multistep Development. Fig. – Multistep pathway from normal cell to metastatic colon carcinoma. The rate-limiting step is postulated to be the loss of the APC gene on chromosome 5. Individuals with familial adenomatous polyposis (FAP) inherit the first mutation (APC gene) and only need to acquire a second mutation. Other individuals need to acquire both mutations in the same cell lineage. Additional genetic damage is accumulated, including loss of genes on 17p and 18q as well as other changes. These can occur in any order and result in progressively abnormal cells, eventually capable of metastasis (figure provided by Bert Vogelstein).

cancer cell requires the activation of at least one oncogene (e.g. *myc*, *ras*), the loss of one or several suppressor genes (e.g. → *RB*, → *p53*, → *p16*, → *APC*), activation of a gene that raises the apoptotic threshold (→ *Bcl-2*) or, alternatively, the loss of genes required for apoptosis (e.g → *ARF* or *p53*, → *Bax*), immortalization (e.g. → telomerase activation) and angiogenic activity. Highly immunogenic, DNA virus-associated tumors may escape rejection in immunodefective hosts. Cellular escapes may be due to the down regulation of rejection inducing tumor antigens or of presenting MHC class I proteins.

Successive steps in tumor development have now been defined as specific changes in DNA sequences or, less frequently, functional changes imposed by methylation. Distinct molecular events have been linked to progression related phenotypic changes in carcinomas of the colon and rectum, prostate, lung, breast, skin, in gliomas and tumors of the hemopoetic system. The loss of tumor antagonizing (growth arresting and/or apoptosis promoting) genes appears to be more frequent than the activation of proliferation-driving genes but this may be due, at least in part, to their easier detectability, e.g. by loss of heterozygosis (LOH). The recently developed microarray techniques that can measure the expression of hundreds of genes in the same sample will give a more complete picture.

Colorectal tumors [→ colon cancer] progress through a series of well defined phenotypic changes, from hyperplasia, through different stages of adenomas, to malignant cancers. Two familial forms, hereditary non polyposis colon cancer (→ HNPCC) and familial adenomatous polyposis (FAP [→ APC gene in Familial Adenomatous Polyposis]) are initiated by germline mutations in mismatch repair genes in the former and the APC gene in the latter condition. The multifunctional APC protein is involved in cellular adhesion, signal transduction and apoptosis. The development of the hereditary forms of colorectal cancer is associated with the inactivation of the second allele and a series of sequential changes during somatic development. The latter are also found in the sporadic form. They include activation of *Ki-ras*, or *N-ras* [more: → RAS], inactivation of both copies of p53 and loss of a gene on chromosome 18 that is identical with or closely linked to → *DCC*. Later stages of progression are associated with extensive aneuploidy, particularly in the FAP, but less in the HNPCC based tumors. The changes do not occur in the same order in different tumors, confirming the notion of alternative pathways.

About 15 – 20% of → breast cancers have a family history. About 5% can be attributed to dominant susceptibility genes. Two of them → *BRCA1* and → *BRCA2* are responsible for 40 – 50% and 30 – 40% of inherited breast cancers. The subsequent development and the biology of sporadic breast cancers is very diverse, indicating the existence of different progressional pathways. A gamut of growth factors and receptors (e.g. EGFR, HER-2/neu), signalling molecules (Ras, Src) and other intracellular growth regulators (e.g. Myc, Cyclin D) can be activated. Tumor suppressor genes (e.g. *p53*, *RB*) and adhesion molecules (e.g. cadherin) may be set out of function. Many as yet unidentified genes may be involved in addition, as indicated by the numerous LOHs. Amplification of → *HER-2/neu* is a bad prognostic sign. However, the protein product of the amplified gene can be used as therapeutic target.

→ Prostatic carcinoma provides a similarly diverse panorama. In addition to mutations of *Ras*, *p53*, *RB1*, *CDKN2* and other known oncogenes and suppressor genes, cadherin down-regulation and LOHs affecting chromosomes 5, 6, 8, 10 and 16 have been detected. No single gene or chromosome region is affected in all prostatic carcinomas. Progression from hormone dependence to independence is often associated with the amplification of the androgen receptor gene on the X chromosome.

Lung cancer in heavy smokers (→ tobacco carcinogenesis) is often a multifocal disease. Deletions effecting the short arm of chromosome 3 are found already at the hyperplastic or dysplastic precursor stage. Subsequent progression may feature 5q deletions, *K-ras* mutations, *p16*, → *FHIT*, *p53* or *RB1* mutations. The deletions and rearrangements on chromosome 3 may become more extensive, probably affecting several genes within a cluster. During late stages

of small cell lung cancer (SCLC) progression, → amplification of the *myc*-family genes (*c*-, *N*-, or *L-myc*) is a bad prognostic sign.

→ Astrocytomas show an increasing number of changes with ascending grade. The least agressive forms show loss of wild type *p53* and a number of LOHs. → Glioblastoma, the most malignant form, has a large number of genetic abnormalities.They include losses from chromosomes 6, 10, 13 and Y, gains on chromosomes 7 and 19, and structural abnormalities of chromosomes 1 and 9. Frequent double minutes indicate gene amplification. Homozygous deletions on 9p, affecting the *CDKN2A*, *CDKN2B* (*p15/16*) and *p14 ARF* loci, are found in 40 – 50%. The inactivation of *p15/16* deranges *RB* dependent cell-cycle control, whereas the deletion of the alternatively spliced *p14* product of the same gene cripples the *p53* dependent apoptotic pathway. Understandably, homozygous deletions of the *p15-16-14* cluster is found in tumors that show no abnormalities of *CDK4*, *RB1*, *MDM2* or *p53* and vice versa. This illustrates the existence both of alternative pathways of progression, or more specifically alternative modes of inactivating the cell-cycle regulating and apoptosis controlling genetic systems. This can be further exemplified by the fact that 90% of the glioblastomas have either no wild type *p16/p15* or wild type *pRB1* or overexpress *CDK4*. In 75% of the glioblastomas, the apoptotic pathway is either inactivated by the lack of wild type *p53*, the inactivation of *p14 ARF* or over-expression of *MDM2*. Tumors with amplified *MDM2* have two wild type *p53* alleles. These examples illustrate how genetic aberrations may target different members in the same cellular control pathway and create similar phenotypes. Additional changes in glioblastomas include *EGFR* amplification and over-expression (35%) and allelic loss from chromosome 10 (90%), probably acting through the inactivation of *PTEN/MMAC1* (>40%), a putative tumor suppressor that encodes a dual specificity phosphatase.

The hereditary form of → renal carcinoma is often initiated by a mutation in the → *VHL* gene at 3p26. The *VHL* gene is also frequently mutated in sporadic clear cell RCC. A cluster of as yet unidentified genes at 3p21.3 are frequently deleted during tumor progression, both in the hereditary and the sporadic form. When an intact human chromosome 3 is introduced into mouse tumor cells and the monochromosomal hybrids are grown in immunodefective mice, a corresponding region, designated as CER1 (commonly eliminated region 1) is regularly eliminated, indicating the presence of one or several tumor antagonizing genes. In contrast, a gene cluster at the telomeric end of the long arm (3q) is frequently retained. Hereditary papillary renal cell carcinoma (HPRC) shows no association with chromosome 3 losses or *VHL* mutations.

In skin cancer [→ basal cell carcinoma], allelic losses in basal cell carcinomas are almost entirely confined to the *Gorlin* locus at 9q [→ Gorlin syndrome]. Squamous cell carcinomas show allelic losses in 25 – 30%, affecting chromosomes 3, 9, 13 and 17.

→ Pancreas carcinoma shows a distinct mutation profile. *K-ras* is activated in 90% of the tumors by point mutations in codon 12. Among the suppressor genes, *p16*, *p53* and *DPC4* are frequently inactivated, *RB1* less frequently. *DPC4* is biallelically inactivated in almost 50% of all pancreatic cancers, but only rarely affected in other tumors.

In endometrial cancer, microsatellite instability, *K-ras*, *PTEN* and *p53* mutations are most common. *p53* overexpression is correlated with poor prognosis.

In carcinoma of the uterine cervix (→ cervical cancer), the HPV [→ human papillomaviruses] encoded transforming proteins E6 and E7 play an important early role (early HPV proteins). This is at least partly due to their inactivating effect on p53 and RB1, respectively. This promotes genetic instability, counteracts apoptosis and abolishes part of the cell-cycle regulatory controls. In addition, amplification of *myc* and *HER-2/neu*, as well as loss of genes from chromosomes 1, 3, 5 and 11 may play important roles. 3p losses are particularly interesting, since the same regions are deleted in renal, lung and nasopharyngeal carcinomas.

In → bladder cancer, chromosome losses are frequent. Monosomy of chromosome 9 is the most common abnormality. In addition, deletions are common on 9p, 17p and 13q. *p53*

and *RB* mutations are correlated with poor prognosis. Multiple primary tumors may be generated by the spread and further progression of a single progenitor clone.

Hepatocellular carcinoma [→ liver cancer, molecular biology] is initiated by the DNA virus hepatitis B virus (HBV). Sequential changes include *p53* losses. The virally encoded transactivator HBV-X is believed to induce the overexpression of several oncogenes.

Hemopoetic tumors [→ hematological malignancies] are often initiated by chromosomal translocations, leading to gene fusions (in CML and a wide variety of childhood leukemias), juxtaposition of an oncogene to an Ig locus (B-cell lymphomas) or to a TCR locus (T-ALL). Sequential events affect mainly the apoptosis regulating systems, with up-regulation of *bcl-2* or inactivation of *p53*, *p19 ARF* or *BAX*. In T-ALL, *p16* may remain unaffected, whereas *ARF* is disrupted or deleted. In the absence of *ARF*, *p53* usually remains in wild type configuration. A similar alternative mutational relationship has been found between *p16* and *RB*, as in other tumors.

Clinical relevance

For diagnosis, oncogene activation by chromosomal translocation, with or without gene fusion, is diagnostic for certain leukemias, lymphomas and sarcomas. For prognosis, the identity of the activated oncogene may be decisive for the biological behavior of the tumor. Oncogene amplification, a well defined step in the progression of carcinomas and gliomas, may be a bad prognostic sign; it is being used clinically to determine prognosis in → neuroblastoma and is a parameter for therapy design.

→ Micrometastasis detection by antibodies and/or by molecular techniques, and the identification of residual, translocation carrying leukemia or sarcoma cells by polymerase chain reaction (PCR) are useful for the diagnosis of residual disease and for predicting the probability of recurrence. These parameters may also provide surrogate endpoints for the evaluation of new therapies.

Therapeutic choices are decisively influenced by the molecular findings. This can be exemplified by the identification of the genes involved in the translocations found in ALL and APML. Current therapeutic experimentation aims at the inhibition of pathologically activated signal transduction chains with small peptides, antibodies or DNA constructs. Faulty protein-protein interactions provide other important targets. It is not necessary to correct all genetic aberrations in tumors that have evolved by multiple steps. Inhibition of a dominating driving mechanism or, alternatively, induction of apoptosis may be sufficient.

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Multi-substrate Docking Proteins

Definition

Multi-substrate docking proteins are a family of proteins homologous to the *Drosophila* daughter of sevenless protein (DOS). They contain multiple domains that mediate interactions with activated growth factor receptors and a variety of signaling intermediary molecules. This group of proteins includes DOS, → Gab1, Gab2, IRS-1 (insulin-responsive substrate-1) and IRS-2. Typically, these proteins contain; a → pleckstrin homology domain, which interacts with lipids involved in signal transduction (phosphoinositides); multiple tyrosines, which recognize src-homology-2 domains of signaling proteins; domains which directly or indirectly associate with tyrosine kinase receptors; and a phosphatidylinositol-3′-kinase binding domain.

MUNC 13/1

Definition

Munc 13/1 is the mammalian homologue of unc-13 from *C. elegans*. Unc-13 encodes a protein kinase that exhibits sequence homology to C1 and C2 domains of protein kinase C (PKC).

Mutagenic

Definition

Mutagenic means causing a heritable change in DNA, the genetic material.

Mutation

Definition

A mutation is a permanent, heritable change in the genetic information of a cell or organism that can be acquired or hereditary [→ acquired or → hereditary mutation]. It may involve an altered base sequence in DNA or a deletion or rearrangement of chromosomes. Mutations in genes are often associated with a different phenotype.

Mutation Rate

Definition

The mutation rate is the rate at which a particular mutation occurs, usually given as the number of events per gene per generation.

Mutator Phenotype

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Definition

Mutator phenotype refers to the increase in mutation rate of cancer cells. The Mutator Phenotype Hypothesis was formulated to account for the disparity between the infrequency of spontaneous mutations in normal cells and the large numbers of mutations observed in human tumors. The hypothesis states that an increase in mutation rate is an early event in tumorigenesis. Some of the random mutations produced throughout the genome are located in genes that normally function to guarantee the accurate transfer of genetic information with each cell division. The expression of this 'mutator phenotype' leads to a cascade of mutations throughout the genome including mutations in other genes required for the mainte-

nance of genetic stability. Among the many mutations produced are ones that promote growth, invasion and metastasis, the hallmarks of cancer.

Characteristics

Mutations and cancer

The ability of cancer cells to continually mutate may be central to how tumors evolve. In the case of solid tumors, there is approximately a twenty-year interval from the exposure of an individual to a carcinogen until the clinical detection of a tumor. During this interval, cancer cells acquire properties that allow them to flourish in a changing environment. By the time a tumor is detectable, the cancer cells are able to divide where normal cells do not, to invade adjacent cellular architectures, to metastasize and eventually to kill the host. In addition, tumors have the capacity to rapidly develop resistance to a variety of chemotherapeutic agents. Each of these phenotypes can be mimicked by mutations in normal cells.

Chromosomal alterations in human cancers

Mutations can be defined as a change in the nucleotide sequence in DNA. Mutations in cancer cells cover a wide spectrum from chromosomal alterations that encompasses millions of nucleotides to point mutations that involve only a few nucleotide substitutions in single genes. Multiple somatic chromosomal alterations are diagnostically associated with cancer cells and involve translocations, deletions, amplifications and aneuploidy (a change in the number of chromosomes in individual cells). Unique chromosomal changes occur at high frequencies in certain tumors and are of diagnostic significance. However, there is a striking heterogeneity of chromosomal alterations in cancer cells within individual tumors. In general, there is a positive correlation between the number of chromosomal changes within a tumor and the malignant potential of that tumor. As molecular techniques are becoming more sensitive, more and more chromosomal abnormalities are being reported in different tumors. In some tumors there is evidence for a sequential order in chromosomal mutations during tumor progressions. Measurements of the number of copies of segments of the genome in tumor cells (DNA copy number) and the loss of pieces of DNA (loss of heterozygosity) have established that many tumors harbor as many as 40 chromosomal alterations. It should be emphasized that these methodologies only score a very small fraction of the genome and as a result may greatly underestimate the number of small chromosomal rearrangements within the genome.

Point mutations in human cancer

Only recently has evidence accumulated indicating that cancer cells not only contain multiple chromosomal alterations, but also contain thousands of smaller changes in nucleotide sequence. These studies have provided strong support of the mutator phenotype hypothesis involving a reduction in the fidelity of DNA replication and/or a decrease in the efficiency of DNA repair. An early clue to the large numbers of mutations in cancer cells was the observations that the resistance of cancer cell lines to divergent chemotherapies was mediated by gene amplification. The first direct evidence in support of a mutator phenotype in cancer was provided by the demonstration that cells from patients with HNPCC (hereditary nonpolyposis colon cancer) exhibit microsatellite instability in association with mutations in DNA repair genes. Microsatellites are short repetitive sequences of nucleotides in DNA that are subjected to slippage during copying by DNA polymerases. In normal individuals, mutations in microsatellites are corrected by the DNA mismatch repair system. In HNPCC tumors, the deficits in mismatch repair result in expansions and contractions in the length of repetitive nucleotide sequences. Based on the enormous number of microsatellites in the human genome, it has been calculated that each tumor could harbor more than 100,000 mutations in these sequences alone. Repetitive sequences located within genes are also mutated at high frequencies in these tumors. Changes in the lengths of repetitive sequences

have also been reported in a variety of tumors that are not known to be associated with mutations or deficiencies in mismatch repair, but may be associated with mutations in other genetic stability genes. Thus, repetitive sequences in DNA may be a 'hot-spot' for mutagenesis and serve as a sentinel for the detection of a mutator phenotype in cancer.

Rarity of spontaneous mutations in normal cells

In normal cells, DNA replication and chromosomal segregation are accurate processes. Measurements of mutagenesis of cells grown in culture yield values of approximately 2.0×10^{-7} mutations/haploid gene/cell division. Taking into account this very low frequency of mutations, it seems improbable that the spontaneous mutation rate is sufficient to generate the large numbers of genetic alterations that are observed in cancer cells. If one assumes that stem cells which give rise to a cancer have a similarly low rate of mutagenesis, then it can be calculated that the average stem cell would accumulate only one or two mutations during tumorigenesis. A few stem cells could accumulate as many as 12 mutations and thus account for the inactivation of tumor suppressor genes in retinoblastomas and other tumors. However, the normal spontaneous mutation rate is inadequate to account for thousands of mutations observed in most tumors.

Historical perspective

The hypothesis that cancer cells exhibit a mutator phenotype was proposed more than 25 years ago and was set forth on the postulate that there is an decrease in the accuracy of DNA synthesis during tumorigenesis. These random mutations could arise by mutations in DNA polymerases that render them error-prone during DNA synthesis or by mutations in DNA repair enzymes making them less efficient in correcting DNA damage or mistakes in incorporation by DNA polymerases. The production of more errors and/or their lack of repair could result in an increase in point mutations and, indirectly, in chromosomal abberations in cancer cells. Further mutations in these genes would result in cascading numbers of mutations as tumor cells multiplied. Independently, Nowell postulated that cancer cells accumulate multiple mutations by successive rounds of clonal selection. Analysis was based on chromosomal changes in the evolution of human tumors. Leukemias with minimal chromosome changes were considered to be early in clonal evolution, while highly aneuploid solid tumors were considered to have undergone multiple rounds of clonal selection. Increases in both mutation rate and clonal selection could contribute to an increase in number of mutations in tumors. Recent studies in bacteria suggest that these mechanisms may reinforce one another. Sequential rounds of selection for different mutants yielded populations of bacteria that invariably contained mutations in genes that normally function to maintain genetic stability. The conclusion is that selection for mutant clones simultaneously enriches for mutations in genes that can produce the mutant clones. With successive rounds of selection there is increasing enrichment for cells that express a mutator phenotype.

Tumor evolution

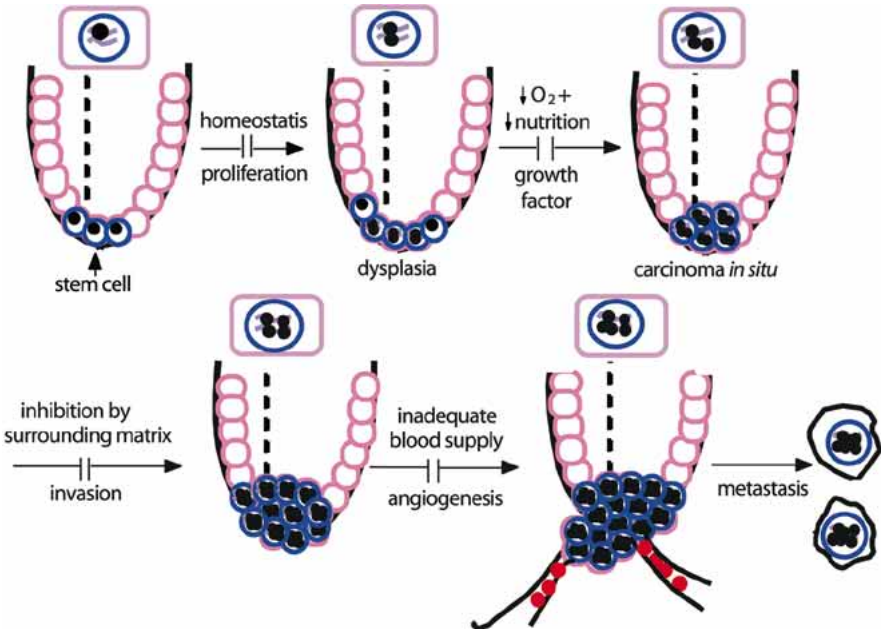
The ability of cancer cells to express a mutator phenotype provides a mechanism for tumor cells to circumvent the host's mechanisms that determine when cells divide and their position within tissues. As tumors expand, they encounter a sequence of restrictive blockages that curtail further expansion. As indicated in Fig., impediments to expansion include the architecture of surrounding tissues, reduced nutrition, decreased oxygen, need for growth factors and inadequate blood supply. Each of these impediments can be overcome by the selective clonal expansion of cancer cells with mutations in genes that impart the required phenotypes. Some of these mutations could be selected from a population of cells harboring multiple mutations. Others could result from new mutations induced by additional mutations that render genetic stability genes error-prone. Thus, with each round of selection there would be a 'piggy-backing' of mutations in genes that in-

crease the overall mutation frequency in cancer cells. In order for a mutator phenotype to account for the many mutations in a cancer cells, it would have to be present early during the course of tumor progression. The continued expression of a mutator phenotype might not be compatible with the enhanced growth of cancer and thus may not be exhibited by cancer cells by the time a tumor is clinically apparent. Nevertheless, the footprints of its presence, multiple mutations, would persist in all cell descendents throughout the course of tumorigenesis (→ multistep development).

Implications of a mutator phenotype

The multiplicity of mutations in cancer cells resulting from a mutator phenotype has implications with respect to pathogenesis, diagnosis, treatment and prevention of human cancer. The presence of large number of mutations in cancer cells implies that the malignant process is irreversible. Once a mutation occurs in a gene it is highly unlikely that a second mutation would occur at the same position to change the mutation back to the wildtype sequence. Also unlikely is the possibility that a second mutation would occur at a different loci and suppress the effects of the first mutation, particularly if the first mutation occurs at a tumor suppressor gene and inactivates that gene. Thus the concept of a mutator phenotype is not compatible with the hypotheses that cancer cells have the potential to differentiate into normal cells. The reports that certain tumors spontaneously regress does not necessarily indicate that these tumor cells have been transformed into normal cells. It is more likely to reflect the selective killing of tumor cells by normal host mechanisms that reject foreign cells.

The heterogeneity of mutations within a tumor provides a population of cancer cells harboring mutants that are resistant to a many chemotherapeutic agents. In addition, the mutator phenotype exhibited by many cancer cells allows them to continuously generate new mutant clones that are resistant to and proliferate in the presence of chemotherapy, immunother-



Mutator Phenotype. Fig. – Accumulation of mutations during tumor proliferation. As each cancer enlarges it encounters barriers to further proliferation. Rare mutants within the tumor have the properties to escape these barriers and to permit clonal expansion. These mutants also contain mutations in DNA stability genes. Thus with repetitive selection for mutant cancer cells exhibiting specific properties there is simultaneous selection for mutators. This repetitive selection could be an important driving force in the establishment of a mutator phenotype in cancer.

apy and even radiation therapy. While such a scenario allows cancer cells to thwart many therapeutic protocols, it might offer new approaches to cancer therapy. It might be feasible to develop drugs that target the genetic instability of tumor cells. For example, nucleotide analogs can be designed that are preferentially incorporated into DNA by mutant error-prone DNA polymerases.

The large number of mutations in → microsatellite sequences in cancer cells may facilitate the early detection of certain human malignancies. Rare tumor cells circulate, can be detected in the blood and could account for the hematological spread of tumor cells to distant sites. In addition, cancer cells in tumors frequently undergo cell death (apoptosis) and shed their DNA into the blood. Thus, methods can be developed for the detection of altered microsatellite sequences in DNA and cells in blood for the early detection of human malignancy and for calibrating the potential of tumors for metastatic dissemination.

If a mutator phenotype is rate-limiting for → tumor progression, it is important to identify the agents and genes involved in its expression. Sources for a mutator phenotype such as mutant DNA polymerases, which are error-prone, or mutations in DNA repair enzymes are not easily corrected. Other sources might be more easily attenuated. Recent evidence indicates that cells contain a number of error-prone DNA polymerases that are induced by DNA damage, and it might be feasible to develop drugs to selectively inhibit these enzymes. More immediately open to experimental analysis is increased DNA damage by normal cellular metabolites, such as oxygen free radicals. These radicals can be scavenged by the administration of specific drugs and vitamins.

If a mutator phenotype constitutes a rate-limiting step for the development of a cancer it might be possible to prevent cancers by delay. Even a two-fold reduction in the rate of accumulation of mutations in cancer cells might have profound effects on the age at which patients succumb to certain adult cancers. Consider primary hepatoma resulting from hepatitis virus B infection that occurs in early infancy and persists chronically. The persistence of chronic hepatitis in individuals increases the risk of subsequent hepatoma by more than 200-fold. It usually takes 40 additional years for the tumor to be clinically manifested. According to the concepts presented in this article, a reduction in the rate of mutation accumulation by only 2-fold could delay the clinical appearance of the tumor from age 50 to age 90. It has been postulated that the persistence of hepatitis results in an inflammatory reaction with generation of oxygen free radicals. Thus, drugs that scavenge oxygen free radicals might have a role in the prevention of primary hepatoma. It should be emphasized that prevention by delay does not affect the rate of initiation of the malignant process, but rather is directed at slowing down the rate of its progression. This approach may offer new direction to reducing the number of cancer-associated deaths.

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mutHLS

Definition

The mismatch repair system in the bacterium *Escherichia coli* is called mutHLS. Five human proteins with homology (hMSH2-6) to the mut S component and four homologues (hMLH1, hMLH3, hPMS1, hPMS2) to mut L have been identified. Germline mutations in some of these genes predispose to hereditary

non-polyposis colorectal cancer (→ HNPCC) syndrome.

MXI1

Definition

MAX-interacting protein 1 (MXI1) is a nuclear protein that associates with MAX. It is a ubiquitiously expressed protein (228 aa and 26kD) member of the basic helix-loop-helix family of transcription factors. MXI1 dimers bind to CAC GTG. MXI1 antagonizes → MYC transcriptional activity by competing for MAX. The gene maps to 10q25, and deletions and mutations are common in prostate cancer.

MYB

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Definition

MYB is a family of genes related to the *C-MYB* proto-oncogene. The name of this gene comes from myeloblastosis, the type of leukemia caused by the *V-MYB* viral oncogene in chickens. All vertebrates contain three related *MYB* genes, *A-MYB*, *B-MYB* and *C-MYB*. Invertebrate animals, including the fruit fly and sea urchin, have only one closely related *MYB* gene. More distantly related genes are present in plants and fungi.

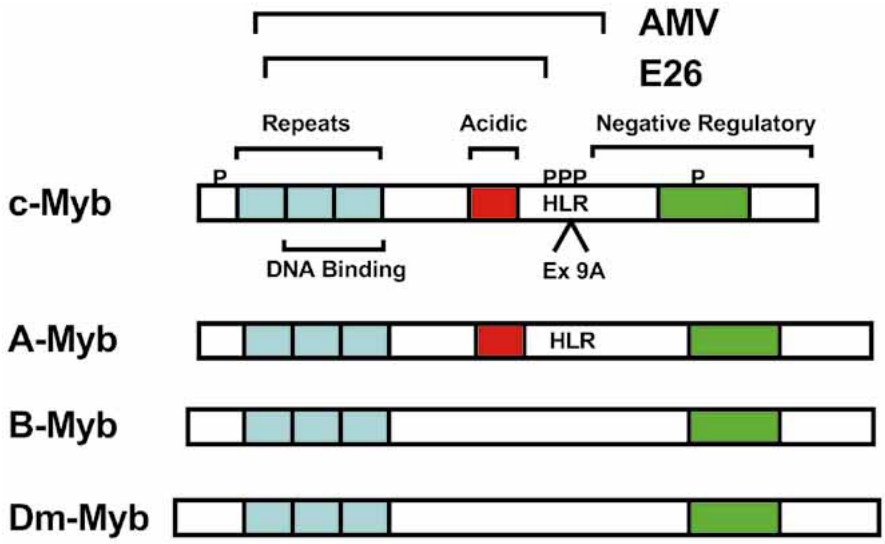
Characteristics

The *MYB* gene family was first discovered because a → retrovirus that causes acute myelogenous leukemia in chickens contains *V-MYB*. Like most other retroviral → oncogenes, *V-MYB* represents an altered form of a normal cellular → proto-oncogene, namely *C-MYB*. A second leukemia virus in chickens contains both *V-MYB* and another oncogene of cellular origin, *V-ETS* [→ ETS transcription factors]. Furthermore, retroviruses without oncogenes can cause leukemias and lymphomas in mice and in chickens by insertion of the virus into the *C-MYB* proto-oncogene. Consistent with the role of *V-MYB* and altered forms of *C-MYB* in causing cancers of blood cells (leukemia and lymphoma), mice that lack a functional *C-MYB* gene die as embryos because they fail to produce blood cells. The normal *C-MYB* gene is turned on (expressed) in immature blood cells and then turns off as these blood cells mature (differentiate). *C-MYB* is also expressed in immature cells in other tissues including skin, gut and lung, but these tissues appear to develop normally in mouse embryos that lack a functional *C-MYB* gene.

By using the *C-MYB* gene as a probe, two additional *MYB*-related genes were discovered in vertebrates. Like *C-MYB*, the expression of *A-MYB* is limited to particular tissues and cell types including testes, brain and germinal center B lymphocytes. Mice that lack a functional *A-MYB* gene survive to adulthood. However, the males do not produce functional sperm. The females are fertile but their mammary glands do not proliferate in response to pregnancy. Mice that express too much *A-MYB* develop an abnormal proliferation of germinal center B lymphocytes.

The *B-MYB* gene appears to be expressed in all dividing cells in vertebrates. Mice that lack a functional *B-MYB* gene die very early in embryogenesis, with a severe defect in cell proliferation. The single *MYB* gene of sea urchins and fruit flies most closely resembles the *B-MYB* gene of vertebrates. This observation suggests that the more specialized *A-MYB* and *C-MYB* genes arose more recently by gene duplication and divergence during the evolution of vertebrates.

All the proteins encoded by these *MYB* genes contain a very similar domain near the amino terminus that binds directly to a specific DNA sequence (AACNG). This DNA-binding domain itself is composed of three tandem repeats of a ~50 amino acid protein sequence. Sequences similar to these Myb protein repeats are also present in a number of proteins that regulate the structure of chromatin and chro-



MYB. Fig. – Animal Myb Proteins. The brackets next to AMV and E26 indicate the portion of C-MYB encoded by the V-MYB oncogenes of these retroviruses. The shaded boxes indicate the most highly conserved domains. HLR indicates a heptad leucine repeat. Ex 9A indicates the position of an alternatively spliced exon in C-MYB. P indicates a known phosphorylation site. Dm-Myb indicates Drosophila MYB.

mosomes including SWI3, ADA2 and the major telomere [→ telomerase] binding proteins of animals, plants and fungi.

All the Myb proteins of animals also contain a conserved domain near the carboxyl terminus that appears to act as a regulatory switch to control protein function. The A-Myb and C-Myb proteins contain an additional central domain that includes an acidic region and a heptad leucine repeat. This central domain causes other genes to be activated when these Myb proteins bind to them. A similar central domain is not present in B-Myb or the Myb proteins of invertebrates, although these proteins have been reported to activate other genes under special circumstances.

Cellular and molecular regulation

The regulation of *B-MYB* gene expression is fairly well understood. *B-MYB* is off when cells are not engaged in the division cycle and is turned on just before actively dividing cells begin to synthesize new DNA (S phase). The expression of the *B-MYB* gene is directly regulated by a critical cellular pathway that includes the p16 tumor suppressor gene product, the → cyclin D oncogene product, the p107/p130 relatives of the → RB tumor suppressor gene and the → E2F transcription factor. This pathway keeps the *B-MYB* gene off until late in the G1 phase of the cell cycle. *B-MYB* is then turned on at the same time as many genes that encode proteins essential for DNA replication. The regulation of the *C-MYB* gene appears to be similar to that of *B-MYB*, but its expression is limited to particular cell types. In contrast, the expression of *A-MYB* does not appear to be similarly regulated during the cell cycle.

The amino and carboxyl termini of C-Myb protein are critical for its normal regulation. Both the V-Myb protein of the avian myeloblastosis virus (AMV) and the V-Myb-Ets fusion protein of the avian E26 leukemia virus (E26) have truncations of both termini of C-Myb. Experiments with recombinant retroviruses have shown that these truncations are important for activating the oncogenic potential of the C-Myb protein. In addition, retroviral insertions into *C-MYB* that cause leukemias and lymphomas in mice and birds also result in the production of truncated versions of the C-Myb protein. Although both C-Myb and V-Myb can regulate the expression of a number of other genes, in

most cases the role of these 'target' genes in oncogenesis remains unclear. One exception is the abnormal indirect activation of a growth factor gene by V-Myb that clearly promotes the growth of leukemic cells.

The C-Myb protein can be phosphorylated by a number of protein kinases including casein kinase II, glycogen synthase kinase III and MAP kinase. An indirect regulation of the C-Myb protein via the pim-1 protein kinase has also been proposed, as has a direct regulation of the Myb DNA-binding domain by prolyl isomerases. The B-Myb protein can be phosphorylated by the cyclin A-CDK2 protein kinase during S phase. However, the physiologic relevance of these suggested modes of regulation remain unclear, pending appropriate genetic analyses.

Clinical aspects

In general, the *MYB* genes have not been identified as targets of frequent and consistent chromosomal translocations, gene amplifications or point mutations in human cancers. However, one recent report suggests that *C-MYB* may be amplified in as many as 10% of all pancreatic carcinomas. Even in the absence of obvious genetic aberrations, the levels of expression of the *MYB* genes have been reported to correlate with clinical prognosis for a number of different cancer types, including breast and colon cancer. Most recently, the use of microarrays to simultaneously measure the expression of many different genes in human cancers has shown that high levels of *C-MYB* expression are very useful for distinguishing acute lymphoid leukemias from acute myeloid leukemias. High levels of *A-MYB* expression are useful for distinguishing a previously unrecognized subclass of diffuse large B-cell lymphoma that resembles normal germinal center B lymphocytes. In addition, increased expression of *B-MYB* correlates with a cluster of proliferation genes that is a predictor of a more aggressive type of breast cancer. As the tools for the analysis of altered gene expression and copy number in human cancers become more refined and widely available, our knowledge of the involvement of the *MYB* genes in a variety of human cancers is certain to become clearer.

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MYC

Synonyms

- *c-myc*
- *C-MYC*
- *MYCC*

Definition

MYC is a the → *v-myc* avian myelocytomatosis viral oncogene homolog. The MYC protein is a nuclear phosphoprotein of 439 amino acids and 48 kD, its gene maps to 8q24.12-13. It participates in the regulation of gene transcription by dimerizing with → MAX; additional members of the MYC nucleoprotein complex include → MAD, → MAZ, → MXI1, → NMI, → MNT. MYC binds to DNA via the sequence 'CAC GTG', the → E-box sequence. The protein belongs to the basic helix-loop-helix (→ BHLH) family of transcription factors. Elevated expression in B-cell malignancies [→ B-cell tumours] results from translocation that juxtapose *MYC* with immunoglobulin genes (mainly the heavy chain locus). Prototypic is the translocation t(8;14)(q24;q32), which interrupts the IgH locus and translocates *MYC* to 14 q^+ chromosome (75% of cases). This → translocation is also observed in B-cell → acute lymphoblastic leukemia. Variant t(8;22)(q24;q11) juxtaposes *MYC* with the gamma locus on $8q^+$, t(2;8)(p13; q24) with the kappa locus on $8q^+$ (together in 5%-10% of cases). Elevated expression results from gene → amplification in a variety of cancer

types, particularly breast cancer. Activation of expression in colon cancers results from → Wnt signalling or from → APC gene mutations. Inappropriate *MYC* expression can trigger cellular → apoptosis.

MYC Family

Definition

The MYC family is a group of → bHLH proteins comprising members MYC, MYCN, MYCL and MYCL2. They are involved in cell-cycle regulation, the control of proliferation and development.

MYCL

Synonyms

- LMYC
- L-myc

Definition

MYCL is a protein of 364 amino acids that dimerizes wih MAX to bind DNA in a sequence specific manner. It belongs to the family of basic region helix-loop-helix proteins, most of which are transcription factors. The gene maps to position 1p34. In a subset of small cell lung cancers, an increase in gene copy number (by amplification) has been reported.

MYCL2

Synonyms

- v-myc avian myelocytomatosis viral oncogene homolog 2

Definition

The protein (357aa, 41 kD) that belongs to the basic helix-loop-helix family of transcription factors. It binds DNA as a heterodimer with MAX. The gene maps to Xq22-28. To date, nothing is known about an association with cancer.

MYCN

Synonyms

- *NMYC*
- *N-myc*

Definition

The *MYCN* gene encodes a nuclear protein of 464 aa, 49 kD Differential phosphorylation of the protein will result in two major components with the relative molecular mass of 62 and 64kD. MYCN is a member of the basic helix-loop-helix family of transcription factors that binds DNA as a dimer with MAX to activate transcription. The gene maps to 2p24; an increase in gene copy number (by → amplification) in is seen in → neuroblastoma (here associated with poor prognosis) and occasionally in small cell lung carcinoma and retinoblastoma; amplified *MYCN* is expressed at an elevated level.

MYF3

Definition

→ Myogenic factor 3.

MYH11

Definition

MYH11 is a smooth muscle myosin heavy chain disrupted by the → inv(16).

MYOD

Definition

Myoblast determination protein 1 (MyoD), also known as myogenic factor (Myf-3) is a → bHLH protein member of the Mrf family of 319 amino acids and 34 kD. The human MYOD1 or MYF3 gene locus maps to human chromosome 11p15.4. MyoD is involved in muscle differentiation, induces fibroblasts to differentiate into myoblasts, activates muscle-specific promoters, and interacts with and is inhibited by the Twist protein.

Myogenesis

Definition

Myogenesis is the embryonic development of muscle tissue.

Myogenic Factor 3

Synonyms

- MYOD1

Definition

Myogenic factor 3, alias MYOD1, is a nuclear protein (319 aa, 34 kD) involved in muscle differentiation. It induces fibroblasts to differentiate into myoblasts, activating muscle-specific promoters. It belongs to the basic helix-loop-helix family of transcription factors. The gene maps to 11p15.

Myoma

Definition

Leiomyoma [→ uterine leiomyoma, clinical oncology].

Myomectomy

Definition

Myomectomy is the surgical removal of individual → fibroids while preserving the uterus; → uterine leiomyoma.

Myometrium

Definition

Myometrium is the thick middle muscular layer of the uterus.

N

N-Acetyltransferase

Definition

N-Acetyltransferase is an acetyl-CoA requiring enzyme which catalyses the acetylation of xenobiotics that are aromatic amines or contain a hydrazine group. It participates in the → detoxification of a plethora of hydrazine arylamine drugs and is also able to bioactivate several known carcinogens.

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NAD(P)H-quinone Oxidoreductase

Definition

NAD(P)H-quinone oxidoreductase, also called quinone reductase or DT-diaphorase, is a flavoprotein that catalyses the two-electron reduction of quinones to hydroquinones.

Naevoid Basal Cell Carcinoma Syndrome

Definition

→ Basal cell nevus syndrome.

Naevus

Definition

Naevus (US: nevus) or mole is a benign neoplasm of pigment-producing cells (melanocytes) in the skin; → melanoma; → mole; → basal cell nevus syndrome.

Nasopharyngeal Carcinoma

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Synonyms

- lymphoepithelioma
- Rigaud and Schmincke types of lymphoepithelioma
- transitional carcinoma

The term lymphoepithelioma is misleading and should not be used because this tumor derives entirely from epithelial origin. The benign lymphoid component is secondarily associated.

Definition

Nasopharyngeal carcinoma (NPC) is an epithelial cancer that has a worldwide distribution. It is endemic in southeast China, particularly in the Cantonese population of the Guangzhou area (up to 80 cases per 100,000 people per year). An intermediate incidence is found in other parts of Southern Asia, North Africa and among Eskimos in Alaska and Greenland

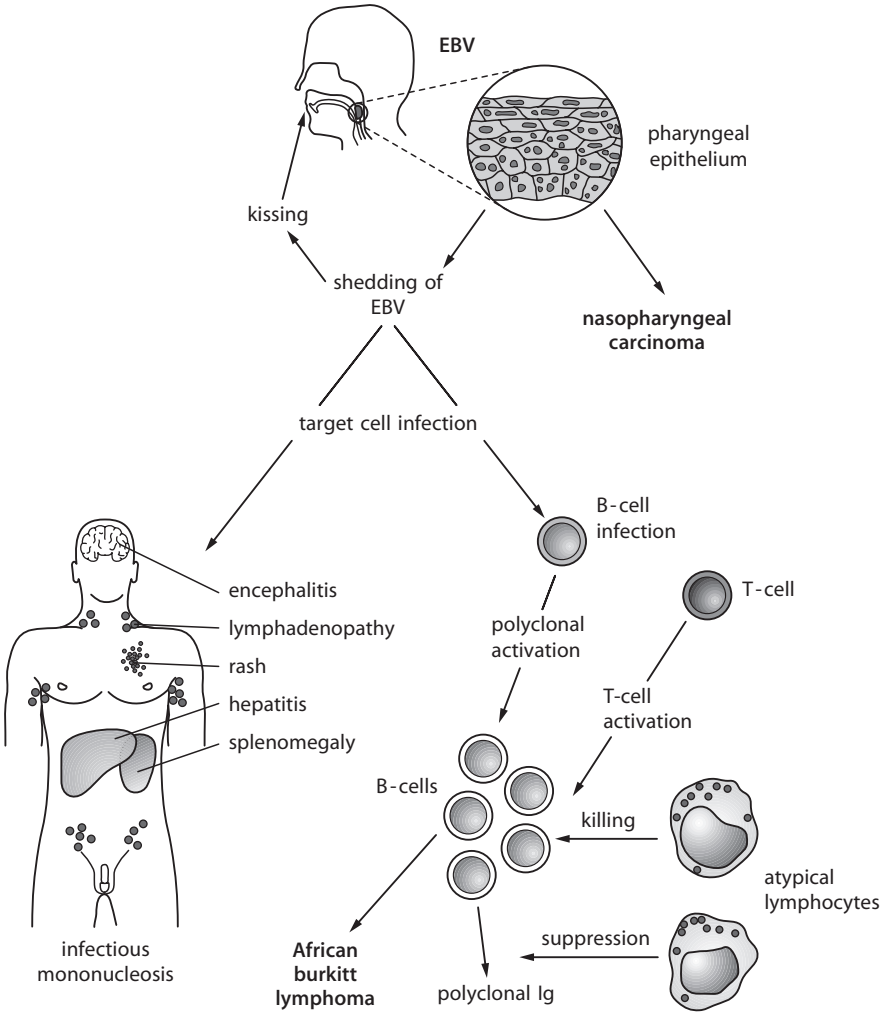
(8 to 12 cases per 100,000 people per year). The incidence of NPC is extremely low in the rest of the world.

The World Health Organization (WHO) has proposed a classification of NPC based on the degree of differentiation:

- **WHO type 1:** keratinizing squamous cell carcinoma (SCC)
- **WHO type 2:** nonkeratinizing epidermoid carcinoma
- **WHO type 3:** nonkeratinizing undifferentiated carcinoma (UNCT).

Characteristics

NPC is a variant of squamous cell carcinoma that has been serologically linked to the presence of → Epstein-Barr virus (EBV) since 1966. EBV is a member of the human herpesvirus family and is the etiologic agent of infectious mononucleosis (Fig.). EBV consistently associ-



Nasopharyngeal Carcinoma. Fig. – Role of the Epstein-Barr virus (EBV) in infectious mononucleosis, nasopharyngeal carcinoma, and Burkitt lymphoma. EBV first invades and replicates within the salivary glands and the pharyngeal epithelium, and is shed into the saliva and respiratory secretions. In some people, the virus can transform the pharyngeal epithelium, leading to nasopharyngeal carcinoma. In people that are not immune from childhood exposure, EBV causes infectious mononucleosis. EBV infects B lymphocytes which undergo polyclonal activation. These B cells stimulate the production of atypical lymphocytes which kill virally infected B cells and suppress the production of immunoglobulins. Some infected B cells are transformed into malignant lymphocytes of Burkitt lymphoma.

ates with the less differentiated types of NPC such as the nonkeratinizing and undifferentiated types of nasopharyngeal squamous carcinomas (WHO types 2 and 3). The → human papillomavirus (HPV), a double stranded DNA member of the papovavirus family, causes proliferative lesions of squamous epithelium such as common warts, flat warts, plantar warts, anogenital warts (condyloma acuminatum) and laryngeal papillomatosis, and has been found in some keratinizing squamous carcinomas (WHO type 1).

Genetic and geographic factors play an important role in the genesis of NPC. Although the incidence of NPC among Chinese people decreases after immigration to low incidence areas such as the United States, the risk profile of the Chinese immigrants remains higher than that of the indigenous population. HLA-A2, HLA-Aw19, HLA-B17, HLA-Bw46 and HLA-Bw58 → major histocompatibility loci have all been associated with an increased risk of developing NPC, whereas the HLA-A11 locus is associated with a decreased risk of NPC in the Chinese population. Conversely, the haplotype HLA-A2 has been correlated with a lower incidence of NPC in whites living in the United States, especially if homozygous. This suggests that different environmental insults may combine with specific genetic predispositions making the pathogenesis of NPC geographically distinct.

Additionally, deletions of the short arm of chromosome 3 as well as of 9p21-22 have been associated with NPC, which suggests that inactivation or deletion of a putative → tumor suppressor gene located in these loci may be implicated in the pathogenesis of this disease. One of the candidates for this genetic implication is the → p16 gene, which encodes for an inhibitor of the cyclin-dependant kinase that directly regulates the retinoblastoma tumor suppressor gene [→ retinoblastoma, cancer genetics] (RB). Homozygous deletions of the p16 gene that maps to chromosome 9p21 have been reported in 35% of the cases of primary UCNT (WHO type 3). Additionally, several reports showed that mutations of the 'guardian of the genome', p53, which occur frequently in SCC, are present in only 10% of patients affected with NPC. Recently, point mutations in the retinoblastoma-related gene → RB2/p130 have been described in 30% of NPC patients. RB2/p130 is a novel tumor suppressor gene that maps to chromosome 16q.12.2, an area that is frequently altered in several human neoplasias including breast, ovarian, hepatic, prostatic and endometrial carcinomas. Interestingly, → amplification of the → Bcl-2 oncoprotein, which inhibits → apoptosis (programmed cell death), has been found mostly in NPC WHO types 2 and 3 and less frequently in the differentiated type 1.

Keratinizing NPC accounts for approximately 25% of all NPC and is more frequent after the age of 40 years. The nonkeratinizing nasopharyngeal carcinoma is the least common, representing only 12% of all NPC. The undifferentiated carcinoma is the most common, representing almost 60% of all NPC, and being the most frequent in younger age.

NPC has been linked by epidemiological and experimental evidence to certain environmental factors such as dietary and life style habits. In fact, a series of studies have suggested that continuous exposure to salted fish and other preserved food containing volatile nitrosamines, which are well-known carcinogens, constitutes a significant cause of NPC in the Chinese population. Tobacco use and alcohol consumption have been linked to an increased incidence of WHO type 1 NPC in white North Americans in proportion to the intake amount.

Because of their location, most NPC remain asymptomatic for a prolonged period and present at an advanced stage. Palpable cervical node metastases are most often (75 – 90%) the first sign of the disease, even if there are no specific local complaints from the patients. This is due to the growth pattern of this neoplasia, which is not a large space occupying mass, but instead is an infiltrating and organ-substituting tumor. This growth pattern results in a variety of neurological symptoms such as hearing loss, tinnitus, → ansomia, → epistaxis, a feeling of an obstruction, → dysphagia, → odynophagia, → dysphonia, → hoarseness and → diplopia.

Because NPC remain asymptomatic for a long time, an early diagnosis is difficult. Molecular biology and cytogenetic techniques have

improved the detection of EBV. Southern blot analysis distinguishes between latent and infectious EBV involvement, whereas *in situ* hybridization allows the localization of single virally infected NPC cells. PCR of the Zebra *EBV* gene on fine-needle biopsies in patients with neck nodal involvement and unknown primary lesions may help in the identification of the nasopharyngeal origin of the disease. Serological analysis of different EBV → antigens, such as screening for anti-EBNA → IgG, anti-EA IgG and IgA, anti-VCA IgG and IgA and anti-Zebra IgG, may be used to identify early cases of NPC as well as relapses. Increased anti-VCA, anti-EA IgA and IgG are used as markers to monitor the disease, the response to therapy and may suggest recurrence, but are neither highly predictive nor accurate. In fact, many long-term disease-free patients show persistently high levels of these markers.

Diagnosis

Diagnosis and staging of NPC is reserved to imaging techniques such as → CT scanning and → magnetic resonance imaging (MRI), which are essential for accurate evaluation of tumor extension and spreading and for guiding radiation treatment planning and evaluation of possible recurrences.

Treatment

Treatment of local NPC is based essentially on external → radiation therapy, because of its radiosensitivity and because of its anatomic location. In fact, surgical resection of the tumor adjacent to the skull base with tumor-free margins is often impossible. The overall control rate of localized lesions with radiotherapy is generally about 70 – 90%. At 5 years, local relapse ranging between 15 to 54% has been reported. Tumor control rate of more advanced lesions is instead dramatically decreased. Future improvement of NPC treatment may result from advances in radiation therapy techniques such as three-dimensional conformal therapy, proton-beam therapy or intracavitary → brachytherapy, which allows the delivery of higher radiation doses to the tumor. Combined radiation therapy and → chemotherapy have been suggested to improve both local control and metastatic spreading of the disease. Novel approaches and future directions may include testing the appropriateness of adjunctive → gene therapy oriented to either improve local control, by reducing the tumor volume prior to radiation therapy, and/or to decrease the probability of distant metastasis.

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NAT

Definition

→ N-acetyltransferase.

Natural Killer Cells

Definition

Natural killer (NK) cells are large granular lymphocytes that kill virus infected cells and tumor cells and also mediate antibody-dependent cell-mediated cytotoxicity.

NBCCS

Definition

Naevoid basal call carcinoma syndrome (NBCCS) is an autosomal dominant cancer predisposition syndrome that is characterised by multiple developmental defects. The gene responsible was localised to chromosome 9 and is now known to be the human homologue of the *Drosophila* segment polarity gene → *patched*; → basal cell nevus syndrome.

NBS

Definition

Nibrin, p95; → Nijmegen breakage syndrome.

NEBD

Definition

→ Nuclear envelope breakdown.

NEDD2

Definition

Neural precursor cell expressed, developmentally down-regulated 2; → caspase 2.

NEMO

Synonyms

- → IKBKG
- inhibitor of k light polypeptide gene enhancer in B cells, kinase γ
- IKK-γ

Definition

Nuclear factor κ essential modulator.

Neoadjuvant Therapy

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Synonyms

- primary chemotherapy
- primary systemic therapy
- pre-operative chemotherapy or systemic therapy
- protoadjuvant therapy

Definition

Neoadjuvant therapy is an auxiliary therapy (e.g. androgen ablation in → prostate cancer) administered prior to another therapy (e.g. surgery or radiation). It is given prior to surgery in an attempt to decrease the size of the tumor. It is performed in an attempt to make the tumor easier to remove and to increase the chances that removal of the tumor is curative.

Characteristics

In most solid tumors, surgery remains the initial primary modality of treatment. With the increasing effectiveness of systemic therapies (chemotherapy and hormonal therapy) in advanced disease stages and also in the post-operative 'adjuvant' setting, studies were instituted in many solid tumor types using systemic therapies prior to surgery. Advantages of this approach may include:

- downstaging of the tumor allowing for less extensive surgery to be performed;
- ability to quantitatively measure *in vivo* sensitivity to the systemic agent that would not be possible in the post operative 'adjuvant' model;
- the ability to measure pre- and post-systemic therapy biologic markers (e.g. by fine needle aspirates or core biopsies) providing valuable insights into the mechanisms behind systemic therapeutic effects;

- earlier initiation of systemic therapy to eradicate systemic micrometastasis.

In addition to the above mentioned benefits, if neoadjuvant chemotherapy could clearly be shown to produce an overall survival benefit compared with postoperative systemic therapy, it could produce a major paradigm shift in multidisciplinary cancer care. Thus, if systemic therapy, used primarily, increased cure rates and/or produced convincing and consistent increases in overall survival compared with postoperative use, then local therapeutic modalities such as surgery and/or radiation therapy would become 'adjuvants' to primary systemic therapy.

Primary (neoadjuvant) systemic therapy is being studied in a number of tumor types, and its use in breast cancer serves as an example. Neoadjuvant chemotherapy has been used effectively in women with locally advanced breast cancer, frequently converting cases where mastectomy was the only realistic option to a situation where breast conservation surgery could be accomplished. In one study series, women with tumors greater than 5 cm in size were able to undergo breast conservation surgery instead of mastectomy 62% of the time. However, most published series indicate a breast preservation rate of about 30% for such large tumors. Despite the ability to induce significant antitumor responses in 75 – 80% of patients, in general 90% of women continue to have viable tumor within the breast. In addition, most series have not yielded a survival advantage compared with the use of the conventional therapeutic sequence of surgery, chemotherapy and then possibly radiation therapy. If (and when) more effective systemic therapies emerge, more widespread use of primary systemic therapy is likely to become a reality.

Neoangiogenesis

Definition

Neoangiogenesis is the capacity of a tumor cell to induce the surrounding tissue to form blood vessels; → angiogenesis.

Neovasculature

Definition

Neovasculature is newly formed blood vessels at a certain tissue site; → angiogenesis.

Nephroblastoma

Definition

→ Wilms tumour.

NER

Definition

→ Nucleotide excision repair.

NETs

Definition

→ Neuroendocrine tumors.

NEU

Definition

→ HER-2/neu.

Neural Ectoderm

Definition

The neural ectoderm is a group of cells that lie adjacent to the posterior part of the developing brain and spinal cord in the embryo, which migrate to form diverse tissues including those that are involved in → MEN 2.

Neuroblastoma

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Definition

Neuroblastomas are childhood embryonal tumors of migrating neuroectodermal cells, derived from the neural crest and destined for the adrenal medulla and sympathetic nervous system.

The term neuroblastoma is commonly used for all types of neuroblastic tumors. The International Neuroblastoma Pathology Committee suggests to distinguish between four types of neuroblastic tumors:

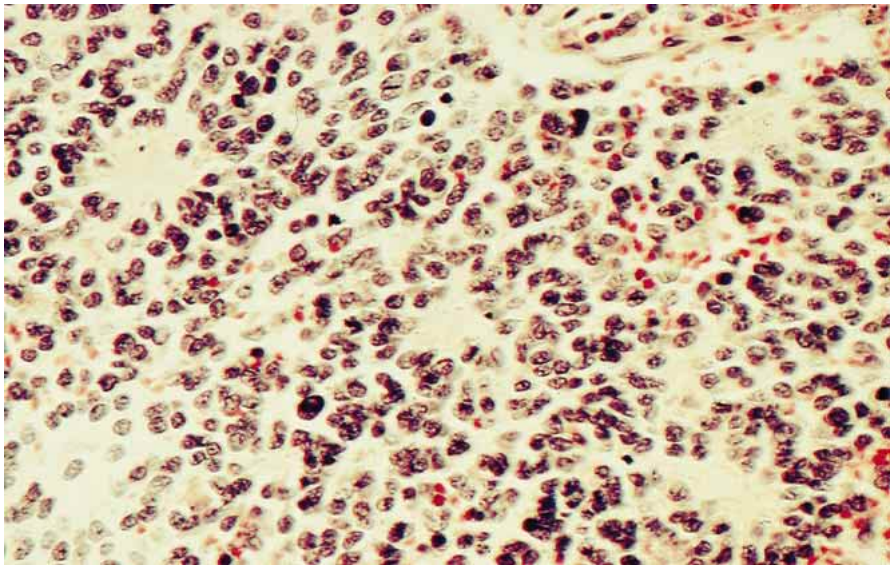
- Neuroblastoma (Schwannian stroma-poor).
- → Ganglioneuroblastoma, intermixed (Schwannian stroma-rich).
- → Ganglioneuroma (Schwannian stroma-dominant).
- Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor.

Characteristics

Neuroblastic tumors are the most common solid extracranial malignant tumors during the first two years of life. In the United States of America there are seven new cases per million population per year are detected in black children and 9.6 per million in white children. More than 90% of cases occur in the first decade of life. Neuroblastoma has been detected in the fetus *in utero* by prenatal ultrasound examination. There is no sex predilection.

The primary sites include the adrenals (40% of neuroblastic tumors), followed by the abdominal (25%), thoracic (15%), cervical (5%), and pelvic sympathetic ganglia (5%). These structures are derivatives of migrating neuroectodermal cells originating from the neural crest. It has been suggested that in ganglioneuroblastoma (Schwannian stroma-rich) the diploid Schwann cells are reactive, in contrast to triploid ganglion cells. All ganglioneuromas (Schwannian stroma-dominant), the fully mature form of neuroblastic tumors, were once neuroblastomas (Schwannian stroma-poor).

Neuroblastic tumors have favorable prognosis in infants less than one year of age. Adrenal neuroblastic tumors have a worse prognosis than extra-adrenal tumors, particularly thor-



Neuroblastoma. Fig. – Schwannian stroma-poor. A tumor composed of neuroblastic cells forming groups or nests.

acic tumors. Children with stage 1 and 2 neuroblastic tumors survive longer than those with stage 3 and 4 tumors. Spontaneous regression is most apparent in patients with stage 4S tumors, i.e. infants with a local stage 1 neuroblastoma or unknown primary with involvement of the liver, skin and/or bone marrow with <10% tumor cells. However, even within stage 4S is a subset of tumors with poor prognosis, which is characterized by unfavorable histology or amplified → *MYCN*. Regression also occurs in a subset of patients with other stages of neuroblastoma, but predominantly in infants. It is not clear whether spontaneous maturation or an immunological phenomenon is related to regression.

Prognostic evaluation is based on an age-linked histopathologic classification distinguishing two prognostic groups; the favorable and the unfavorable histology group (→ Shimada System).

Tumors in the favorable histology group include:

- Age up to 1.5 years: neuroblastoma poorly differentiated subtype with low (< 2% or < 100/5,000 cells) or intermediate (2-4% or 100-200/5,000 cells) mitosis-karyorrhexis index (MKI).
- Age between 1.5 and 5 years: neuroblastoma, differentiating subtype with low MKI.
- Ganglioneuroblastoma, intermixed usually seen in older children.
- Ganglioneuroma, maturing and mature subtypes, usually seen in older children.

Tumors in the unfavorable group include:

- Any age: neuroblastoma, undifferentiated subtype.
- Age between 1.5 and 5 years: neuroblastoma, poorly differentiated subtype.
- Any age: neuroblastoma with high (> 4% or > 299/5,000 cells) MKI.
- Age between 1.5 and 5 years: neuroblastoma with intermediate MKI.
- Age 5 years or older: all neuroblastoma subtypes.
- ganglioneuroblastoma, nodular.

Although the aetiology of neuroblastoma is unkown it appears unlikely that environmental exposure plays a major role. However, cases of neuroblastoma have been described that were associated with the fetal hydantoin, phenobarbitol or alcohol syndromes, suggesting that prenatal exposure to these substances may increase the risk of neuroblastoma. Other studies have been suggesting a weak association between neuroblastoma and paternal occupational exposure to electromagnetic fields, or maternal use of hair coloring products, but none of these associations has been confirmed. Moreover, no prenatal or postnatal exposure to drugs, chemicals or radiation has been strongly or consistently associated with an increased incidence of neuroblastoma.

Although most neuroblastomas appear to be sporadic, a number of reports have been describing familial neuroblastoma, as well as bilateral or multifocal disease, that are consistent with hereditary predisposition.

Constitutional chromosomal abnormalities have been described in lymphocytes of patients with neuroblastoma, even though there is no apparent pattern. An interstitial → deletion and a reciprocal → translocation t(1;17), both affecting 1p36, were observed in patients with neuroblastoma. However, it is unclear if these 1p36 rearrangements were contributing to a neuroblastoma predisposition.

Somatic genetic changes associated with neuroblastoma progression include → *MYCN* → amplification, → allelic loss at 1p, 11q, 14q and gain of 17q.

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NeuroD

Definition

NeuroD (neurogenic differentiation factor), also known as Beta2 and NeuroD1 (neurogenic differentiation 1), is a mammalian → bHLH protein (356 amino acids, 39 kD) member of the Atonal family. It is a transcriptional activator and acts as a differentiation factor during neurogenesis. It also binds to the insulin gene → E-box. Efficient DNA binding requires dimerization with an other bHLH protein; it forms heterodimer with E47. The human NEUROD1 or NEUROD gene locus maps at 2q32 and the mouse neurod1 or neurod gene locus at chromosome 2 (50.00 cM).

NeuroD2, also known as Ndf2 (neurogenic differentiation factor 2) is a mammalian → bHLH protein (381 amino acids, 41 kD) member of the Atonal family. The human gene locus maps at chromosome 17q12 and the mouse at chromosome 11 (57.00 cM).

NeuroD3, also known as Neurogenin and Math4c, is a mammalian → bHLH protein (237 amino acids, 25 kD) member of the Atonal family. The human gene locus maps at chromosome 5q23-q31 and the mouse at chromosome 13 (35.00 cM).

Neuroendocrine Carcinoma

Definition

→ Gastrinoma.

Neuroendocrine Tumors

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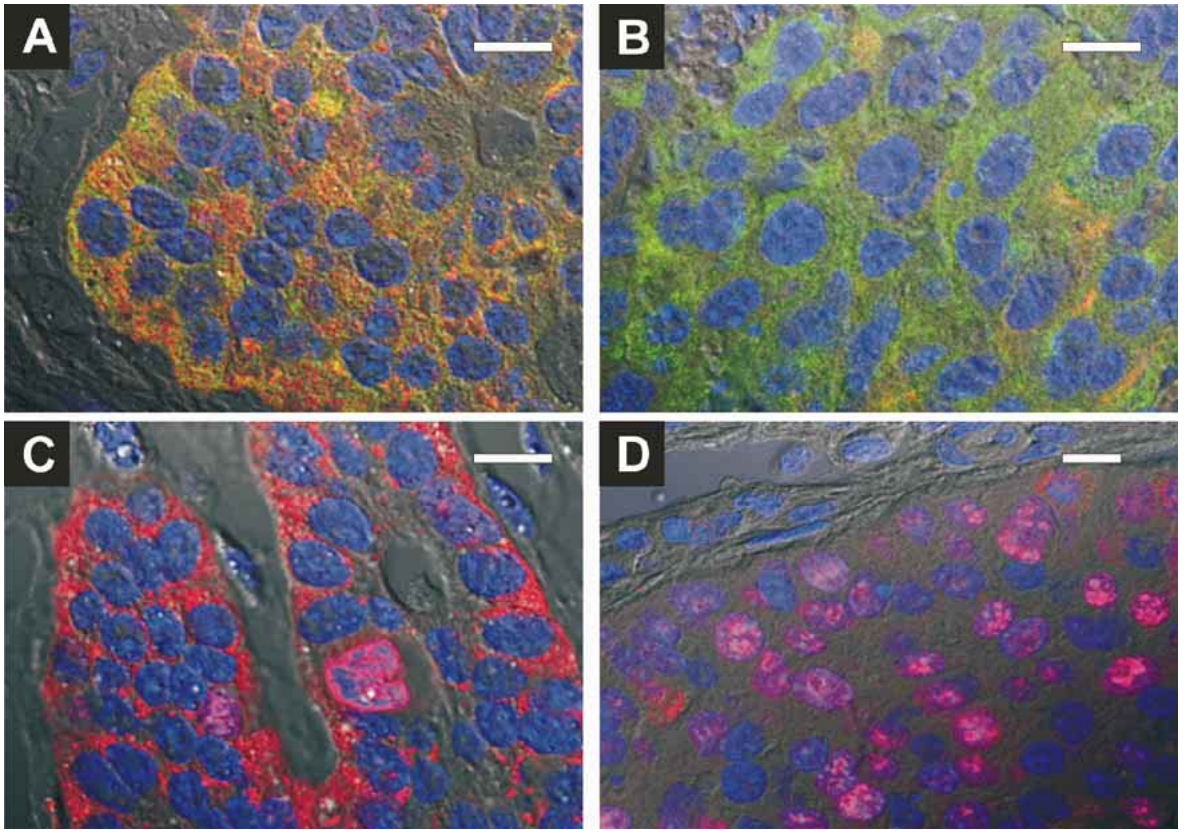
Synonyms

- Carcinoid tumors (at the rather indolent pole)
- small cell carcinoma (at the highly aggressive pole of the spectrum)
- apudoma (refering to tumors capable of amine precursor uptake and decarboxylation)
- argentaffinoma (related to positive reactions to silver stains)

Definition

Neuroendocrine tumors (NETs) are neoplasms with a broad range of morphologic patterns, grade of differentiation and biological behavior that share common features of neuroendocrine programming. NETs contain various amounts of molecules involved in the regulated release of neuropeptides, neurotransmitters and hormones, and may be associated with hyperfunctional syndromes or be nonfunctioning. Most NETs are of endodermal (fore-gut, mid-gut and hind-gut) derivation and rare entities of true neural crest origin. They may occur in every topographic location, with a predeliction of the lung, the gut and the pancreas. Other primary organs include the skin, salivary glands, prostate and various sites in the urinary, genital and bilary tracts.



Neuroendocrine Tumors. Fig. – Confocal laser scanning microscopy images of well- differentiated (A, C) and poorly differentiated (B, D) intestinal neuroendocrine tumors (NETs). Binding of appropriate primary antibodies was assessed by Alexa Fluor 488 conjugated goat-anti-rabbit antibody (green) that detects synaptophysin and of Cy3 goat-anti-mouse antibody (red) that detects both the cytoplasmic protein chromogranin and the nuclear antigen Ki-67 (MiB-1). Nuclei were decorated by Hoechst nuclear stain (blue). **A** The well-differentiated NET shows a strong expression of both chromogranin A and synaptophysin, while the poorly differentiated carcinoma (**B**) is preferentially stained with anti-synaptophysin and contains rare cells coexpressing chromogranin A. **D** Nuclear Ki-67 (MiB1) is present in a small percentage of nuclei (pink) of chromogranin-positive tumor cells of the low-grade NET. **C** The large majority of cells of the poorly differentiated NET is devoid of chromogranin A but shows nuclear Ki-67/MiB-1 expression, indicating a high proliferative activity.

Two major groups of NETs are recognized:

- The epithelial group traditionally known as carcinoid, atypical carcinoid, small or oat cell carcinoma, islet cell (pancreatic endocrine) tumor, Merkel cell carcinoma of skin, parathyroid neoplasms and pituitary tumors.
- The neural group including paraganglioma, pheochromocytoma, neuroblastoma and primitive neuroectodermal tumor.

Here, only the epithelial-type NETs are considered.

Characteristics

Diagnostic criteria for entering the NET category

Neuroendocrine marker molecules reported in NETs reside in different cellular compartments: Synaptophysin and the R-SNARE proteins (→ SNAREs) synaptobrevin 1and 2 (VAMP1, 2) are localized in synaptic vesicle-like microvesicles (→ SLMVs), granins, cytochrome b_{561}, somatostatin or hormones in large dense-core vesicles (→ LDCVs), neuron-specific enolase and protein gene product 9.5 in cytoplasm, and

the cell adhesion molecules (NCAMs) or Q-SNARE proteins (syntaxin 1A, SNAP-25) in the plasmalemma [→ SNAP receptors].

Expression of somatostatin receptors, which belong to the superfamily of G protein-coupled receptors, is found in the majority of NETs. The effective binding of somatostatin-analogues (octreotide, lantreotide) is of therapeutic relevance. Serotonin and its metabolites are considered to cause the → carcinoid syndrome. Traditional histochemical techniques based on silver reduction and electron microscopy, showing LDCVs and SLMVs, have now given way to immunolabeling techniques. Immunohistochemically, the positivity for synaptophysin, synaptobrevin1,2/VAMP1,2 and chromogranin A is generally the best way to provide evidence of neuroendocrine differentiation. The synaptophysin and synaptobrevin1,2/VAMP1,2 immunostains are the most reliable since they are positive even in poorly differentiated NETs, which may be practically devoid of LDCV constituents (Figs. A-D). Generally, the presence of at least 2 neuroendocrine markers in the majority of tumor cells coexpressed with cytokeratin-type intermediate filaments is necessary for the diagnosis of an epithelial tumor of the NET category. In mixed exocrine-endocrine tumors, the neuroendocrine population should represent >30%. Tumors with exocrine and endocrine features present in identical cells are designated as amphicrine. A minority of neuroendocrine cells, which may have prognostic implications, may be identified in tumors that do not belong to the NET category.

Prognostic classification concepts of NETs

Among various classification systems using different terminologies, the new World Health Organization (WHO) classification reflects the present state of knowledge. It is applied to gastrointestinal and pancreatic NETs in addition to adenohypophysal, adrenal, paraganglial and neural NETs, but does not include lung or thyroid tumors. The simplified WHO classification applies the term 'endocrine' instead of 'neuroendocrine' and defines 5 major categories:

1. well-differentiated endocrine tumor,
2. well-differentiated endocrine carcinoma,
3. poorly-differentiated endocrine carcinoma,
4. mixed endocrine-exocrine tumor,
5. tumor-like lesions.

The term well-differentiated endocrine tumor/carcinoma now largely replaces carcinoid or islet cell tumor. Functioning tumors may still be designated according to the hormonal syndromes, such as insulinoma or gastrinoma. Organoid growth patterns, forming islands, uniform nests, ribbons of tumor cells, are characteristic features of well-differentiated NETs. High-grade cellular atypia, high mitotic index/proliferative activity, focal necroses and an aggressive course are indicative of poorly-differentiated (large cell-form and small cell-form) NETs of category 3. The greatest difficulty concerns the distinction between lesions of benign or uncertain behavior included in category 1 and low-grade endocrine carcinoma (category 2). Features associated with malignancy are local extension, angioinvasion, cellular atypia, increased mitotic index/proliferative activity, → Ki-67 labeling index and the presence of metastases. Tumor size and tissue-appropriate or -inappropriate hormonal products in conjunction with the primary tumor site have to be taken into consideration, since different degrees of aggressiveness may occur within each tumor category. In general, well-differentiated NETs measuring 1 cm or less rarely metastasize, while lesions >2cm frequently metastasize, but are usually rather indolent with a median survival of ≥2years. A mitotic count of >2 per 10 high power field (HPF) or a Ki-67 index >2-5% are correlated with a malignant behavior. The majority of poorly-differentiated endocrine carcinomas are metastatic at the time of diagnosis and have a poor prognosis with a medial survival <1 year. A correct clinicopathological evaluation may predict the clinical behavior and is essential for the management of patients.

Genetics

Most NETs are sporadic neoplasms, but subsets occur as a part of heriditary cancer syndromes

including → MEN-1, → MEN-2 syndromes or → VHL. Allelic deletions and mutations at 11q13 have also been documented in sporadic gastroenteropancreatic NETs, indicating that the MEN-1 gene and perhaps other tumor suppressor genes at 11q13 may play a role in their tumorigenesis. Furthermore, loss of heterozygosity (LOH) of chromosome 3p25-26, 16q and 17p distal to the p53 locus have been documented. DNA sequence copy number changes include losses of various chromosomal regions at 3pq, 6pq, 10pq, 11pq, Xq and the Y chromosome and gains at 5q, 7pq, 9q, 12q, 17pq, 20q and Xp. Thus, the data obtained by comparative genomic hybridization (CGH) show different patterns of genetic aberrations, with an increasing number of genomic changes with tumor size and disease stage.

NETs of the pancreas

Pancreatic NETs ('islet cell tumors'), which are considerd in the WHO classification, are accompanied by hormonal symptoms in about 80% of cases and may be a part of MEN-1. They occur solitary or multiple and may produce insulin (hyperinsulinemic hypoglycinemia), glucagon (glucagonoma syndrome), gastrin (→ Zollinger-Ellison syndrome), somatostatin, pancreatic polypeptide, vasoactive intestinal polypeptide (Verner-Morrrison syndrome), growth hormone releasing factor, adrenocorticotropic hormone (Cushing syndrome), serotonin (carcinoid syndrome) or be non-functioning. A tumor diameter of <2 cm, a low mitotic index of ≤ 2 per 10 high power fields and a $\leq 2\%$ Ki-67 labeling are generally associated with a benign behavior of pancratic NETs. Survival is significantly decreased when the Ki-67 index is $>4\%$ The large majority of insulin-producing tumors are benign, while most other functioning and non-functioning tumors are of uncertain behavior or are low-grade malignant carcinomas. The well-differentiated neoplasms (category 1 and 2) are composed of uniform cells with no, mild or moderate atypia and show a trabecular or a solid-medullar (insular) growth pattern. The category of poorly-differentiated NETs encompasses tumors composed of small to intermediate sized cells growing in solid aggregates, often with central necrosis. True mixed endocrine-exocrine neoplasms, with at least one-third endocrine cells, are extremely rare. The prognosis depends on the exocrine component.

NETs of the stomach and the esophagus

Most well-differentiated gastric endocrine tumors are enterochromaffin-like (ECL) cell tumors (carcinoids) and may be classified on the basis of the underlying gastric pathology. Hypergastrinemia is involved in the pathogenesis of ECL cell tumors type I and II. They often arise as multiple lesions on a background of endocrine-cell hyperplasia in chronic atrophic gastritis (type I tumor) or with MEN-1 or the ZES syndrome (type II tumors), and are benign or low-grade malignant. Sporadic (type III) tumors develop in the absence of hypergastrinemia and behave more aggressively. ECL cell tumors are composed of trabecular, adenoid or solid nests of endocrine cells. Well-differentiated tumors confined to the mucosa or submucosa without angioinvasion and a diameter of ≤ 1 cm are considered to be benign. Tumors of ≤ 1 cm with angioinvasion or 1-2 cm without angioinvasion are of uncertain behavior ('borderline'), and tumors of 1-2 cm with angioinvasion or of >2 cm without angioinvasion are low-grade malignant. Poorly-differentiated carcinomas showing tightly packed small- to intermediate-sized cells with frequent mitoses are high-grade malignant and have a poor outcome.

NETs of the esophagus are rare neoplasms of the middle and lower thirds. Most cases either belong to category 3 of poorly-differentiated NETS of small cell type or are combined with squamous cell or adenocarcinoma (category 4). They are generally non-functioning and have a poor prognosis with frequent systemic dissemination.

NETs of the intestine

The majority of intestinal NETs are well-differentiated slow-growing neoplasms (carcinoids) of category 1 or 2, which may metastasize to mesenteric lymph nodes and liver. Poorly-dif-

ferentiated carcinomas of category 3 are rare. Intestinal NETs are often associated with production of ectopic hormones including serotonin, pancreatic polypeptide, glucagon, gastrin, somatostatin, cholecystokinin, calcitonin and bombesin. Secretion of vasoactive amines by tumors that are metastatic to the liver may lead to the → carcinoid syndrome in about 10% of cases. NETs arising in the duodenum have a lower metastasizing rate than those of the jejunum and ileum. About 40% of duodenal NETs produce gastrin and may be associated with MEN1 and/or ZES. Somatostatin-producing tumors tend to involve the papilla, include psammoma bodies and may be associated with neurofibromatosis. Gangliocytic paragangliomas, composed of epithelial and neuromatous elements, may arise in the duodenal wall and are generally benign. Serotonin- and substance P-producing enterochromaffin cells are the predominant component of most NETs arising in the jejunum, ileum, appendix and proximal colon (mid-gut), while enteroglucagon-producing cells are often present in NETs of the distal colon and rectum (hind-gut). Most intestinal NETs arise in the ileum, are frequently multiple and develop metastases in about 35% of cases. NETs of the appendix are usually benign and rarely metastasize. The majority resemble its counterparts in the ileum, and are composed of serotonin- and substance P-producing EC cells arranged in solid nests. Enteroglucagon-producing tumors of the hind-gut type may show a glandular or ribbon-like pattern. The so-called goblet-cell carcinoids of the appendix are composed of clusters of mucin-containing cells with scattered endocrine cells and are now considered as low-grade adenocarcinomas, which may involve lymph nodes and liver or spread to peritoneal surfaces. Well-differentiated NETs rarely occur in the large bowel and may present as small mucosal nodules. Poorly-differntiated NETs or composite adenocarcinoma-small cell carcinoma (category 4) arise more frequently in the colon and rectum than in the small intestine and may present with an extensive local and metastatic tumor growth. The histologic appearance of intestinal NETs (category 1, 2) show solid nests, trabeculae or tubules with peripheral palisading or a ribbon type. Tumor cell nests tend to be surrounded by a dense fibrous stroma. Poorly-differentiated carcinoma (category 3) may resemble its small cell counterparts in other organs and show clusters of anaplastic cells with high mitotic activity, necrosis and lymphatic and vascular invasion, indicating an aggressive metastatizing behavior with a poor outcome.

NETs of the lung

Well-differentiated neuroendocrine carcinoma (carcinoid tumor).

These centrally or peripheral localized NETs are composed of round or ovoid cells with a finely dispersed chromatin and up to 2 mitoses per 10 HPF. They have a well defined architecture of nests, cords, strands or microacinar structures in a fibrovascular stroma. They are usually indolent with a cure obtained by surgical resection in most cases. Since about 15% develop lymph node metastases, they should be considered as low-grade malignant tumors.

Moderately-differentiated neuroendocrine carcinoma (atypical carcinoid tumor).

These are NETs with a morphology and clinical course between typical carcinoids and small cell carcinoma. Lymph node metastases develop in 30 – 50%.

Poorly-differentiated neuroendocrine carcinoma.

The high-grade NETs encompass 4 types; small (oat) cell, intermediate, combined and large cell type. Small cell carcinomas, which are strongly associated with smoking, are composed of oval cells with scanty cytoplasm, dispersed chromatin and are often spindly with molding. Pseudoreosettes may be present. Mitoses, necroses and crushing artefacts are frequent. The intermediate type yields larger cells with more abundant cytoplasm. Small cell tumors containing a subpopulation of larger cells correspond to the combined type. The diagnostic criteria for large cell NETs are not yet reliably defined. Neuroendocrine differentiation may only be detected by immunohistochemistry in carcinomas showing an undifferentiated non-small cell or basaloid morphology. Poorly-

differentiated neuroendocrine carcinoma constitutes about 20% of lung cancer and are highly aggressive diseases with early and rapid dissemination with lymph node and extrathoracic organ metastases. Patients may present endocrine symptoms (Cushing, inappropriate antidiuretic hormone production, Eaton-Lambert syndrome).

NETs of the thymus

The classification system of pulmonary NETs is also applied to thymic neoplasms ranging from well- or moderately-differentiated tumors (carcinoids and atypical carcinoids) to poorly-differentiated small cell or large cell carcinomas. MEN-1 associated carcinoids account for about 25% of thymic carcinoids and are more aggressive than sporadic tumors.

C-cell (medullary) thyroid carcinoma

This tumor of parafollicular C-cell differentiation may be sporadic (about 80%) or hereditary. Heriditary tumors are often multicentric and bilateral and develop on a background of C-cell hyperplasia. The RET protooncogene plays an important role in cases, occurring in MEN-2A and MEN-2B or in familiary thyroid carcinoma. Somatic point mutations in the RET gene are also found in sporadic cases. Tumor cells express calcitonin in addition to various amounts of other neuroendocrine marker proteins, and are stained by CEA-antibodies.

The histolology may be classic, with sheets of polygonal tumor cells, or display various patterns (follicular, anaplastic, small cell). Amyloid deposits are present in about 80%. The tumor is rather indolent, but lymph node metastases in the neck and the upper mediastinum may be found at initial presentation.

NET (Merkel cell carcinoma) of the skin

This cutaneous type of NET is an aggressive neoplasm composed of small basophilic cells that are arranged in nests within the dermis. The tumor cells contain neuroendocrine markers in addition to cytokeratin 20 (CK20). About 60% of small cell carcinomas of the salivary gland express CK20, indicating a close relationship to Merkel cell carcinoma.

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Neuroepithelioma

Definition

Neuroepithelioma is a → Ewing sarcoma-related tumor with limited neuroectodermal differentiation. The term is primarily used in the United States synonymously to → pPNET.

Neurofibromatosis 2

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Synonyms

- NF2
- bilateral acoustic neurofibromatosis
- central neurofibromatosis

Definition

Neurofibromatosis 2 (NF2) is an autosomal dominant disorder characterized by the bilateral development of vestibular schwannomas, along with the occurrence multiple other benign intracranial, spinal cord and peripheral nerve tumors. Cataracts are nontumorous manifestations of NF2.

Characteristics

Diagnostic criteria and clinical aspects

The diagnostic criteria for NF2 are the observance of bilateral eight-nerve masses (vestibular schwannomas) with appropriate imaging techniques (e.g., → CT or → MRI), or the existence of a first-degree relative with NF2 and either a unilateral vestibular schwannoma or two of the following: meningioma, neurofibroma, glioma, schwannoma or juvenile posterior subcapsular lenticular opacity.

Vestibular schwannomas, formerly termed acoustic neuromas, are a universal feature of NF2. They are slow growing, benign tumors that form in the internal auditory canal where the eighth nerve and facial nerve are close together. Initial symptoms of NF2 usually begin subtly but progress as the slow-growing tumor expands, causing tinnitus, hearing loss, and balance problems. Schwannomas sometimes occur on other cranial nerves, more often on sensory than motor nerves, and on peripheral nerves, with skin tumors that can be particularly painful. The schwannomas of NF2 rarely, if ever, become malignant. About half of all NF2 patients also develop intracranial, and occasionally spinal meningiomas, although neither shows a preferred location of occurrence. Spinal schwannomas arise in about two-thirds of NF2 patients and can be extremely debilitating. Astrocytoma and ependymoma, occur in a small minority (5 – 10%) of patients. Decreased visual acuity due to cataracts, which may occur even before tumors are evident, affects at least one-third of NF2 patients.

The mean onset age for NF2 is approximately 21 years but the disorder has been seen as early as age 2 and as late as age 70. The first manifestations of disease are usually related to vestibular schwannomas causing hearing loss, tinnitus, or loss of balance but may include facial weakness, visual impairment, and painful skin schwannomas. The progression of NF2 shows extreme variability as it depends on tumor number, location and growth rate. The mean survival from diagnosis is currently 15 years, leading to an average age of death of 36 years for NF2 patients, although this life expectancy is likely to increase with advances in diagnostic and surgical techniques.

Genetics

NF2 affects approximately 1 in 40,000 individuals due to germline mutation in a gene on chromosome 22q that encodes a protein named merlin (schwannomin). About half of cases are new mutations and half show a family history. The disorder is inherited in an autosomal dominant pattern, but has a recessive mechanism of action at the level of individual tumor cells, making the NF2 gene a classic tumor suppressor locus. NF2 involves an inherited inactivating mutation that only leads to tumor for-

mation when a somatic mutation inactivates the remaining copy of the *NF2* gene in an appropriate target cell, leading to absence of functional merlin. Almost all NF2 mutations, both germline and somatic, involve evident inactivation of the gene due to deletion, chromosomal loss or rearrangement, or truncating mutations. A handful of missense mutations or small in-frame deletions have been described, which presumably impact critical functional domains of merlin. Somatic mutations that inactivate both *NF2* copies in target cells also account for essentially all cases of sporadic unilateral vestibular schwannoma and more than half of all sporadic meningiomas in the general non-NF2 population.

The *NF2* gene spans 110 kilobases with 16 constitutive exons and one alternatively spliced exon. It encodes two major merlin isoforms that differ only at the extreme carboxy terminus. Isoform 1, encoded by exons 1-15 and 17, comprises 595 amino acids. Alternative splicing of exon 16 eliminates the final 16 amino acids and replaces them with 11 novel residues to produce merlin isoform 2. Germline *NF2* mutations have been found in all exons except exons 16 and 17, suggesting that both major isoforms may have tumor suppressor activity. Nonsense mutations due to C to T transitions in CGA codons are particularly frequent. There is some degree of correlation between mutation type and disease severity as missense mutations in exons 7, 11, and 15 as well as large deletions are generally associated with mild disease manifestations. By contrast, nonsense mutations throughout the gene, and especially in exon 6, cause more severe disease. Somatic mutations also occur throughout the gene except in exons 16 and 17, and deletions are more frequent than in germline. Complete loss of chromosome 22 leading to readily detectable loss of heterozygosity in the tumors is an especially frequent mechanism of somatic alteration.

Merlin protein

Merlin was named for its similarity with three known proteins, moesin, ezrin and radixin (merlin =moesin, ezrin, radixin-like protein). These closest relatives of merlin are known collectively as → ERM proteins, and all four are members of the protein 4.1 family, which also includes erythrocyte protein 4.1, talin, certain unconventional myosins, several protein tyrosine phosphatases and several anonymous proteins. The protein 4.1 family is defined by the presence of a conserved → FERM domain, spanning ~270 amino acids, that is believed to mediate interactions with integral membrane proteins. In merlin and the ERMs, this domain is located in the NH2-terminal half of the protein, and is followed by a long alpha helical segment and a charged COOH-terminal domain. Protein 4.1, the prototype for this family, helps to maintain membrane stability and cell shape in the erythrocyte where it connects the spectrin-actin cytoskeletal lattice with the integral membrane proteins, glycophorin, and the anion channel. The ERMs, closer relatives to merlin, also bind integral membranes proteins, such as CD44, via the FERM domain and to actin via a COOH-terminal binding site. The ERM proteins all show intramolecular interaction whereby the COOH-terminal region binds to a more NH2-terminal segment, effectively blocking the binding site for actin and certain other interactors. This 'closed' conformation can be altered to an 'open conformation' by specific phosphorylation or phosphoinositides, permitting binding to the actin cytoskeleton. Each of the ERMs is approximately 78% identical to the others and they may have overlapping functions.

Merlin is approximately 63% identical to the ERM proteins across the FERM domain and the immediate downstream region but is much more divergent across the long alpha helical domain, which has a notable interruption by a cluster of proline residues. Merlin is the only one of these four proteins that displays alternate COOH-termini, creating two major isoforms. Interestingly, merlin isoform 1 undergoes the same 'closed conformation/open conformation' regulation as the ERMs due to intramolecular interaction, although no conserved actin binding site is present near its COOH-terminus. By contrast, merlin isoform 2 does not exhibit 'head to tail' intramolecular binding and remains constitutively in the 'open conformation'.

The precise normal function of merlin remains to be defined, although it is likely to involve linking cytoskeletal and membrane elements like protein 4.1 and the ERMs. Indeed, in cultured cells, where merlin is expressed as a protein with an apparent size of approximately 66 kD in SDS-polyacrylamide gel electrophoresis, the protein is found in areas of membrane remodeling, particularly membrane ruffles, where it co-localizes with actin. However, merlin is not associated with cytoplasmic actin stress fibers. To date, a number of proteins capable of interacting with merlin have been described, including among others F-actin, β2 spectrin, → CD44, → hepatocyte growth factor-regulated tyrosine kinase substrate, β1 integrin and ERM proteins. Perhaps the most intriguing interactor to date is NHERF, which also binds to ERMs in the 'open conformation'. NHERF is a 358 amino acid protein with two PDZ protein interaction domains upstream from a C-terminal region that binds to the unmasked N-terminal half of merlin and the ERMs. It was originally described as an adaptor protein required for protein kinase A regulation of the kidney sodium-hydrogen exchanger (NHE3). NHERF binds to NHE3 *via* the second of the PDZ domains and to several other membrane proteins, such as the β2 adrenergic receptor, the purinergic P2Y1 receptor and the cystic fibrosis transmembrane conductance regulator, via the first PDZ domain. potentially tying these proteins, and merlin/ERMs to sodium-hydrogen exchange or to protein kinase A regulation.

That growth control in Schwann cells and meningeal cells is disrupted by the loss of merlin function, implies that lack of merlin subtly alters some aspect of intracellular signaling, resulting in a slow proliferation. Despite having an overall structure similar to the ERMs, merlin is the only one of these four proteins demonstrated to have a specific tumor suppressor function, suggesting that it affects one or more signaling pathways different from the ERMs. Although the tumors of NF2 are benign, merlin mutations can also be involved in progression of non-NF2 tumors, most notably malignant meningiomas, suggesting that loss of the protein can have different effects in different circumstances. For example, in the mouse, merlin deficiency is associated with the development of highly malignant tumors such as osteosarcomas and rhabdomyosarcomas. The NH2-terminal half of merlin has been implicated in growth control in *Drosophila*, where inactivation of the NF2 homologue leads to cellular overproliferation. Thus, it seems likely that merlin deficiency can affect different aspects of the web of signal transduction that connects the cell membrane, actin-cytoskeleton and the nucleus. Indeed, any signaling pathway in which NHERF can act as a cofactor and any of the growing list of proteins with which NHERF physically interacts are already candidates for disruption in the absence of merlin. The delineation of merlin's full complement of interactions and their effects in the target cells, Schwann cells and arachnoidal cells of the meninges, will probably be required to understand merlin's tumor suppressor activity in NF2

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Neuropeptide

Definition

Neuropeptides are chemical messengers often colocalized with classic neurotransmitters within nerve endings in the central nervous system (CNS). Over 50 neuropeptides have been characterized and grouped into 10 large families. Several of these (e.g. angiotensin, gastrin, vasoactive intestinal peptide) act as hormones outside the CNS. In contrast to small-molecule neurotransmitters, neuropeptides are synthesized from larger precursor proteins (polyproteins) in the neuronal cell body, packaged in vesicles and then transported to the axon terminals by fast axonal transport.

Neurotrophin

Definition

Neurotrophin is a nerve growth factor (NGF) family of neurotrophic factors that include NGF, BDNF, NT-3 and NT-4/5; → TRK.

Neurotrophin-3 (NT-3) belongs to the nerve growth factor family of currently five known neurotrophins. It functions as a noncovalent homodimer and exerts its effect via the high affinity receptor trkC, but can also activate → TRK-A and → TRK-B. It is an important regulator of neuronal precursor cells.

Nevus

Definition

Naevus (UK: naevus) or mole is a benign neoplasm of pigment-producing cells (melanocytes) in the skin; → melanoma; → mole; → basal cell nevus syndrome.

NF2

Definition

→ Neurofibromatosis 2.

NFκB

Definition

Nuclear factor κ B is a transcription factor complex that is composed either of a member of the → rel family of proteins (p65 or c-rel) and a p50 or p52 protein or of p50/p52 complexes without a rel protein. NFκB is activated during embryogenesis, during immune response by cytokines (by tumor necrosis factor or interleukin-1) or during neoplastic transformation or apoptosis-inhibiting responses by tumor promoters or other agents. NFκB is sequestered in the cytoplasm by a member of the IκB family of inhibitory proteins. It masks the nuclear localization signal of NFκB, thereby preventing nuclear localization. Phosphorylation of IκB by → IκB kinases induces their → ubiquitination and degradation by → proteasomes, allowing free NFκB to translocate into the nucleus, to bind cognate DNA sequences and to induce gene expression.

NFκBIKA

Definition

Conserved helix-loop-helix ubiquitous kinase.

NFκBIKB

Definition

Inhibitor of κ light polypeptide gene enhancer in B-cells, kinase β.

NFYB

Synonyms

- NFYβ
- nuclear transcription factor Y, β
- CBF-B
- CBF-β

Definition

CCAAT-binding transcription factor; nuclear protein 207 aa, 22kd, stimulates the transcription of various genes by binding to a CCAAT motif in promoters, for instance of albumin and β-actin genes; forms dimers with NFYA; gene maps to chromosome 12q22-q23.

NGR

Definition

Asparagine-glycine-arginine.

NHEJ

Definition

→ Non-homologous end joining.

NHL

Definition

Non-Hodgkin lymphoma [→ Hodgkin disease].

Nibrin

Definition

Nibrin is the protein defective in → Nijmegen breakage syndrome. It is involved in the early stages of recombination repair including the processing of DNA damage, and exists in a multi-function complex with → Rad50 and → Mre11.

Nick Translation

Definition

Nick translation is an enzymatic *in vitro* reaction to label DNA, in particular with radioactive or fluorescent nucleotides. It usually employs the ability of *Escherichia coli* polymerase I to use a nick as a starting point from which one strand of a duplex DNA can be degraded and replaced by re-synthesised new material, thereby incorporating the labeled nucleotides. *In situ* nick translation (ISNT) can be used to determine the degree of DNA fragmentation as a measure of apoptosis.

Nicotine

Definition

Nicotine is a compound present in tobacco and tobacco smoke that is responsible for the addictive properties of these products; → tobacco carcinogenesis.

Nijmegen Breakage Syndrome

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Synonyms

- Nijmegen breakage syndrome (NBS)
- ataxia telangiectasia variant 1 and variant 2
- Berlin breakage syndrome
- Semanová II syndrome

Definition

Nijmegen breakage syndrome is a genetic disease characterised by an extreme sensitivity towards ionising radiation and a high risk for the development of lymphatic tumours.

Characteristics

Nijmegen breakage syndrome (NBS) is a rare genetic disease,with less than 100 families ascertained world-wide, the majority in eastern Europe. Inheritance follows an autosomal recessive modus with complete penetrance (1). The majority of patients have the same 5 bp deletion in the *NBS1* gene, but five further mutations, all of which lead to a severely truncated protein, have also been described (2). Although birth weight and size of homozygotes are normal, microcephaly and growth retardation soon become apparent and are the major symptoms of the disorder. Progressive mental retardation is typical. However, patients with normal intelligence are known and IQ is often within the normal range during early childhood. A sloping forehead and receding mandible give NBS patients a characteristic facial appearance that becomes more pronounced with age. NBS patients are immunodeficient in both cellular and humoral immune systems, leading to frequent infections particularly of the respiratory tract. Approximately 40% of NBS patients develop cancer before the age of 21. B and T cell lymphomas are the most common malignancies seen, although rhabdomyosarcoma, haemoblastoma and neuroblastoma have all also been observed.

The protein product of the *NBS1* gene, nibrin, is involved in the repair of DNA damage, as demonstrated by the chromosomal instability observed in patient cells (3). The cytogenetic aberrations seen include chromatid breaks and recombination figures, which frequently involve chromosomes 7 and 14 where immunoglobulin and T-cell receptor genes are located. The frequency of chromosome breaks is greatly increased in patient cells after irradiation or treatment with radiomimetic drugs. Indeed, their radiosensitivity makes treatment of malignancy in these patients problematic.

Cellular and molecular features

The DNA lesion caused by ionising radiation, to which NBS patients and their cells are most sensitive, is the double strand break (DSB). Unrepaired DSBs increase the mutation rate in NBS cells and thus lead to neoplasia. Furthermore, the continued replication of DNA despite an ionising radiation challenge, so called radioresistant DNA synthesis (RDS), is another major mutagenic effector charcterstically found in NBS cells.

Several repair systems are available for the repair of DSBs in eukaryotic cells (4):

- non-homologous end joining (NHEJ) by the DNA-PK complex,
- single-strand annealing (SSA) by the RAD50/MRE11 complex,
- repair exploiting homologous recombination mediated by the RAD52 complex or the → BRCA1/2 complex.

NHEJ/SSA systems are active particularly prior to DNA replication, whilst repair after or during DNA replication uses homologous recombination.

Nibrin is an essential component of the RAD50/MRE11 SSA pathway. This repair process requires the degradation of single-stranded DNA from the DSB to regions of so-called microhomology, which can then anneal as a first step in DNA resynthesis and

break repair. The exonuclease activity for SSA is provided by the RAD50/MRE11 proteins. DSB repair by the RAD50/MRE11/nibrin complex following ionising radiation can be visualised microscopically in cells by immunostaining with antibodies to MRE11, RAD50 or nibrin. Discrete nuclear foci, the sites of DSB repair, can be seen in normal cells but are completely lacking in cells from NBS patients (Fig. 1). RAD50 and MRE11 are still complexed together, even in NBS cells, so that nibrin is clearly responsible for the recruitment of these repair enzymes to the sites of DSB repair. Indeed, much of the RAD50/MRE11 complex is not even correctly localised in the nucleus of NBS cells (Fig. 1). Mutations in *MRE11* lead to a genetic disease with a similar phenotype to NBS, but mutations in RAD50 have not yet been observed in humans.

In accordance with its function in recruiting repair enzymes, nibrin is activated, by phosphorylation, in response to the presence of DSBs in DNA (5). This phosphorylation is performed by the protein ATM, which is deficient in patients with the genetic disease ataxia-telangiectasia (AT). Cells from AT patients show a similar phenotype to NBS cells in terms of chromosomal instability, radiation sensitivity and reduced DSB repair foci. As in NBS cells, AT cells fail to stop semiconservative DNA synthesis or prevent entry into S-phase in response to a mutagenic challenge, and in both cases activation of p53 is abnormal and downstream genes are not correctly induced. Thus nibrin presumably also functions in signal transduction to the p53 checkpoint system.

Clinical aspects

Reduced cell proliferation and increased apoptosis, due to the accumulation of DNA damage, explain the growth retardation and microcephaly of NBS patients. Similarly, their immundeficiency stems from the involvement of nibrin in the processing of DSBs that are formed as a natural intermediate during immunoglobulin gene-rearrangements. In terms of somatic mutation rate, the NBS cell, which due to the loss of nibrin function is unable to perform SSA, must use alternative but inappropriate pathways to repair DSBs, possibly by error-prone homologous recombination.

The cancer incidence in NBS heterozygotes is reportedly high, and the impact of NBS1 mutations within the general population is therefore of some concern. Sequence variations in NBS1 have been found in children with → acute lymphoblastic leukemia (ALL) and this suggests that nibrin may be also involved in the pathogenesis of this disease.

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NIK

Definition

NF-κB inducing kinase; → NF-κB.

NK

Definition

→ Natural killer cells.

NM23 Metastasis Suppressor Gene

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Synonyms

- NME, (non-metastatic)
- *awd*, abnormal wing discs (Drosophila)
- *ndpk* or *ndk*, nucleoside diphosphate kinase

Definition

Non-metastatic cDNA clone #23, identified on the basis of its quantitatively higher expression in low metastatic murine melanoma cell lines as compared to related, highly metastatic cell lines. The gene exhibits metastasis suppressor activity upon transfection into several metastatically competent tumor cell lines and also induces differentiation. In the human, it is currently a family of six related genes (*nm23-H1* to *-H6*).

Characteristics

Expression pattern

The *nm23* cDNA was identified on the basis of its reduced mRNA and protein levels in two tumorigenic, but poorly metastatic, murine K-1735 melanoma cell lines, as compared to five related, more highly metastatic K-1735 melanoma cell lines. This pattern of reduced expression coincident with high tumor metastatic potential has been observed in additional, but not all, experimental animal model systems of metastasis.

In human tumor cohort studies, low tumor cell Nm23 expression has significantly correlated with indicators of high tumor metastatic potential, such as decreased patient disease-free or overall survival, the presence of lymph node metastases, poor tumor grade, in breast, ovarian, cervical, gastric, hepatocellular and other carcinomas and melanomas. Reduced Nm23 expression is not considered an independent prognostic factor.

A significant exception to this trend is in unicellular diseases such as leukemias, where increased Nm23 expression correlates with proliferation and advanced disease. Nm23 may also act extracellularly in these cancer types.

Mutations

Mutations in the *nm23* coding sequence have been infrequently observed, but are typically found in cancer types that do not exhibit reduced expression coincident with high metastatic potential. Mutations of a serine 120 and a leucine 48 have been reported in high grade human neuroblastomas.

Metastasis suppressive activity

Metastasis suppressor gene function is demonstrated by transfection of the gene of interest into a metastatically competent tumor cell line, with a resultant significant decrease in tumor metastatic potential *in vivo* with no effect on tumorigenicity. Nm23 cDNA has been transfected into eight human tumor cell lines and metastasis suppressor activity reported. The cell lines tested are principally breast carcinoma and melanoma, but also include colon and esophageal carcinomas. The percent of metastasis suppression compared to vector-alone control transfectants ranged from 45 – 96%, indicating that this gene alone does not control metastasis.

Transfectants expressing *nm23* exhibited altered activity in several *in vitro* assays of the tumor metastatic process. These included reduced motility in Boyden chamber assays, invasion through extracellular matrix coated filters and reduced colonization in soft agar, either alone or in the presence of TGF-β.

Where-ever tested, the proliferative capacity of the *nm23*- and control transfectants *in vitro* were equivalent. Likewise, the tumorigenicity *in vivo* were comparable, separating this metastasis suppressor gene from classic tumor suppressor genes.

Differentiation

The Drosophila homolog of Nm23, *awd*, causes aberrant differentiation and lethality postmetamorphosis when reduced in expression or mutated. Much of the aberrant differentiation is localized to the wing discs, the areas of presumptive adult epithelia, as well as the brain and ovaries.

Transfection of *nm23* into human breast carcinoma and phaeochromocytoma cell lines resulted in the acquisition of differentiated characteristics *in vitro*. These characteristics include the development of ductal morphology and the synthesis and basolateral secretion of basement membrane proteins, as well as sialomucin synthesis for the breast carcinoma line. Neurite extension and decreased proliferation were observed in the *nm23*-transfected phaeochromocytoma cells. Given the similarity of embryonic and metastatic cells, it is hypothesized that the 'normal' function of *nm23* may be in differentiation, which is reversed by its reduced expression in metastasis.

Biochemical activities

The biochemical pathway whereby *nm23* suppresses tumor metastasis or induces differentiation is unknown. The Nm23 proteins exhibit a number of biochemical activities as a nucleoside diphosphate kinase, histidine protein kinase, serine protein kinase and possibly a DNA binding activity. The histidine protein kinase activity is poorly understood in mammalian cells, but well known as the 'two-component' signal transduction system in prokaryotes, yeast and plants.

Clinical relevance

Loss of heterozygosity or allelic deletion of *nm23-H1* and *nm23-H2*, both situated on chromosome arm 17q23, is frequently observed in → breast cancer and other carcinomas. Allelic deletion of both alleles have been infrequently observed. Low Nm23 protein level but not allelic deletion was found to be the best predictor of high tumor metastatic potential. It is hypothesized that transcriptional regulation of *nm23* expression is relevant, and the promoters of several *nm23* genes have been reported and are under characterization.

Metastatic Colonization

Tumor metastasis is a major contributor to cancer patient morbidity and mortality. The identification of a molecular 'map' of the tumor metastatic process will lead to the development of therapeutic interventions for the individual steps in the process.

For breast and many other cancers, only a proportion of patients have detectable distant metastases at the time of diagnosis and surgery. It is not known in these patients the degree to which tumor cells have completed the metastatic process, i.e., invaded from the primary tumor, intravasated and extravasated the circulatory system and established themselves as small, occult micrometastases in a distant organ. To the degree that this process has occurred, the development of therapeutic interventions against the early steps in the metastatic process will be ineffective. For patients without detectable distant metastases, two aspects of the metastatic process remain open for therapeutic intervention: angiogenesis, which permits growth beyond the size serviced by diffusion; and colonization, the formation of a tumor colony in an environment different from that of the primary tumor.

Clinical Hypothesis

It is hypothesized that a proportion of aggressive breast and other carcinomas exhibit low Nm23 expression, and that elevation of tumor cell Nm23 expression would be expected to reduce the motility, invasiveness and colonization of metastatic cells, as well as induce their differentiation. Current efforts are directed toward identifying compounds that will induce nm23 transcription in metastatic breast tumor cells.

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NMI

Definition

N-myc (→ MYCN) interactor (NMI) also interacts with STAT proteins, with MAX, MYC, MXI1 and several other transcription factors that share a Zip, HLH or a Zip-HLH motif. In response to IL-2 and interferon-γ, the NMI protein augments STAT-mediated transcription without having its own transactivation domain [STAT [→ signal transducers and activators of transcription in oncogenesis]]. It also enhances the association of CBP/p300 coactivator proteins with STAT5 and STAT1, and may serve as an adaptor protein that stabilises dimerization. The protein of 307 amino acids is predominantly cytoplasmic. The gene maps to 22q13.

N-Nitrosamine

Definition

N-nitrosamine are compounds having a nitroso group (-N=O) bound to the nitrogen of a secondary amine.

NNK(4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone)

Definition

NNK(4-(methylnitrosamino)-1-(3-pyridyl)-1-butanor is a tobacco-specific carcinogen that induces lung tumors in laboratory animals; → tobacco carcinogenesis.

NOL3

Definition

→ Nucleolar protein-3.

Non-homologous End Joining

Definition

Non-homologous end joining (NHEJ) is the joining of broken DNA directly at its ends, mediated by DNA-dependent protein kinase. It is the ligation of double-stranded DNA ends with no need for sequence homology between the ends joined. Several proteins are known to be involved in this process, which is considered the major double-strand break (→ DSB)-repair pathway in mammalian cells; → Nijmegen breakage syndrome.

Non-syntenic

Definition

Non-syntenic refers to genes or genetic loci that lie on different chromosomes, i.e. are not genetically linked.

Non-ulcer Dyspepsia

Definition

Non-ulcer dyspepsia is a persistent upper abdominal symptoms in the absence of any identifiable lesions of the upper digestive tract.

Normoxia

Definition

Normoxia is the physiological (normal) O_2 partial pressure distribution in a defined tissue, allowing unrestricted function and activity of cells making up the tissue (or organ). Normoxic refers to a physiologically adequate supply of oxygen.

Normoxic

Definition

Normoxic is having a physiologically adequate supply of oxygen.

Northern Blotting

Definition

Northern blotting is a technique for transferring RNA from an agarose gel to a nitrocellulose filter on which it can be hybridized to a complementary DNA.

NOTCH IC

Definition

Notch IC is a form of Notch that is comprised only of the intracellular (IC) domain of the protein. It is thought that this is the active form of Notch that is produced as a result of the proteolytic processing induced by ligand binding. This is also a form of the protein that is found in human leukemia as a result of a chromosomal translocation with the T-cell receptor; → Notch/Jagged signaling in neoplasia.

NOTCH/JAGGED Signaling in Neoplasia

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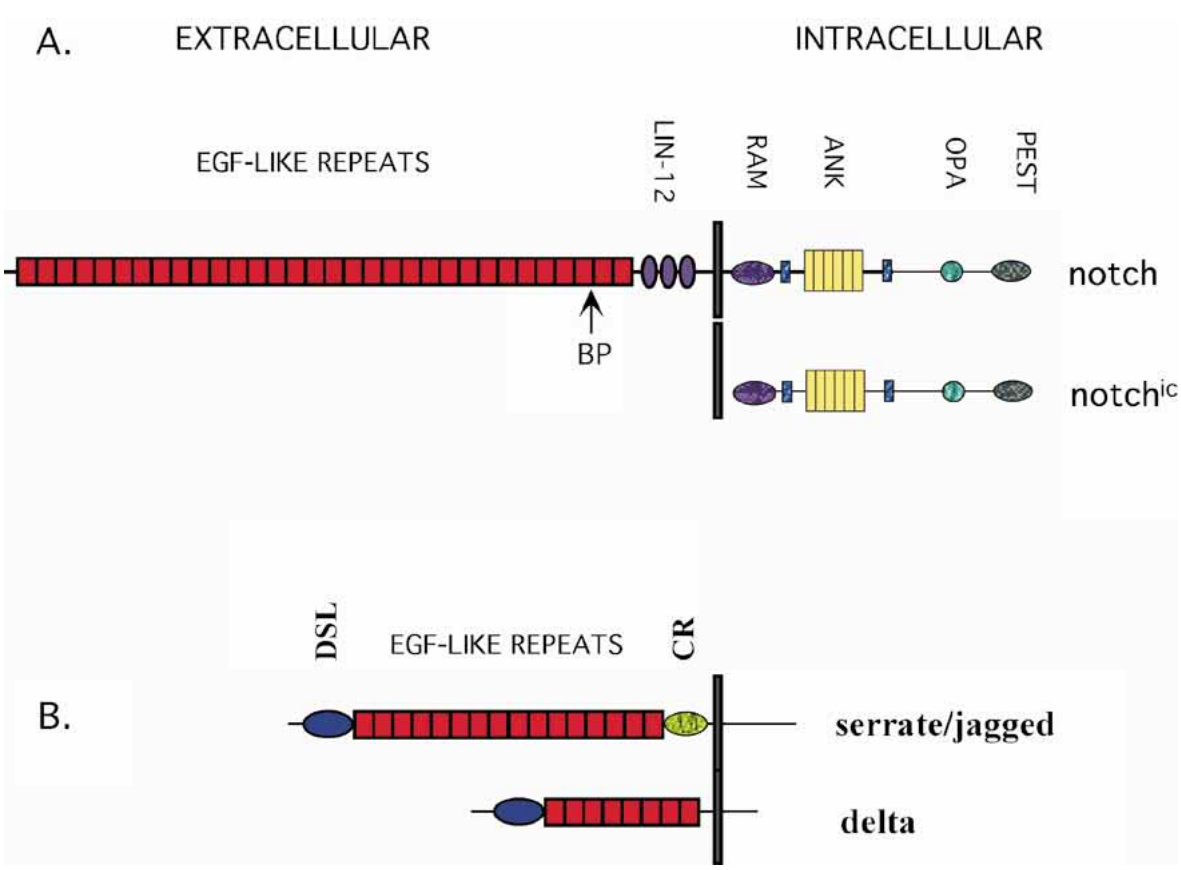
Definition

Notch and Jagged proteins define an evolutionarily conserved signal transduction pathway that is critically important for cell fate decisions in metazoan development. Notch and Jagged are single pass membrane-spanning proteins that are believed to share a receptor/ligand relationship, respectively. *Jagged* is the mammalian ortholog of the *Serrate* gene of *Drosophila*. Collectively, the Notch ligands are referred to as the DSL proteins (Delta/Serrate/Lag). Mutations in this pathway underlie several inherited disorders as well as cancer. Aberrant expression of the intracellular portion of Notch proteins ($Notch^{ic}$) is associated with the oncogenic transformation of cells.

Characteristics

There are four known mammalian Notch proteins, termed Notch1-4. These proteins are related through a high degree of sequence identity and structural organization. Notch proteins are membrane-spanning receptors with molecular weights of approximately 300 kD (Fig. 1A). The extracellular domain of Notch1 is composed of approximately 1750 amino acids, which include 36 tandem repeats of a sequence resembling epidermal growth factor (EGF) and three repeats of a motif designated as lin-12 repeats. The cytoplasmic domain comprises a sequence of approximately 750 amino



NOTCH/JAGGED Signaling in Neoplasia. Fig. 1 – Structure of Notch and DSL Proteins. A Notch proteins are transmembrane-spaning receptors for DSL Proteins (B). Notch^{ic} is thought represent an activated Notch molecule. In T-cell leukemia, the chromosomal translocation t(7;9)(q34;q34.3) fuses the T-cell receptor β locus to the Notch1 locus at the indicated break point (BP). The result of this translocation is the constitutive production of Notch^{IC}-like molecules. RAM, binding domain for CSL transcription factors; ANK, 6-tandem copies of ankyrin repeats; OPA, Glutamine-rich region; PEST, a domain thought to be involved in protein turnover. Putative nuclear localization sequences are indicated by small boxes and flank the ANK domain. **B** Notch ligands are encoded by the Serrate/Jagged and Delta genes. DSL, a 45 amino acid sequence unique to DSL proteins thought to be involved in interactions with Notch. CR, a cysteine-rich domain of unkown function used to classify DSL proteins as either Serrate/Jagged or Delta. The intracellular sequences of the DSL proteins is of variable length and contains no recognizable motifs.

acids with no apparent enzymatic activity, but contains 6 tandem copies of an ankyrin-like repeat (CDC10/ANK), a region rich in glutamine (OPA) and a region rich in proline, glutamate, serine and threonine (PEST). The CDC10 and OPA repeats are thought to mediate protein-protein interactions, whereas the PEST domain may target the proteins for degradation. The RAM domain is located between the ANK repeats and the innerface of the plasma membrane. This domain is the primary binding site for the transcription factor CSL (CBF1/supressor of hairless/Lag1).

Proteins that are encoded by the *Serrate* and *Delta* genes and are collectively referred to as DSL proteins. As with the *Notch* genes, the fruit fly *Drosophila melanogaster* possesses a single *Serrate* and *Delta* gene. However, mammalian genomes posses at least 2 *Serrate* and 4 *Delta* genes. The primary amino acid sequence of Serrate and Delta reveals a striking similarity to the Notch proteins. Serrate and Delta are large ($>$ 100 kD) single pass transmembrane-spanning proteins (Fig. 1B). Their extracellular domains are composed of between 1-16 tandem copies of an EGF-like repeat. One common fea-

ture shared among the ligand proteins is a 45 amino acid sequence rich in cysteine, termed the DSL domain and located N-terminal to the EGF-like repeats. This domain is thought to play a role in ligand-receptor interactions. Unlike Notch proteins, the intracellular sequences of Serrate and Delta are short (ca. 100-150 amino acids) and contain no sequence homology to any other proteins in the database. The function of the intracellular sequences of Serrate and Delta is not understood. However, the integrity of these sequences are important, since deletion of the intracellular domain from either Delta or Serrate leads to a phenotype similar to loss of Notch signaling in the fly. In addition, Serrate proteins contain a cysteine-rich domain located between the EGF-like repeats and the outerface of the plasma membrane. The presence of this domain is used to classify ligand proteins as either Serrate- or Delta-like.

Cell and molecular parameters

Notch proteins are synthesized as precursor proteins of approximately 300 kD. During trafficking through the Golgi complex, Notch undergoes proteolytic processing that produces the mature receptor. This processing step appears to be carried out by a furin-like protease, and the resulting mature Notch receptor is composed of two fragments, the extracellular fragment and an intracellular fragment that remains embedded in the plasma membrane. One of the major questions in Notch signaling is, what is the mechanism of ligand activation of Notch? One proposed mechanism is, the association of Notch with a DSL ligand on a neighboring cell induces a proteolytic event that releases the intracellular domain of Notch from its membrane tether (Fig. 2). This ligand-induced processing is thought to be carried out by a presenilin-dependent γ-secretase-like protease. Currently, it is not understood how ligand induces this processing event or if activation of Notch can occur through interactions with ligands present in the same cell. Moreover, there is still debate over the possibility of signaling from membrane tethered forms of Notch that do not require additional proteolytic processing.

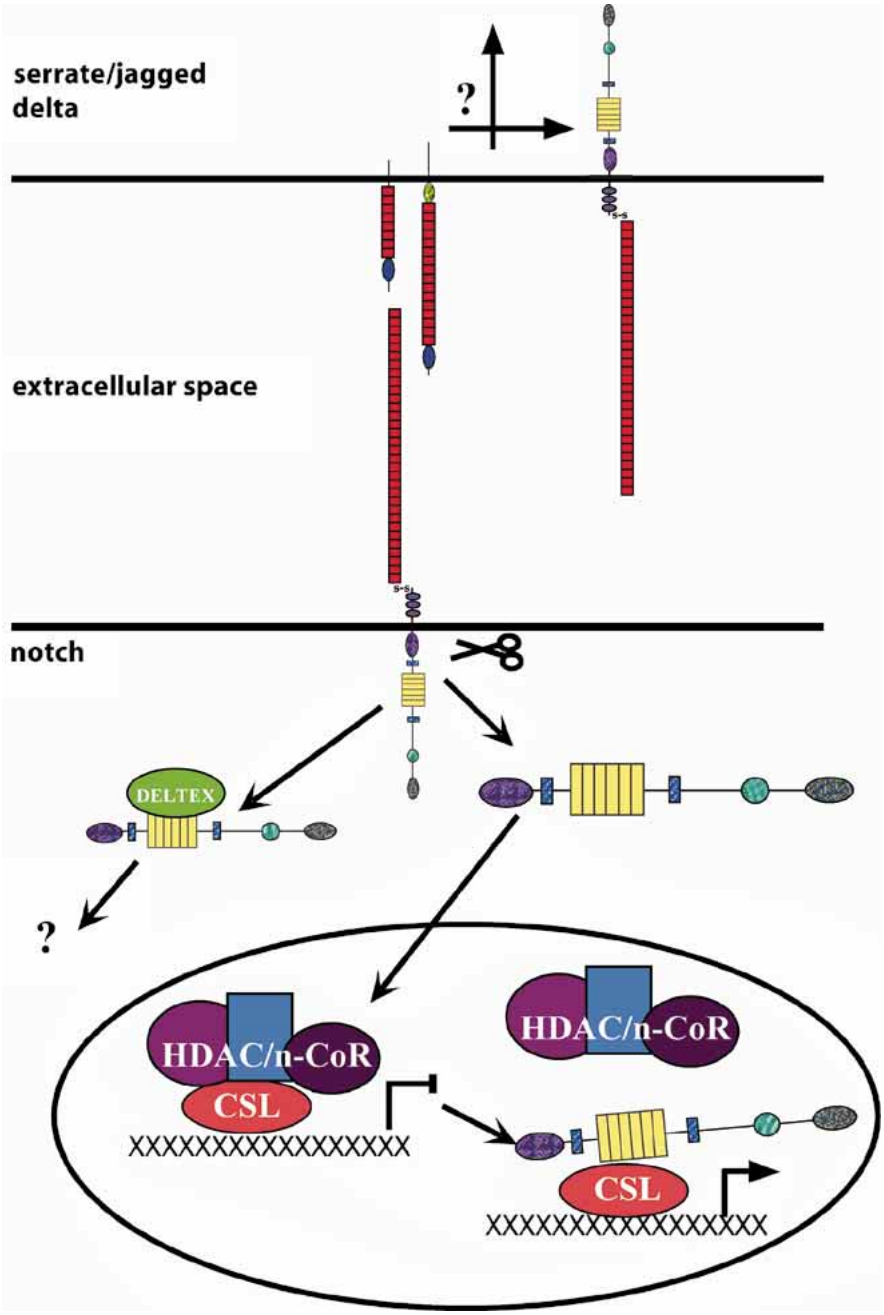
Signaling mechanisms

The mechanism of signaling downstream of ligand activation remains unclear. Once released from the plasma membrane by ligand activation, → Notch^{IC} (comprising only the intracellular (IC) domain of the protein) moves to the nucleus and participates in the regulation of gene expression, which in part serves to govern cellular processes such as differentiation, proliferation and apoptosis. The major question is, what is the mechanism by which Notch proteins exert the pleiotropic effects that are observed in distinct cellular contexts?

Over the last several years many proteins have been identified that can physically interact with Notch^{IC}. However, a clear picture has not emerged to describe the mechanism of Notch signaling. For simplicity these proteins will not be discussed in this essay as they are discussed in several of the referenced reviews. Rather, this essay will focus on Deltex and CSL, two proteins that appear to play a major role in Notch signaling, as shown by genetic analysis in *D. melanogaster* and *C. elegans*.

Deltex genetically acts as an enhancer of Notch signaling and has been to shown to physically associate with Notch through the ankyrin repeats. Deltex is a cytoplasmic proline-rich protein with no known biochemical function. The significance of the interaction between Notch and Deltex, with respect to the mechanism of Notch signaling, remains unclear. One model suggests that Deltex serves to couple Notch signaling to the inhibition of the → Ras pathway. Increased expression of Deltex in certain T-cell lymphomas underscores a role for Deltex signaling in neoplasia.

CSL is the collective term for the mammalian counterparts to Su(H) and lag. CSL proteins are transcriptional regulatory proteins that repress transcription under non-induced conditions. Repression by CSL is thought to be mediated by a histone deacetylase (HDAC) complex containing n-CoR/Smrt. Upon ligand activation of Notch, Notch^{IC} probably translocates to the nucleus and binds to CSL, thereby displacing the HDAC complex and in turn providing a transcriptional activation function. Recent evidence suggests, however, that there are CSL-indepen-



NOTCH/JAGGED Signaling in Neoplasia. Fig. 2 – Model for Notch Signaling. Notch signaling is thought to occur following the interaction of DSL proteins and Notch proteins on neighboring cells. This interaction triggers a series of proteolytic processing events (indicated by scissors). Once Notch is cleaved, it is released from its membrane tether and translocates to the nucleus. In the nucleus Notchic displaces the HDAC/co-Repressor complex and interacts with the transcription factor CSL, and thereby activates gene expression. Notch^{IC} interacts with a cytoplasmic protein called Deltex indicating a potential cytoplasmic signaling cascade. The current model for Notch signaling does not account for DSL-Notch interactions in the same cell (shown at top, ?). HDAC/n-CoR, histone deacetylase repressor complex. CSL, a transcriptional regulator. '?' indicates unknown function or mechanism.

dent changes in gene expression. Moreover, Notch mutants that are defective in binding to CSL are able to transform cells in culture, indicating that transformation might be through an alternate signaling pathway. However, it is clear that $Notch^{IC}$ must be localized to the nucleus and effect transcription in order to transform cells (5).

Other proteins have been identified that appear genetically to inhibit Notch function. These proteins are Dishevelled (Dsh) and Numb. Dsh is a protein with unknown biochemical function that is a component of the Wnt signal transduction system. Dsh has been shown to physically interact with the carboxy-terminal portion of Notch. Numb also appears to influence Notch signaling. Numb is a cytoplasmic protein that has homology to phosphotyrosine binding (PTB) domains and may provide a link to protein tyrosine kinase signaling cascades. The molecular basis of these interactions and the mechanism of inhibition of Notch signaling is not known.

Clinical aspects

T-Cell Leukemia.

The human homologue of *Notch1* (*TAN1*) was cloned from a T-cell acute lymphoblastic leukemia (T-ALL) that harbored the chromosomal translocation t(7;9)(q34;q34.3). This translocation joins a portion of *NOTCH1/TAN1* to the T-cell receptor β locus. This translocation generates aberrant Notch proteins that lack most of the extracellular domain and are not tethered to the plasma membrane. As described above, these forms of Notch are thought to be constitutively active.

Cervical cancer.

In situ expression studies on normal and neoplastic cervical epithelium provide evidence for a role for aberrant Notch activity in the development of → cervical cancer. In normal cervical tissue, expression of Notch is limited to the basal layer of the stratified epithelium. However, in dysplastic tissues, both Notch and ligand expression is increased compared to normal tissues, indicating that inappropriate activation of Notch signaling might play a role in the generation of cervical neoplasia. It is interesting to note that a large percentage of cervical neoplasms are positive for → Human Papillomavirus (HPV) E6 and E7. $Notch^{IC}$ cooperates with the → adenovirus oncoprotein E1A to transform baby rat kidney epithelial cells. Since E6 and E7 have some analogous roles to E1A, the possibility exists that in the transformation of cervical epithelium, infection by HPV cooperates with somatic mutations in genes of the Notch pathway to drive tumorigenesis.

B-Cell leukemia.

→ Epstein-Barr Virus (EBV) is the etiologic agent of Burkitt lymphoma [→ malignant lymphoma, hallmarks and concepts]. The latent viral protein EBNA-2 is a transcriptional activator that functions by binding to host cell CSL proteins and displacing the HDAC repressor complex in an analogous manner to the model for $Notch^{IC}$. Therefore, in transformation of B-cells by EBV infection, EBNA2 may provide a function similar to $Notch^{IC}$ by usurping CSL proteins.

Mouse mammary tumor virus (MMTV) induced mammary carcinoma.

Infection of mice with MMTV has been used as an insertional mutagen to identify genes that contribute to the generation of mammary carcinoma. Among the genes affected by such mutations is *Int-3*, a *Notch* gene family member now termed *Notch4*. The mutations result in aberrant expression of truncated Int-3 proteins, comprising only the intracellular portion of the molecule.

Feline leukemia virus (FeLV) induced T-cell leukemia.

Infection of cats with replication competent feline leukemia virus (FeLV) yielded T-cell leukemia that harbored recombinant FeLV, which had transduced a portion of the feline *Notch2* gene. The transduced gene encodes a Notch2 protein analogous to that expressed in human T-ALL.

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NPC

Definition

→ Nasopharyngeal carcinoma.

NQO

Definition

→ Quinone reductase.

NT-3

Definition

Neurotrophin-3 [→ neurotrophin].

NTRK1

Definition

→ TRK-A.

NTRK2

Definition

→ TRK-B.

NTRK3

Definition

→ TRK-C.

Nuclear Envelope Breakdown

Definition

The nuclear envelope consists of two concentric lipid layers fused to one another that are punctured by nuclear pore complexes, which allow passive and regulated transport of proteins and RNA between the nucleus and cytoplasm. Underlying the inner lipid bilayer is the nuclear lamina, composed predominantly of polymerised intermediate filaments known as nuclear lamins. Nuclear envelope breakdown (NEBD) requires vesicularisation of the lipid bilayers, depolymerisation of the nuclear lamins to disassemble the nuclear lamina, and the falling apart of nuclear pore complexes. The best understood of these three processes, nuclear lamina disassembly, is caused by a multisite phosphorylation of the nuclear lamins. The number of kinases directly involved in depolymerisation of nuclear lamins is unclear, however at least cyclin B1/CDK1 and → protein kinase C are likely to be involved.

Nuclear Export Signal

Definition

The nuclear export signal is a short amino acid sequence (LxxLxϕ, ϕ being L, I, M or V; → amino acid single letter code) located in a protein and sufficient to promote exit from the nucleus.

Nuclear Matrix

Definition

The nuclear matrix is a network of fibers surrounding and penetrating the nucleus.

Nucleolar Protein-3

Definition

Nucleolar protein-3 is an → apoptosis repressor, an inhibitor protein of apoptosis that interacts selectively with → caspases.

Nucleolus

Definition

The nucleolus is a membraneless organelle in the nucleus where rRNA is transcribed and ribosomal subunits are assembled.

Nucleoside Diphosphate Kinase

Definition

Nucleoside diphosphate kinase is an enzymatic activity in which the terminal (γ) phosphate is removed from a nucleoside triphosphate and transferred to a nucleoside diphosphate.

Nucleosome

Definition

The nucleosome is the basic structural subunit of chromatin, consisting of about 200 bp of DNA and an octamer of histone proteins. It is a chromatin particle that by electron microscopy has a beaded appearance. It is connected by thin fibers to the neighbouring nucleosomes, like 'beads on a string', and consists of approximately 200 bp of DNA packaged by an octamer of histones (H2A, H2B, H3 and H4).

Nucleotide Excision Repair

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Definition

Nucleotide excision repair (NER) is a complex biochemical mechanism that recognizes alterations in the chemical structure of DNA due to base modification by physical agents (most notably ultraviolet radiation) or endogenous or exogenous chemicals. After recognition of the alteration in the structure of DNA, the damage is removed by excision of the oligonucleotide that contains the damage. The resulting gap is filled in by DNA polymerase using the complementary (undamaged) strand as template and finally ligated. The process is essentially error-free. There are at least 27 polypeptides required to complete the recognition, excision and gap-filling phases.

Characteristics

NER was initially characterized in *E. coli* in the 1960s and was identified by the ability of wild type bacteria to remove ultraviolet light (UV)-induced photoproducts from large molecular weight DNA. The kinetics of this process correlated well with the resumption of DNA replication and led to the idea that removal of such

damage was directly related to the recovery of DNA synthesis and improved survival. Since that time, NER processes in mammalian cells have been documented by a great number of cellular and molecular biology studies. The dissection of the pathway in eukaryotic cells has been greatly facilitated by the availability of mutant cell lines, many of which are derived from individuals who are defective in some aspect of NER and are consequently cancer-prone. During the 1990s, all of the central factors involved in NER were cloned, and the basic 'cut and refill' reactions have been reconstituted *in vitro* from purified components. Despite these advances, however, many important aspects of the regulation of NER and its integration with other basic cell biology processes remain to be elucidated.

Cellular & Molecular Regulation

NER is a versatile and sophisticated pathway for the removal of DNA damage induced by a variety of environmental and endogenous factors. One of the most relevant, and best studied, DNA damaging agents is UV light, which induces dimerization of adjacent pyrimidine bases. The major products of this photochemical reaction (hence the term 'photoproducts') are cyclobutane pyrimidine dimers (CPD) and 6-4 pyrimidine-pyrimidones (6-4s). Both lesions induce structural distortions in DNA, and if left unrepaired, can cause errors in replication that can lead to mutations. In addition to photoproducts, a wide variety of bulky chemical adducts are removed by NER, and the common denominator amongst these diverse lesions is distortion of the DNA helix, which in turn interferes with basic nuclear transactions such as transcription and DNA replication. These lesions present such a potential threat to the integrity of the cell that the basic mechanism for removing them has diverged surprisingly little throughout evolution. In all eukaryotic cells that have been studied, two modes of NER have been identified. These are global genomic repair (GGR) and transcription-coupled repair (TCR). These mechanisms are distinguished primarily by the way in which damage is detected. After the damage is detected, the pathway for excision, removal and gap filling is common to both GGR and TCR.

In the following discussion, many of the proteins were identified by cloning the genes responsible for the NER-defective syndrome xeroderma pigmentosum (XP; see Clinical Relevance). There are seven distinct groups of XP termed XP-A through XP-G, and the general molecular defect associated with each group is outlined in the table.

- *Global Genome Repair.* The global genome is defined as that part of the genome that is not transcribed, which is estimated to be 95% of the human genome. The XPC protein is specifically required for repair of lesions in the global genome, and cells with nonfunctional XPC are completely deficient in such repair. XP-C cells, however, are capable of removing lesions from the transcribed strand of active genes. The exact role of XPC in GGR has been obscure, but recent advances have elucidated its function more fully. XPC is a 125 kD protein that acts as a heterodimer with one of two homologs of the yeast protein Rad23 (these homologs are termed hHR23A and hHR23B). It is now thought that the XPC-hHR23B (or A) complex acts at the very earliest stages of NER in the global genome in a damage recognition fashion. In ways that are not yet understood, this complex senses and binds to the damaged DNA. In this process, it locally distorts the double helix and then recruits the core repair apparatus. The rate of GGR is strongly dependent on the type of lesion, which presumably reflects the degree of helical distortion and the affinity of the XPC heterodimer. The XPC complex has been shown to have high affinity for DNA that contains a 6-4 photoproduct, and these lesions are repaired very rapidly. Recognition of CPD in the global genome probably requires the XPE protein; the XPE-CPD complex is then recognized by the XPC complex. Due to this two-stage mechanism, repair of CPD in the global genome is much slower than that of 6-4s.
- *Transcription-Coupled Repair.* Elongating RNA polymerase II stalls at a wide variety

Nucleotide Excision Repair. Table – Molecular defects in XP complementation groups. [a]Cells from complementation groups signified with a hyphen (XP-A); the corresponding proteins are not hyphenated (XPA). [b]Patients are very severely affected and may have developmental defects.
[c]Proteins also found in transcription factor TFIIH. Patients are likely to have developmental abnormalities.
[d]Defective transcription-coupled repair of oxidative damage suggests a second, non-endonucleolytic function for this protein. These patients may also have developmental defects, possibly due to reduced repair of oxidative damage.

protein[a]	defect
XPA[b]	no lesion recognition
XPB[c]	reduced helicase activity that unwinds DNA 3′ to 5′ of the lesion
XPC	reduced recognition of lesions in global genome, normal transcription-coupled repair
XPD[c]	reduced helicase activity that unwinds DNA 5′ to 3′ of the lesion
XPE	? mutant UV-DNA damage binding protein
XPF	mutant endonuclease that cuts 5′ to the lesion
XPG[d]	mutant endonuclease that cuts 3′ to the lesion

of lesions and must act as a damage sensor, although the mechanism by which it does so remains obscure. In addition to the stalled polymerase, at least two other proteins are required for TCR. These are termed CSA and CSB, and are thought to act in a structural rather than in a catalytic fashion. Depending on the type of lesion, damage in the transcribed strand of an active gene can be repaired either by TCR or GGR. For instance, UV-induced 6-4s in active genes is primarily repaired by GGR because of the very high affinity of the XPC complex for these lesions. UV-induced CPD on the other hand, is repaired by TCR. TCR is highly conserved in both prokaryotes and eukaryotes, presumably because blocked RNA polymerases seriously interfere with cellular metabolism. TCR has also been shown to specifically reduce the frequency of mutations in active genes; mutations in such genes would also be expected to have a deleterious effect on cellular biochemistry and cell-cycle control.

- *Excision and Gap Filling.* After the damage is initially sensed, the XPA protein plays a critical early role in NER. XPA is a DNA binding protein with high affinity for damaged DNA, and nonfunctional XPA leads to a complete loss of NER capacity. The protein was long thought to be the initial sensor of DNA damage, but more recent data implicates the XPC complex in this process. Current thinking is that XPA verifies that the DNA is damaged and acts to position the repair machinery correctly around the lesion. The human single strand binding protein, RPA, acts in concert with XPA and probably helps to maintain the open conformation. Through its extensive network of protein binding sites, XPA recruits the basal transcription factor TFIIH to the lesion. TFIIH is composed of 5 polypeptides, including the XPB and XPD helicases, which unwind the DNA on both sides of the lesion. Two structure-specific endonucleases, XPF-ERCC1 and XPG cut the DNA on either side of the lesion to release an oligonucleotide 24-32 bases in length that contains the damage. The 3′ terminus acts as a primer, and the gap is filled in by either DNA polymerase d or e, together with cofactors; the relative contribution of each polymerase remains to be determined. The final step in the process is ligation of the 5′ terminus, which is probably carried out by DNA ligase I. Since the gap to be filled is short, and the fidelity of the polymerase complex is very high, NER is considered to be an error-free process.

Clinical Relevance

The central role played by DNA repair in the prevention of cancer was first appreciated in 1968 when Cleaver recognized that patients with the skin cancer-prone disorder xeroderma pigmentosum (XP) had a defect in the repair of UV-induced damage (4). XP patients have a greater than 1000-fold increase in the incidence of sunlight-associated skin cancer, and if they are exposed to any sunlight they usually succumb to metastatic skin cancer. The cells of XP patients are also defective in the repair of bulky chemical adducts, such as those induced by cigarette smoke and endogenous metabolic processes. Evidence is emerging that XP patients who live longer, because they have compulsively avoided the sun, have an increased risk of internal cancers, particularly if they smoke. Many XP patients also undergo progressive neurological deterioration, and this is thought to be due to the accumulation of damage induced by reactive oxygen species. Repair of such damage is thought to require some of the NER proteins acting in pathways that are independent of NER. In addition, patients in XP groups that have a molecular defect that affects transcription in addition to NER (groups B and D), are likely to have developmental abnormalities that are presumably related to subtle defects in transcription.

There are two additional syndromes that manifest defects in excision repair but are curiously not cancer-prone. These are Cockaynes syndrome (CS) and trichothiodystrophy (TTD). CS patients exhibit severe mental retardation and varying degrees of photosensitivity. Cells from these patients are defective in TCR because they lack a protein that couples the blocked RNA polymerase to the repair machinery. It has been postulated that the reason these patients do not develop skin cancer is the increased level of apoptosis induced in these cells following UV. The exaggerated apoptotic response may be due to the persistently blocked RNA polymerase II molecules, which cause accumulation of p53. TTD patients exhibit many of the same symptoms and signs of CS patients, but the molecular defect affects the same protein responsible for the XPD phenotype, namely the XPD helicase in TFIIH. The reason that one syndrome is cancer-prone while the other is not is puzzling, but recent studies have shown that mutations in the XPD gene occur in different regions in TTD and XP-D cells. It has been postulated that the mutations in TTD patients differentially affect transcription to a greater degree, and that subtle defects in transcription prevent cellular transformation. It has also been shown that TTD cells are quite proficient in repairing 6-4s while XP-D cells are not. The development of cancer in the latter cells may be related to the greater mutagenic potential of 6-4 photoproducts.

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NWTSG

Definition

National Wilms Tumour Study Group (NWTSG) is an organisation that has co-ordinated clinical trials for the treatment of Wilms tumour in North America since the early 1970s. They have data on epidemiological, genetic and clinical features together with treatment outcome on over 6,000 children treated for Wilms tumour.

O

OCT

Definition

→ Optical coherence tomography.

Octreotide

Definition

Octreotide is a chemical compound used in nuclear medicine for localization of → gastrinoma.

ODC

Definition

→ Ornithine decarboxylase.

Odynophagia

Definition

Odynophagia is pain during swallowing.

Oligodendrocytoma

Definition

Oligodendrocytoma is a tumor apparently derived from oligodendrocytic glia; → brain tumors.

Omeprazole

Definition

Omeprazole is an antisecretory agent, which is a proton pump inhibitor that is commonly used for suppression of gastric acid output in patients with → Zollinger-Ellison syndrome; → gastrinoma.

Oncogene

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Synonyms

- transforming gene

Definition

An oncogene is any gene that has the ability to stimulate cellular growth. Oncogene products can transform eukaryotic cells such that they grow in a way analogous to tumor cells. The definition was originally applied to the transforming genes acquired by RNA tumorviruses through → transduction of oncogenes; today the term is used rather broadly.

Characteristics

Oncogenes were originally isolated from → RNA tumor viruses, where they are responsible for the ability of rapid tumor induction after infection of an animal host. In the viral genome the on-

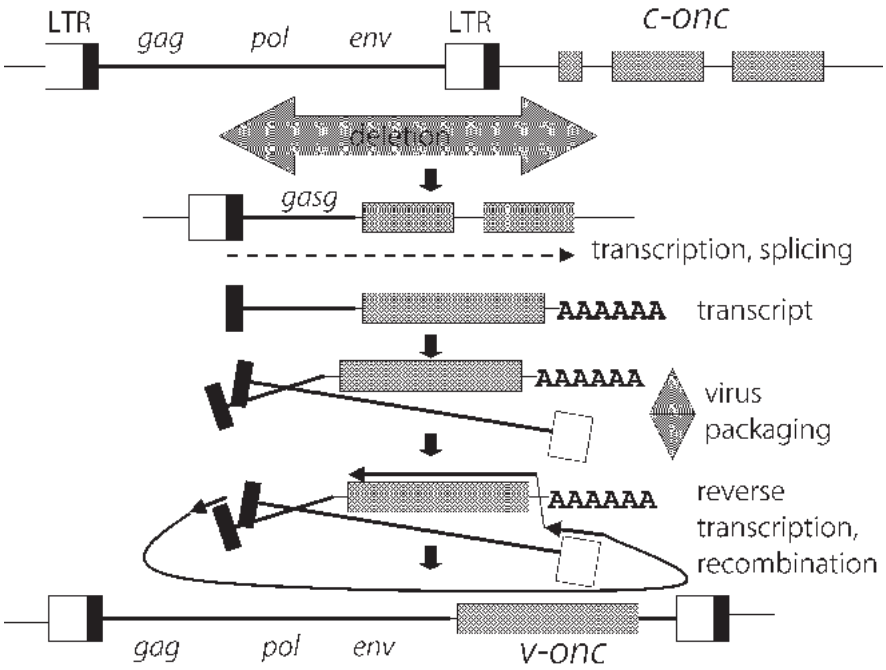
cogene was referred to as a viral oncogene or *v-onc*.

It was soon established that the *v-onc*s are actually derived from the genome of the host cell. They are captured by the virus after infection of the cell by a process called transduction (Fig. 1; → transduction of oncogenes). Transduction appears in a wide range of animal species from chickens to monkeys, it has not been observed in humans. The cellular counterparts, from which the *v-onc*s are derived, are referred to as proto-oncogene, or *c-onc*. Proto-oncogenes are normal constituents of the cellular genome and are highly conserved among all eukaryotic organisms.

This original rigid definition has softened in subsequent years. Broadly speaking, the term oncogene now includes any gene that has a growth stimulatory effect on cells, with the result of:

- conferring sustained cellular multiplication
- advancement of → cell cycle progression
- decreased requirement for → growth factors
- focus formation under conditions of cell culture
- ability of cells to grow under more restricted experimental conditions, such as in soft agar
- tumorigenic conversion, such as in experimental animals
- conversion of cells to form tumors that undergo → metastasis
- escape from → apoptosis.

The precursors of oncogenes (proto-oncogenes) are present in their normal structure



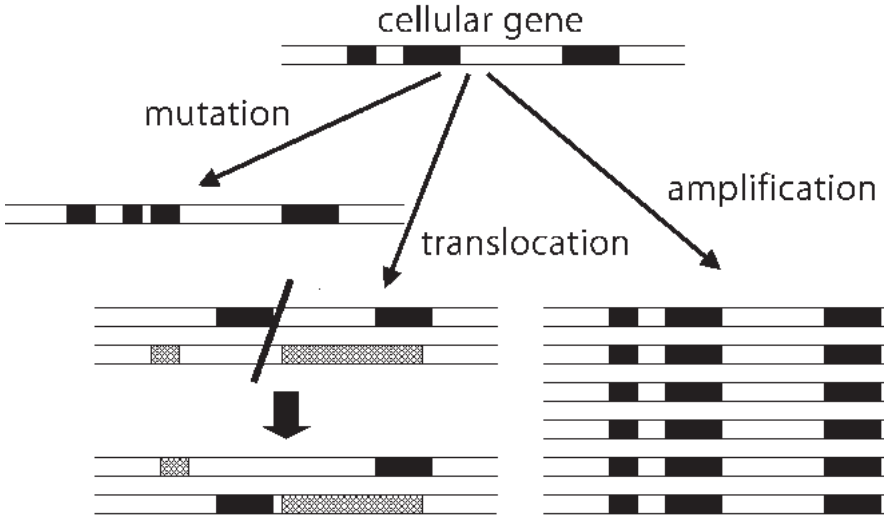
Oncogene. Fig. 1 – Model for the transduction by retroviruses. The process begins when the provirus of a retrovirus integrates into the vicinity of a cellular oncogene. The characteristic 'long terminal repeats' (LTR) are drawn as black and white boxes. A deletion enables the *c-onc* and the provirus to fuse into a single genetic unit, one of the proviral LTR plus regions of both the provirus and the *c-onc* , are deleted. Transcription from the hybrid unit generates a hybrid RNA, introns have been removed by splicing. This RNA can be packaged, together with a wild type retroviral RNA, to form a virion (retroviruses have two RNA molecules). Infection of a cell initiates reverse transcription of viral RNA. Beginning at the 5′-end, the reverse transcriptase can jump to the 3′-end to continue chain propagation along the retroviral genome. Reverse transcriptase can also jump to the hybrid RNA and generate a new retrovirus genome, containing the *c-onc*.

and expression activity in all eukaryotic cells, they represent potential 'enemies within', but perform normal, usually vital, functions. Their oncogenic potential can be activated by any one of the following mechanisms (Fig. 2):

- expression deregulation, by which the normal expression pattern is altered by a variety of mechanisms
- mutation within the gene, which results in an abnormal protein that has a different biological activity (e.g. → RAS)

Oncogene. Table – Retroviral genes in different animal species and oncogenes.

virus	name	species	tumor	oncogene
Rous sarcoma	RSV	chicken	sarcoma	*src*
Harvey murine sarcoma	Ha-MuSV	rat	sarcoma	*H-ras*
Kirsten murine sarcoma	Ki-MuSV	rat	erythroleukemia	*K-ras*
Moloney murine sarcoma	Mo-MuSV	mouse	sarcoma	*mos*
FBJ murine osteosarcoma	FBJ-MuSV	mouse	chondrosarcoma	*fos*
simian sarcoma	SSV	monkey	sarcoma	*sis*
feline sarcoma	Pi-FeSV	cat	sarcoma	*sis*
feline sarcoma	SM-FeSV	cat	fibrosarcoma	*fms*
feline sarcoma	ST-FeSV	cat	fibrosarcoma	*fes*
avian sarcoma	ASV-17	chicken	fibrosarcoma	*jun*
Fujinami sarcoma	FuSV	chicken	sarcoma	*fps*
avian myelocytomatosis	MC29	chicken	carcinoma, sarcoma & myelocytoma	*myc*
Abelson leukemia	MuLV	mouse	B cell lymphoma	*abl*
reticuloendotheliosis	REV-T	turkey	lymphatic leukemia	rel
avian erythroblastosis	AEV	chicken	erythroleukemia & fibrosarcoma	*erbB* (*erbA*?)
avian myeloblastosis	AMV	chicken	myeloblastic leukemia	*myb*



Oncogene. Fig. 2 – Molecular pathways for activating the oncogenic potential of cellular oncogenes. Translocation defines exchange of genetic material between two non-homologous chromosomes.

- translocation, by which DNA of two genes on two different chromosomes can recombine with the result of a fusion gene and a fusion protein (e.g. *BCR/ABL* [→ BCR-ABL1]) or a deregulated gene expression, when gene expression signals are replaced by other DNA sequences
- → amplification, where the number of gene copies multiplies, with the consequence of enhanced gene expression (e.g. → *MYCN*)

Oncogene cooperation

Experimental approaches have shown that alteration of a single oncogene is insufficient to achieve full tumorigenic conversion of a normal cell. Only when at least two altered oncogenes are introduced can a normal cell assume a tumorigenic phenotype. Oncogene cooperation is well in line with the multiple genetic changes that a tumor acquires during its evolution to metastatic disease (→ multistep development). Oncogenes can also cooperate with → tumor suppressor genes.

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Oncogene Transduction

Definition

→ Transduction of oncogenes.

Oncoprotein

Definition

An oncoprotein is a protein, usually encoded in genes, possessing one or more mutations that can induce malignant transformation of target cells.

Optical Coherence Tomography

Definition

Optical coherence tomography (OCT) is an optical imaging modality of superficial tissue layers at almost microscopic resolution; → laser diagnostics.

Optical Mammography

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Synonyms

- Light tomography
- breast transillumination

Definition

Optical mammography is an imaging modality using transilluminated light in the visible and near infra-red wavelength range, which is used to detect or to differentiate neoplastic lesions of the breast.

Description

Many attempts to detect breast cancer by transillumination have failed in the past due to the strongly diffusive nature of light transport in tissues (1). Lasers might initiate a technical revolution in this field. The use of several wavelengths may enable differential spectroscopy, leading to functional and tissue composition information. High frequency intensity modulation or pulsed laser systems provide the additional information of the length of the photon path in a tissue, eventually enabling differentiation between absorption and scattering properties of breast tissue or even 3-dimensional reconstruction of optical properties.

Two-frequency domain instruments have been clinically evaluated in Germany. The

Fig. demonstrates the Carl Zeiss instrument 'LIMA' that uses two laser diodes at 690 nm and 815 nm, and a modulation frequency of 110 Mhz. A clear improvement in image quality over conventional imaging was demonstrated (2). Furthermore, the approach enabled the characterization of individual tumors with regard to size, position and tissue-optical properties. However, no significant improvement in sensitivity appears to result from the frequency domain approach.

Actual research focuses on time domain instruments using laser pulses in the picosecond range. Entire flight time distributions are registered. By using only the first photons leaving the tissue, contrasted pictures of differentially absorbing tissue formations may become possible. However, the probability for a photon to pass a 4-7 cm layer of breast tissue without any scattering (ballistic photon) is zero. Thus, image contrast will always be inferior to X-ray images. On the other hand, additional and quantitative information can be obtained by the use of different wavelengths such as tissue oxygenation parameters or tissue composition information, which may compensate for the lack of contrast and detail resolution. Such time domain instruments are entering initial clinical testing (3). Furthermore, for the purpose of enhanced specificity in detectable lesions, contrast agents may prove useful in the future (4).

Optical Mammography. Fig. – Prototype of a Frequency Domain optical mammograph. The instrument uses two intensity modulated (110 MHz) laser beams at wavelengths of 690 and 810 nm to scan a breast. Images are calculated from amplitude and phase data.

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Orchiectomy, bilateral

Definition

Bilateral orchiectomy is the surgical removal of both testicles or only the hormone-secreting portion of both testicles.

Organotypic Cultures

Definition

Organotypic cultures are the growth of epithelial cells (keratinocytes) embedded in a collagen

matrix, first submerged within culture medium. Once the matrix is lifted to float on the liquid ('raft'), the cells become exposed to the air pressure and show remarkable similarity in differentiation (multi-layered morphology, expression of keratins, etc.) to epithelial tissues.

Ornithine Decarboxylase

Definition

Ornithine decarboxylase 1 (ec 4.1.1.17) is a protein of 461 amino acids and 51 kD. The human ornithine decarboxylase 1 (ODC1) gene locus maps at 2p25 and the mouse odc gene locus at chromosome 12 (6.00 cM). Odc catalyses the first step and also the rate-limiting step in the pathway of polyamine biosynthesis. It is the rate-limiting enzyme in the biosynthesis of putrescine and polyamines, spermine and spermidine. ODC is activated by tumor promoters and activation appears to stimulate cellular growth but not cell division.

Osteoma

Definition

Osteoma is a benign, local growth usually confined to the facial skeleton of FAP patients, in particular in mandibular and maxillary bones. It is often visible by X-ray, more seldom as external swellings, and is a good diagnostic predictors for FAP. → APC gene in Familial Adenomatous Polyposis.

Ovarian Cancer

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Definition

A heterogeneous group of malignant tumors derived from the ovary (there are also a wide assortment of benign tumors derived from the ovary). Approximately 90% of malignant ovarian tumors originate from the ovarian surface celomic epithelium and are therefore designated epithelial ovarian cancer (EOC). Germ cell tumors and stromal tumors account for the remaining cases.

EOC can be divided according to histological appearance:

- serous, the most common type (composed of epithelium resembling that of fallopian tubes)
- endometrioid (composed of epithelium resembling that of the endometrium)
- clear cell (composed of clear cell epithelium and resembling gestational endometrium)
- transitional cell tumors (composed of epithelium resembling urothelial cells)
- mucinous (composed of epithelium resembling that of the endocervix)
- squamous cell (squamous epithelial cells)
- mixed epithelial tumor (mixture of two or more of the above subtypes)
- undifferentiated (no recognizable differentiation features)

Ovarian cancer is staged according to the following International Federation of Gynecology and Obstetrics (→ FIGO) guidelines. Grading of EOC is based on degree of differentiation, cytologic → atypia and mitotic index.

1. Stage I: restricted to the ovaries.
2. Stage II: involvement of ovaries and pelvic extension.
3. Stage III: involvement of ovaries with peritoneal implants outside the pelvis.

4. Stage IV: involvement of ovaries with distant metastasis, such as liver, lung or brain.

Characteristics

In westernized countries, ovarian cancer is the sixth most common cancer in women. Over half of the women diagnosed with ovarian cancer are over 65 years of age. The age-adjusted incidence for ovarian cancer is approximately 15 per 100,000 and the total number of cases is expected to increase as the overall population ages. Overall, ovarian cancer prognosis is poor. In spite of the recent introduction of aggressive treatments, five year survival rates for patients with advanced ovarian cancer (stage III and IV) has remained low and ranges between 5 and 30%. However, the outcome is much more favorable for stage I patients where 5-year survival can reach 90 – 95%. Unfortunately, because of lack of symptoms, only 25% of the women with ovarian cancer are diagnosed with stage I disease. These facts make ovarian cancer a disease for which early detection represents an intervention of choice in reducing morbidity. Several putative ovarian cancer serological markers have been identified, including → CA125, → TATI, → CEA and → CA19-9. Of those, CA125 has proven to be the most clinically useful. Unfortunately, studies have exhibited mixed results, with some studies detecting only 23% of stage I ovarian carcinoma with CA125. CA125 may be useful when used in combination with pelvic ultrasound assessment, and CA125 has been used in monitoring recurrence in patients with CA125-positive tumors. Overall, CA125 lacks the specificity and sensitivity required for the screening of the general population.

The etiology of ovarian cancer is not well understood but the following risk factors have been identified:

- age: half the cases occur in women of 65 years or older
- menstrual history: ovarian cancer risk increases with increasing numbers of menstrual cycles
- birth control pills: the use of which, for at least five years, lowers the risk of ovarian cancer
- pregnancy and breastfeeding: lowers the risk of ovarian cancer
- family history

Typical treatment for ovarian cancer is surgery followed by chemotherapy. The exact details of treatment depend on the tumor type, grade and stage, the age, as well as the general health of the patient.

In spite of recent advances in the field of cancer genetics and molecular biology, little is known about the mechanisms of ovarian tumorigenesis. Chromosome abnormalities are frequent in ovarian cancer and allelic losses have been observed in chromosomes 4p, 6p, 6q, 7p, 7q, 8p, 8q, 9p, 11q, 12p, 12q, 13q, 16p, 16q, 17p, 17q, 19p, 19q, 22q and Xq. These observations suggest the presence of several tumor suppressor genes important in ovarian cancer, but very few have been unambiguously identified. The tumor suppressor p53 is inactivated in a large number of ovarian cancers. Although the tumor suppressor genes → *BRCA1* and → *BRCA2* have been implicated in familial breast and ovarian cancer syndromes, the vast majority of ovarian cancers are sporadic and may have a different natural history.

The *K*-→ *Ras*, → *Her-2/neu* and c-→ myc oncogenes have all been implicated in EOC. The frequencies of alteration of these different → oncogenes varies according to the subtype of ovarian cancer. No clear association has been reliably observed between the activation of these oncogenes and survival. Indeed, stage and grade have been the only factors consistently shown to predict outcome of ovarian cancer.

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Oxidative DNA Damage

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Definition

Oxidative DNA damage represents free radical damage to DNA. Oxidation essentially involves the addition of oxygen or removal of hydrogen atoms from a molecule. Oxidation of DNA may simply result in a small change to one of the bases, or a deoxyribose in the backbone of the molecule may be altered to such an extent that the continuity of the backbone is broken. Single-strand breaks are more common than double-strand breaks.

Characteristics

DNA is thought of as a very stable molecule and yet it readily undergoes damage, by a variety of agents that are both endogenous and exogenous in origin. Ionising radiation (e.g. X-rays), ultraviolet radiation and various chemicals, including some present in tobacco smoke (→ tobacco carcinogenesis), cause the release of free radicals, and if DNA is not protected, oxidative damage can occur. Free radicals (→ reactive oxygen species) also arise within the cells of the body, being released as a minor product during the cycle of oxidation of carbohydrates in the mitochondria. The hydroxyl radical, *OH, is particularly reactive with DNA.

In addition to single- and double-strand breaks, many different oxidation products of the four bases (→ adducts to DNA) have been identified in DNA treated with radiation or other free radical-generating chemicals. Some of these modified bases are potentially capable of giving rise to a → mutation. For instance, → 8-oxoguanine, if present in the DNA when it is replicating, may lead to the incorporation of adenine rather than cytosine into the newly synthesised complementary strand, thus changing the DNA sequence.

Oxidative DNA Damage. Fig. – The comet assay. Cells are embedded in agarose on a microscope slide, lysed, and electrophoresed at high pH. This view is of the DNA from one cell, stained with DAPI and visualised by fluorescence microscopy. The percentage of DNA fluorescence in the tail of the 'comet' is proportional to the frequency of breaks - such as breaks introduced at 8-oxoguanine sites by the enzyme FPG.

Some oxidation is detectable in the DNA of normal human cells. It is usually measured by gas chromatography (→ GC-MS) or high performance liquid chromatography (→ HPLC). Normally for GC-MS, the DNA is acid-hydrolysed to bases (guanine etc.), while for HPLC the DNA is enzymically hydrolysed to nucleosides (deoxyguanosine (dG) etc.). Both of these methods have given relatively high values for the extent of conversion of guanine to 8-oxoguanine, with up to 300 or more 8-oxo-guanines for every 10^6 unaltered guanines. However, recently it has been recognised that guanine tends to oxidise during preparation of samples for analysis and so the early estimates of 8-oxoguanine are considered to be excessive. Values as low as 3 per 10^6 have been reported from HPLC analysis of anaerobically prepared samples.

There is another approach to measuring oxidised bases using bacterial repair endonucleases, which recognise the damage and make a corresponding break in the DNA.

The enzyme FPG (formamidopyrimidine glycosylase) recognises 8-oxoguanine. DNA breaks can then be measured in various ways, including the → comet assay. This approach gives values for 8-oxoguanine that are even lower, with around 0.5 per 10^6 guanines.

The extent of background DNA oxidation in normal cells remains an important question. Methodological problems must be solved before a consensus can be reached.

Cellular & Molecular Regulation

Measurement is made of the steady state level of DNA damage, which is a dynamic equilibrium between input of damage and its repair. In the case of oxidative damage, input is controlled by → antioxidant defences. The tripeptide glutathione is present at high concentration in the nucleus and 'mops up' free radicals before they can cause damage. Superoxide dismutase and catalase are enzymes that convert superoxide and hydrogen peroxide (two reactive forms of oxygen) ultimately to non-harmful products. Other enzymes combine various organic free radicals with glutathione, thus inactivating them. Fruits, vegetables and grains in the diet are a source of antioxidants, including vitamin C, vitamin E, carotenoids and flavonoids. These natural chemicals have the ability to quench or scavenge free radicals; whether they act as antioxidants *in vivo* depends on whether they are taken up from the gut in sufficient amounts, and has been the subject of recent human intervention trials. In general, it is possible to detect a significant decrease in the steady state level of base oxidation (and/or an increased resistance to *in vitro* oxidation of DNA) in white blood cells of volunteers taking individual antioxidant supplements or antioxidant-rich foods, ranging from fried onions to kiwifruit.

However effective the antioxidant defences, some DNA oxidation does occur. The turnover of this damage is achieved by → repair of DNA. Small base damage, which includes base oxidation, is repaired primarily by base excision repair. Here, the damaged base is removed, followed by the base-less sugar-phosphate residue and perhaps a few neighbouring nucleotides. A small repair patch of new nucleotide(s) is inserted and ligated. Single-strand break repair is accomplished by ligation.

As well as being present in cellular DNA, 8-oxodG is detectable in urine as free nucleoside. The idea that urinary 8-oxodG represents the accumulated product of DNA repair in all the cells of the body is attractive but flawed; base excision repair releases the base not the nucleoside. Even if this 8-oxodG originates in the oxidation of broken down DNA from dead cells passing through the kidneys, it still reflects oxidative stress, and it has given useful information about oxidative stress related to exercise, smoking and nutrition. Most impressively, consumption of 300g a day of brussels sprouts led to a decrease of 28% in urinary 8-oxodG concentration.

Clinical Relevance

It is commonly stated that oxidative DNA damage is a significant cause of cancer, and that fruits and vegetables protect against cancer because the antioxidants they contain decrease the amount of base oxidation in the cellular DNA. However, there is little evidence that this is true. In two large scale human intervention trials, smokers and/or asbestos workers were given β-carotene (a carotenoid antioxidant) daily for several years. The lung cancer incidence was actually higher in these subjects compared with those taking placebo (or other supplement).

In an experimental animal system, a high level of oxidative DNA damage is not necessarily marked by an elevated cancer risk. In a → knock-out mouse model, which is defective in the murine equivalent of FPG, there is a slight increase in the steady state level of 8-oxoguanine, but no increase in cancer incidence. It seems that there is a back-up repair pathway that deals (more slowly, but adequately) with oxidative damage.

→ Oxidative stress is a feature of many other diseases, including heart disease, diabetes, cataract, and rheumatoid and arthritic conditions. It may be a cause of the clinical condition, or an effect. A common theory of ageing argues that the accumulation of free radical-induced da-

mage to biomolecules - lipids, proteins and nucleic acids - is responsible for the general cellular dysfunction and deterioration of body processes in later life.

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Oxidative Stress

Definition

Oxidative stress can result from.

- diminished antioxidant status, e.g. by lowered antioxidant defence enzymes, depletion of glutathione or dietary antioxidants in a cell or an organism
- increased production of reactive oxygen and nitrogen species, e.g. by exposure to elevated O_2 or due to excessive activation of phagocytic cells in chronic inflammatory diseases.

Persistent oxidative stress is thought to play an etiopathological role in many chronic degenerative diseases including cancer.

8-Oxoguanine

Definition

8-oxoguanine is one of many products of the oxidation of DNA. It differs from guanine by replacement of a hydrogen atom with a hydroxyl group. 8-oxodeoxyguanosine occurs when combined with deoxyribose.

Oxygenation of Tumors

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Synonyms

- tumor oxygen partial pressure distribution
- tumor oxygen tension distribution
- tumor oxygenation status and tumor oxygen level

Definition

Tumor oxygenation, which reflects the distribution of oxygen (O_2) partial pressures (pO_2 values) or O_2 concentrations, results from O_2 availability (O_2 supply) to the tumor tissue, the → diffusional flux of O_2 from the microvessels to the cells and the → respiration rate (O_2 consumption rate) of the parenchymal and stromal cells making up the tissue. O_2 supply is mainly influenced by the efficacy of blood flow and microcirculatory function.

Characteristics

Whereas in normal tissues the O_2 supply meets the metabolic demands, in many solid tumors the respiration rate may outweigh an insufficient O_2 supply and result in the development of tissue areas with very low O_2 levels (→ hypoxia, O_2 depleted tissue areas) or even areas completely lacking O_2 (→ anoxia).

Considerable evidence demonstrates that in most human tumors oxygenation is compromised as compared to normal tissues, which are characterized by 'normal' O_2 partial pressure distributions (→ normoxia). Oxygenation is extremely heterogeneous within an individual tumor (intra-tumor heterogeneity). Furthermore, considerable heterogeneity of oxygen-depleted areas (hypoxic areas) has been shown between tumors of the same clinical size, stage, grade or histological type (inter-tumor heterogeneity). Tumor oxygen supply is not regulated according to the metabolic de-

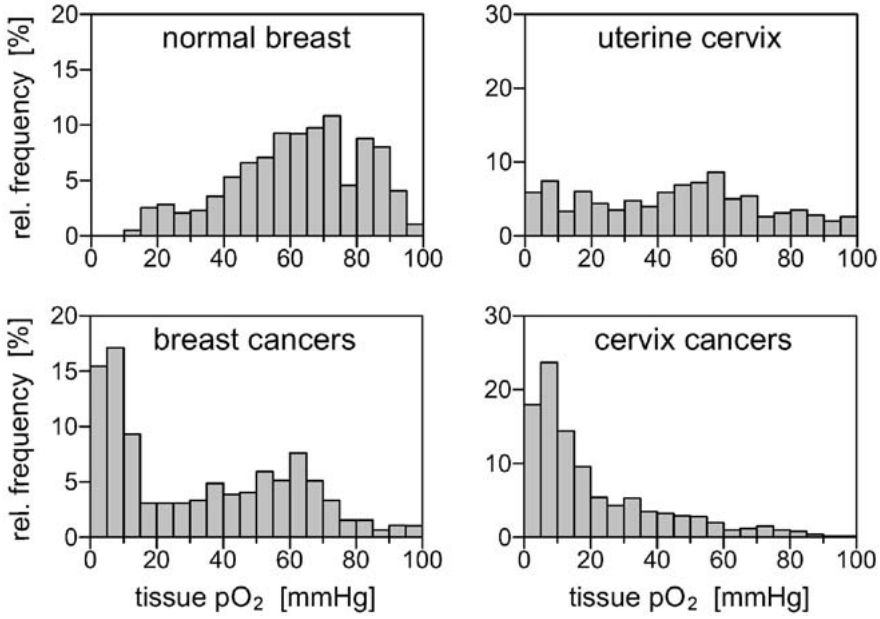
mand, as is the case in the physiological situation. On average, the median pO_2 values in tumors are lower than in normal tissues at the site of tumor growth (Fig.). Tumor oxygenation is independent of clinical size, stage, grade, histology and various oncologic parameters or patient demographics. In some cancer entities, the oxygenation status tends to deteriorate with decreasing blood hemoglobin levels. Metastatic lesions seem to have an oxygenation status comparable to that of the primaries, whereas local recurrences have a poorer oxygenation status than the primary tumors, i.e., the fraction of very low pO_2 values $\leq$ 2.5 mmHg ('hypoxic fraction') is substantially higher in recurrent tumors.

Pathomechanisms of tumor hypoxia

Tumor hypoxia results from an inadequate O_2 delivery to the respiring neoplastic as well as stromal cells. Limited and even abolished O_2 supply is due to severe structural and functional abnormalities of the → microcirculation, as well as due to a deterioration of diffusion geometry. In addition, cancer-related and/or (chemo-) therapy-induced → anemia and carboxyhemoglobin formation (in heavy smokers) can lead to a reduced O_2 content of the blood. As a result, areas with very low (down to zero) O_2 partial pressures exist in solid tumors. These very low pO_2 microregions are heterogeneously distributed within the tumor mass and may be located next to regions with pO_2 values corresponding to the normal tissue from which the tumor has been derived.

Methods of measurement

Assessment of the tumor oxygenation status in experimental tumors and in the clinical setting has been performed using various techniques. So far, the most direct and often used method for description of oxygenation is the polarographic measurement of O_2 partial pressures. With this invasive microtechnique, frequency



Oxygenation of Tumors. Fig. – Oxygenation status of normal tissues (upper panel) and solid tumors (lower panel). Frequency distribution of measured pO_2 values (pO_2 histograms) for normal breast tissue and uterine cervix are compared with the respective cancer tissues (clinical stages IIb – IV). Oxygen partial pressures (pO_2) in the tissue were measured with a computerized polarographic microtechnique which enables direct assessment of the pO_2 data with an O_2 sensitive needle-electrode. Oxygen partial pressures were measured along several electrode tracks in each individual tumor. Pooled data presented here are derived from pretreatment measurements in conscious patients.

distributions (→ histograms) of measured intratumor pO_2 values can be obtained with a relatively high spatial resolution. Other direct procedures include fiber-optic O_2 sensors and electron paramagnetic resonance oximetry. This latter technique is minimally invasive requiring only application of the paramagnetic material.

Measurement of intravascular oxyhemoglobin (HbO_2) saturation is another method that has the potential to allow a characterization of the oxygenation status of human tumors. However, it only provides information related to the vascular compartment and thus the situation in the extravascular space can only be inferred.

Tumor oxygenation can be measured in tomographic images in the clinical setting upon inhalation of radiolabeled ^{15}O-gas in positron emission tomography (PET) studies. However, as with magnetic resonance imaging (MRI) procedures, limitations include poor quantification and poor spatial resolution. The parameter measured with these non-invasive techniques is not directly interpretable as a tumor pO_2 value or O_2 concentration.

Non-invasive methods for detection of tumor hypoxia include the binding and the retaining of radiolabeled → bioreductive drugs, such as fluoromisonidazole (labeled with ^{18}F and detected by positron emission tomography) and iodoazomycin-arabinoside (labeled with ^{123}I and detected with single photon emission computerized tomography, SPECT).

Several techniques for assessment of tumor oxygenation require the analysis of tumor biopsy specimens. Using immunohistochemistry with hypoxia markers (such as misonidazole, pimonidazole, etanidazole or nitroimidazole-theophylline), detailed information concerning hypoxia at the cellular level can be obtained. Disadvantages include the need for injection of a hypoxia marker and possible sampling errors.

Clinical relevance

Tumor hypoxia has been considered to be a therapeutic problem since it renders solid tumors resistant to sparsely ionizing radiation (X- and γ-rays), some forms of chemotherapy (e.g. cyclophosphamide, carboplatin) and photodynamic therapy. Oxygen levels may furthermore influence a series of biological parameters, which in turn may markedly increase the malignant potential of a tumor irrespective of tumor treatment modalities.

Sustained hypoxia in a tumor may cause molecular changes that can result in a more malignant phenotype. During the process of malignant progression, tumors develop an increased potential for invasive growth and tumor cell spread.

The most striking molecular mechanism during malignant progression is a genomic change in the neoplastic cells. This leads to an increased genomic instability with a rise in the selection pressure for certain tumor cell clones.

Hypoxia-associated resistance to radio- and chemotherapy is multifactorial. Besides direct effects (e.g. DNA damage) and hypoxia-induced genome and proteome changes, indirect effects may also play a pivotal role. For example, inhibition of cell proliferation or tissue → acidosis which often coincides with hypoxia in tumors that show a high glycolytic rate.

Tumor oxygenation as a prognostic parameter

Clinical trials have recently been conducted in more than 600 patients with locally advanced cervical cancer, in squamous cell carcinomas of the head and neck and in soft tissue sarcomas. In these studies, pretreatment tumor oxygenation has turned out to be a very powerful prognostic factor for locoregional tumor control and patient survival. Tumor hypoxia adversely affects locoregional control, disease-free survival and overall survival. This effect is independent of other known prognostic variables. Hypoxia also diminishes disease-free and overall survival in soft tissue sarcoma.

Modulation of tumor oxygenation

Manipulations of the tumor oxygenation status for therapeutic benefit have been performed for many years. A number of strategies to improve tumor oxygenation and to increase its uniformity have been considered. These include en-

hancing O_2 availability and/or reducing cellular respiration rate. The former can be achieved either by increasing the O_2 content in the tumor microvessels or by improving tumor blood flow. A reduction in the O_2 consumption rate would permit further diffusion of O_2 from blood vessels into distant cell layers to meet the needs of those tumor cells far away from the 'O_2 source', i.e., from microvessels perfused with red blood cells.

Considering the data accumulated so far on strategies to improve tumor oxygenation, it seems unlikely that there is a single solution to overcoming tumor hypoxia. A more rational approach may be to combine a number of these means since many of the factors contributing to tumor hypoxia work in parallel rather than in series with one another.

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P

p14ARF

Definition

p14ARF is a protein that binds to, and promotes, degradation of → MDM2, thereby releasing functional → p53.

p16

Definition

p16 is an inhibitor of the cyclin dependant kinases (cdk4 and 6). It functions to inhibit the phosphorylation and inactivation of the retinoblastoma gene RB/p105 and serves as a tumor suppressor gene; → CDKN2A.

p16INK4A

Definition

p16INK4a is a → tumor suppressor gene that encodes a protein of 16 kD. p16INK4a is a member of the INK4 family of cyclin-dependent kinase (CDK) inhibitors (→ CKIs); → CDKN2A.

p21 (WAF1/CIP1/SDI1)

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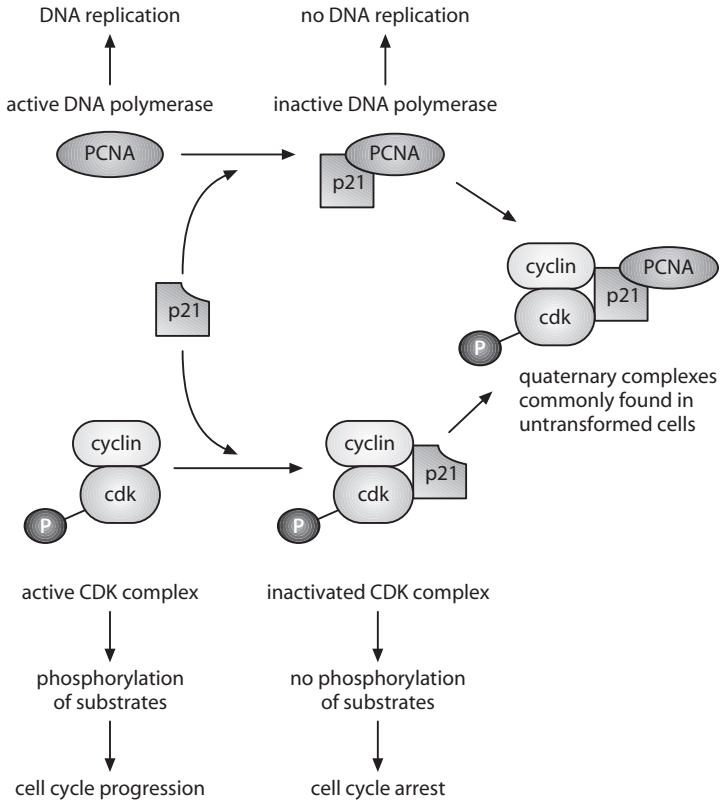
Synonyms

- Waf1
- Cip1
- Sdi1
- CAP20
- mda-6

Definition

The p21 protein is an inhibitor of cyclin-dependent kinases (CDKs) and of PCNA (proliferating cell nuclear antigen, which is a subunit of DNA polymerase). The activity of CDKs are required for cell cycle progression, while PCNA is essential for DNA replication. Therefore, the inhibition of CDK and PCNA activity by elevated levels of p21 causes cell cycle arrest. Thus, p21 is a negative regulator of cell proliferation. For reasons of its involvement in cell cycle regulation, p21 has been implicated in several related cellular processes such as differentiation, senescence, apoptosis and carcinogenesis. Since p21 has been discovered by diverse experimental approaches, it has received different suffixes including wildtype p53-activated fragment 1 (Waf1), senescent-derived inhibitor-1 (Sdi1), CDK2-interacting protein (Cip1), CDK2-associated 20K protein (CAP20) or melanoma differentiation-associated protein (mda-6). Most commonly, it is called p21Waf1 ($p21^{Waf1}$), p21Waf1/Cip1 ($p21^{Waf1/Cip1}$) or p21Waf1/Cip1/Sdi1 ($p21^{Waf1/Cip1/Sdi1}$).



p21 (WAF1/CIP1/SDI1). Fig. – Targets and function of p21. p21 binds to the DNA polymerase subunit PCNA and inactivates its DNA-replicative function. As a consequence, DNA replication, but not DNA repair, is blocked. p21 also binds to cyclin/cdk complexes and inhibits their enzymatic activity. As a result, the substrates of the cyclin/cdk complexes are not being phosphorylated, and the cells become arrested in the cell cycle. Thus, through the increase of p21 levels, the cells are able to stop proliferation. This process can be reversed: a reduction in the amount of p21 protein will release the inhibition of PCNA and of cyclin/cdk, and the cells resume growth.

Characteristics

The p21 gene is composed of three exons that are located on human chromosome 6 (6p21.2). Its 2.1 kb transcript encodes a 21 kD polypeptide (hence its name), which consists of 164 amino acids and generally is located in the nucleus. Homologues of this gene have been cloned from mouse, rat, and cat, and are likely to exist in organisms as diverse as *Xenopus*, insects, plants and yeast.

The major targets of p21, the → CDKs, are considered the 'engine of the cell cycle', and they direct the events required for cellular proliferation. Each CDK consists of two parts: one part is a cyclin protein (which acts as a regulatory subunit) and the other part is the actual cyclin-dependent kinase (cdk, which constitutes the catalytic subunit). The complex, composed of one cyclin subunit plus one cdk subunit, is called CDK. Several different cyclin proteins (cyclinA, cyclinB, etc.) as well as several different cdk subunits (cdk1, cdk2, etc.) exist and can combine to form different combinations of CDK heterodimers. For cell cycle progression and cell division to occur, the following CDK complexes are absolutely required: cyclinD together with cdk4 or cdk6 (cyclinD/cdk4 and cyclinD/cdk6), cyclinE/cdk2, cyclinA/cdk2, cyclinA/cdk1 and cyclinB/cdk1. The enzymatic activity of all CDK complexes, and thus their ability to drive cells through the cell cycle, is strictly regulated by post-translational modifications (phosphorylation and dephosphorylation) and by their interaction with inhibitory

proteins, the cyclin-dependent kinase inhibitors (abbreviated CKI or CDI).

There are two families of → CKIs:

- one is the Cip/Kip (CDK inhibitory protein) family that is constituted of p21 itself, p27Kip1 and p57Kip2
- the other is the INK4 (inhibitor of kinase 4) family, of which the tumor suppressor p16INK4a is the best studied member.

While the INK4 proteins bind to and inhibit the activity of only two CDKs, namely cdk4 and cdk6, members of the Cip/Kip family are rather indiscriminatory and affect all of the further above mentioned CDK complexes. p21 in particular has been labeled as a universal inhibitor of CDKs.

However, at rather low concentrations, p21 does not inhibit all CDKs with the same efficiency. In the case of cyclinE/cdk2 and cyclinA/cdk2, a single molecule of p21 is sufficient to completely inhibit the respective catalytic activity. Thus, in this instance, p21 fully conforms to its reputation as a potent CDK inhibitor. However, in the case of cyclinD/cdk4 and cyclinD/cdk6 [→ cyclin D], two molecules of p21 are required for inhibition; at equimolar concentrations (one molecule of p21 plus one cyclin/cdk complex) p21 does not inhibit cyclinD/cdk4 or cyclinD/cdk6 activity. Rather, under these conditions, p21 stimulates the assembly and nuclear targeting of these complexes. Moreover, the binding of p21 by cyclinD/cdk4 and cyclinD/cdk6 sequesters this inhibitor away from cyclinE/cdk2 and cyclinA/cdk2, thus indirectly promoting activation of these latter CDK complexes. As a consequence, the relatively low concentrations of p21, which are present in most cells, do not act cell growth inhibitory. For these reasons, the widely accepted definition of p21 as a general CDK inhibitor may have to be modified to accommodate its newly recognized role as a differential regulator of individual cyclin/cdk combinations. The biological rationale behind these different aspects of p21 function is not yet completely understood. Nonetheless, the major critical function of p21, in particular at increased expression levels, is the inhibition of CDK activity, which subsequently causes cell cycle arrest and inhibition of cell division.

In addition to modulating CDK activity, p21 protein is able to inhibit DNA synthesis through its ability to bind to and block the activity of → PCNA. The CDK- and PCNA-inhibitory activities of p21 are functionally independent and reside in separate parts of the protein. The amino-terminal region, which is strongly conserved among the → Cip/Kip family members, contains distinct cyclin- and cdk-interacting domains; residues 16 to 24 constitute the binding site for cyclins, whereas residues 45 to 65 form the binding site for cdk2. Thus, p21 simultaneously binds to both subunits of the CDK complex, whereby the interaction with the cyclin subunit serves to stabilize the ternary complex. As deduced from the crystal structure of the cyclinA-cdk2-p27Kip1 complex, the mechanism through which Cip/Kip proteins inhibit the kinase is through blocking the binding of ATP. This is achieved by a two-fold process: first, the inhibitor inserts a small helix inside the catalytic cleft of the cdk subunit that directly blocks ATP binding; second, the inhibitor causes a conformational change within the amino-terminal region of the cdk subunit that results in the loss of many ATP-interacting elements. Together, these processes result in the efficient, yet reversible, inhibition of kinase activity. It remains to be established why binding of one molecule of p21 to complexes containing cdk4 or cdk6 does not result in a similar inhibition of kinase activity.

The carboxy-terminal domain of p21, which is not conserved among the various members of the Cip/Kip family, contains a high-affinity binding site for PCNA (proliferating cell nuclear antigen, the processivity factor of DNA polymerase δ and ε). In this case, p21 masks contact sites on PCNA that are required for interaction with other proteins of the polymerase assembly. The interaction of PCNA with DNA, however, is not impeded by p21 binding. It is interesting to note that by binding PCNA, p21 inhibits DNA replication but not DNA repair. Rather, it appears that p21 actually stimulates PCNA-dependent nucleotide excision repair. Thus, by interacting with PCNA, p21 executes a two-fold task: it blocks replicative DNA synthesis, which

allows time for DNA repair to take place before the errors are duplicated, and at the same time it helps the repair to be executed.

Cellular and Molecular Regulation

Low amounts of p21 protein are present in most cell types. However, the expression levels can be increased significantly in response to a wide spectrum of diverse external stimuli and through the activation of different intracellular signal transduction pathways. In general, p21 expression can be regulated at multiple levels. The promoter of the p21 gene contains numerous binding sites for various transcription factors, most prominent among these is the tumor suppressor → p53. In addition, enhanced expression of p21 can be mediated post-transcriptionally through the increased stability of its mRNA, and post-translationally through alterations in p21 protein half-life, degradation through the ubiquitin/proteasome pathway and cleavage by caspases. Moreover, the ability of p21 to interact with its respective targets can be modified by phosphorylation of p21, as well as by alterations in its subcellular localization.

The best understood regulation of p21 is in response to stress conditions that generate DNA damage, such as certain chemotherapeutic drugs, oxidants or irradiation. These insults activate the tumor suppressor p53, which binds to the p21 promoter and increases p21 expression. Elevated levels of p21 cause cell-cycle arrest and provide the cells with the opportunity to repair the DNA damage. In this sense, p21 exerts a protective function against stress, which relies on its ability to suppress cell proliferation. Cells that were engineered to lack the p21 gene have been found to inefficiently arrest in the cell cycle after DNA-damaging insults and are much more prone to cell death (apoptosis). Thus, p21 is a major executioner of p53 functions and contributes to cellular survival after exposure to harmful stimuli. In cells that are deficient in p53 function, as is the case in more than 50% of all human cancers, the transcriptional induction of p21 is not observed in response to many DNA-damaging agents.

The expression of p21 is highly modulated during the course of cellular differentiation and development. At least in some tissues the exit from the cell cycle during terminal differentiation is mediated by p21, and this function of p21 is regulated by pathways that are independent of p53. Thus, these differentiation inducing signals act through pathways that target transcription factors other than p53. One such factor is MyoD, which is a central regulator of myogenesis that induces myocytes to exit the cell cycle and fuse to form myotubes. A binding site for MyoD has been identified in the p21 promoter and shown to mediate p21 induction during myogenesis. Furthermore, the ability of p21 to be induced in a p53-independent manner has been established for various differentiation models *in vitro* as well as during development *in vivo*. From these studies it has been determined that the induction of p21 is a primary mediator of differentiation. Moreover, in the case of hematopoietic stem cells, p21 has been shown to be the molecular switch that keeps these cells in relative quiescence; proliferative restriction is critical to the survival of these cells as increased cycling causes their premature depletion.

Most normal cells that are cultured *in vitro* cease to proliferate after a finite number of doublings, a process called → senescence. It has been shown that p21 accumulates as cell cultures age and approach senescence. In parallel with the increase in p21, the enzymatic activity of cyclinE/cdk2 complexes is decreased and PCNA activity is inhibited. In addition, probably through indirect mechanisms, p21 has been implicated in the negative regulation of → telomerase, which is an enzyme that is required for the extension of cellular life span. In this regard, it has been shown that the inactivation of the p21 gene delayed cellular senescence, i.e., the cells underwent more doublings before they reached senescence. In other studies, it was shown that the experimental inhibition of p21 function in early senescent cells stimulated the cells to re-enter S-phase and replicate their DNA, although the cells did not undergo cell division. This finding is consistent with the fact that in early senescent cells, cyclinE/cdk2 complexes and PCNA are inhibited

by p21. Upon removal of p21, both enzymes resume their function and drive cells through S-phase. Overall, p21 appears to be a critical initiator of the senescent process. However, once cells are fully senescent, other proteins, such as the CKI p16INK4a, take over for the long-term maintenance of this arrest.

Clinical Relevance

In theory every CKI should be a tumor suppressor. By virtue of their ability to arrest cells in the cell cycle and prevent their proliferation, CKIs are crucial negative regulators of cell growth. The elimination of CKI activity, for example by mutation or deletion, should release CDK complexes from this form of inhibition and promote unrestricted, inappropriate cellular growth. Conversely, the artificially increased expression of CKIs in tumor cells, for example through a gene therapy approach, should lead to the cessation of tumor cell proliferation.

In reality, a tumor suppressor function of p21 has been difficult to prove. Unlike p16INK4a, which is a *bona fide* tumor suppressor and frequently found inactivated in human cancers, p21 has not yet convincingly revealed such a function. Efforts to find tumors that harbor mutations in the p21 gene yielded such events only at a very low rate. However, polymorphisms of p21 have been observed in various cancers and one of them (at position 149) was found to be associated with human esophageal cancer.

Initially, the generation of mice with a homozygous deletion of the p21 gene yielded disappointing results; these mice did not exhibit an increased rate of spontaneous tumor formation. This was surprising because p21 is one of the major executioners of the tumor suppressor p53, and mice without the gene for p53 have a rapid rate of early tumor formation. Nonetheless, keratinocytes that were established from the p21-deficient mice exhibited increased susceptibility to transformation by the *ras* oncogene, and the resulting tumors grew much more aggressively as compared to *ras*-transformed keratinocytes from mice that contained normal p21 gene. Thus, it appears that the lack of p21 function contributes to the promotion of malignant tumorigenesis, and that the absence of p21 may become obvious and detrimental to cellular function only under conditions of stress, such as in response to genotoxins or aggravated growth-stimuli by oncogenes.

When artificially introduced and overexpressed in either normal or tumor cells, all CKIs tested to date cause cell cycle arrest. This effect could be useful in gene therapy-based treatments of human cancers in the future, after the numerous difficulties associated with gene therapy approaches have been solved. Two different approaches can be envisioned: First, CKIs could be used to complement defects in the tumor cells and either restore normal growth control or completely suppress tumor cell growth. Second, CKIs could be used to selectively, and reversibly, block the growth of only normal cells; this would permit the use of high doses of chemotherapeutic drugs to efficiently kill and eliminate the rapidly dividing tumor cells. The normal cells, due to their transiently arrested state, would be protected from the cytotoxic drugs. After termination of the chemotherapy, the elevated amount of CKI activity would have to return to pre-treatment levels in order to allow resumption of normal cell growth. Although still far from reality, such CKI-based gene therapy approaches could one day supplement our arsenal of anti-cancer weapons.

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p27

Definition

→ Cyclin-dependent kinase inhibitor 1B.

p27KIP1

Definition

p27Kip1 is a cyclin-dependent kinase inhibitor and cell cycle regulator as well as a tumor suppressor; → cyclin-dependent kinase inhibitor 1B.

p53

Definition

→ TP53.

p53 Gene Family

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Definition

The *p53* gene family currently consists of 3 genes:

1. *p53* (chromosome 17p13).
2. *p73* (chromosome 1p36).
3. *p63* (chromosome 3q27-29).
   p63 has multiple synonyms including *p51*, *p40*, *p73L*, and *Ket*.

Characteristics

All three members of the *p53* gene family encode similar nuclear proteins with the following features; an N-terminal transactivation domain, a central DNA-binding domain and a C-terminal oligomerization domain. Unlike *p53*, *p63* and *p73* give rise to multiple mRNAs due to alternative promoter utilization and alternative mRNA splicing. The use of an alternative promoter in intron 3 of *p63* and *p73* directs the synthesis of p63 and p73 isoforms, respectively, that lack the N-terminal transactivation domain and are designated with the prefix 'ΔN'. Alternative mRNA splicing leads to the production of p63 and p73 isoforms with different extensions C-terminal to their respective oligomerization domains, and are designated with the suffix α, β, γ, etc. The C-termini of p63α and p73α both contain a → SAM-domain not found in p53. SAM domains can serve as protein-protein interaction modules. At the level of primary sequence, p63 and p73 resemble one another more closely than either does to p53. The p73 transactivation domain can interact with the coactivator protein p300, and → MDM2. MDM2 prevents p300 from binding to p73 and prevents p73 from activating transcription. In contrast to p53, MDM2 does not appear to target p73 for → ubiquitin-dependent proteolysis. p63 and p73 can bind to canonical p53-DNA binding sites and likely do so as homotetramers, based on the behavior of p53. Notably, the oligomerization domains of p53, p63 and p73 preferentially form homoligomers rather than heteroligomers. p63 and p73 isoforms with intact transactivation domains can activate p53-responsive promoters. The efficiency with which different p63 and p73 isoforms bind to DNA and activate transcription appears to be influenced by the nature of their C-terminal extensions. Preliminary data suggest that the optimal DNA binding sites for p53, p63, and p73 differ because some 'p53 target genes' are differentially activated by p53, p63 and p73 in cells. ΔNp63 species can prevent transactivation competent p53 family members from binding to p53-responsive promoters and thus act in a dominant negative fashion. The same is likely true for ΔNp73.

In addition to activating p53-target genes, specific p63 and p73 isoforms can induce apoptosis when overproduced in tumor cells that lack wild type p53. In a neuroblastoma cell line, p73 promoted cellular differentiation. The physiological signals that regulate p63

and p73 production are still being elucidated. As is true for p53, certain forms of DNA damage, such as following gamma irradiation and genotoxic chemotherapeutic drugs, can induce the accumulation of p73. Here, the accumulation and activation of p73 has been linked to its ability to bind to the → ABL oncogene protein product.

Roles in development

p53 -/- mice are viable but develop a number of tumors including lymphomas and sarcomas. *p73* -/- mice are also viable but are not tumor prone. However, *p73* -/- mice are prone to bacterial infections such as otitis, neurological and pheromonal abnormalities such as hippocampal dysgenesis and hydrocephalus, and exhibit impaired responses to pheromones. *p63* -/- mice die in the neonatal period with profound defects in limb and epidermal morphogenesis.

Biological activity in cancer

Mutations that directly or indirectly compromise p53 function are very common in human cancers. Loss of p53 function has several consequences, including diminished apoptosis in response to oncogene activation, inactivation of cell-cycle checkpoint controls and enhanced genomic instability. Whether aberrations in p63 and p73 play a role in human cancer is currently unclear. Some human tumors have amplified the region of the genome that contains *p63*, or deleted the region of the genome that contains *p73*. It is not clear, however, whether *p63* and *p73* are the biologically relevant targets underlying these alterations or merely bystanders. Some squamous cell tumors produce high levels of ΔNp63. This might suggest that ΔNp63 can act as an oncoprotein, but might also reflect the fact that the cell of origin for these tumors normally produce high levels of this protein. The frequent deletion of 1p36 in human tumors led to the early suggestion that *p73* might be a tumor suppressor gene. Against this idea, however, are the observations that the retained *p73* allele in such tumors is typically wildtype, and p73 mRNA levels are typically higher in tumor cells compared to their normal counterparts.

Clinical relevance

Germ line mutations in *p53* cause the Li-Fraumeni hereditary cancer syndrome [→ Li-Fraumeni syndrome]. Germ line mutations in the *p63* gene are responsible for ectrodactyly, ectodermal dysplasia and facial clefts (EEC) syndrome. Germ line mutations in *p73* have not been reported. Somatic intragenic mutations of *p63* and *p73*, unlike *p53*, appear to be rare in human cancers.

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p53 Protein, biological and clinical aspects

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Definition

p53 is a 53 kD protein a that was discovered in 1979 by virtue of its capacity to bind several viral antigens (SV40 large T antigen, E1B from adenovirus) or by its ability to induce a humoral response in tumor-bearing animals. Mouse and human p53 genes were cloned in 1983 and 1984, respectively. The p53 gene belongs

to the category of → tumor suppressor genes as it fulfils the three main criteria:

- homozygotic somatic alterations in human cancer
- germline mutations are associated with the → Li-Fraumeni syndrome, a rare autosomal-dominant disease whose hallmark is a predisposition to a wide range of cancers among members of a family
- the p53 protein is a negative regulator of cell growth.

In addition, some mutant p53 can exert a dominant negative effect toward wild type p53. Furthermore, it is possible that several p53 mutants harbors a gain in function that suggests that mutant p53 could act as an oncogene.

Characteristics

The human p53 gene is localized on the short arm of chromosome 17 (17p13.1). It is 20 kb long and contains 11 exons. The p53 protein is translated from an open reading frame issued from exon 2 to 11. An alternative splicing which changes the carboxy-terminus of the protein has been reported but its signification is unknown. The p53 gene is phylogenetically conserved from the human to the fly. In 1997, two genes homologous to p53 were identified: p73 localized in chromosome 1p36 and p63 localized in chromosome 3q27. Despite extensive homology in the DNA binding domain, these proteins seems to be involved in different pathways.

The human p53 protein is a 393 amino-acid protein consisting of five different structural and functional regions:

- The acidic amino terminus (1-60) is the transactivation domain. It binds several cellular and viral proteins, including mdm2, TFIID, THIIH, and adenovirus E1B 55kD protein. Mdm2 is involved in the stabilization of p53, whereas TAFIID and TFIIH are required for activation of transcription.
- The proline rich domain (63-97) is involved in a transactivation independent apoptosis.
- The central region (100-300) region is highly conserved in p53 through evolution and in other p53 members. It contains the sequence-specific DNA binding domains. The vast majority of p53 missense mutations in human tumors are clustered within this central region.
- The oligomerization domain (323-356) contains a β-sheet-turn-α-β-sheet motif that can homodimerize and the p53 tetramer contains a pair of such dimers. It also contains a highly conserved leucine-rich nuclear export signal.
- The carboxy-terminus (363-393: It contains three nuclear localization signals and has several biochemical activities. i) It binds non-specifically to different forms of DNA including damaged DNA: ii) it negatively regulates the specific DNA binding activity of p53: iii) it is able to reanneal complementary single-stranded DNA. This region is the target for several cellular proteins such as ERCC2 and ERCC3, two components of TFIIH involved in DNA repair.

The p53 protein is the target for various post-translational modifications such as phosphorylation (at the amino and carboxy-terminus) and acetylation (at the carboxy-terminus).

Biological functions

p53 as the guardian of the genome. The p53 protein is stabilized in response to different checkpoints activated by DNA damage, hypoxia, viral infection or oncogene activation, resulting in diverse biological effects such as cell-cycle arrest, apoptosis, senescence, differentiation and antiangiogenesis. The stable p53 protein is activated by phosphorylation, dephosphorylation and acetylation to yield a potent sequence-specific DNA-binding transcription factor. The wide range of p53 biological effects can be partly explained by its activation of the expression of a number of target genes including p21(WAFI), GADD45, → 14-3-3σ, Bax, BTG2, PIG3, IGF-BP3 and others. p53 can induce or potentiate apoptosis through several mechanisms, both by regulating the expression of genes that can participate in the apoptotic response and through transcriptionally independent means. There appears to be cell type

variability in both the response to p53 expression and in the requirement for p53 transcriptional transactivation for the induction of apoptosis.

In cells expressing a mutant form of p53, neither cell-cycle arrest nor apoptosis occurs in response to DNA damage. Transfection of wild type p53 into these cells restores the choice between cell-cycle arrest and apoptosis, indicating that wild type p53 appears to act as a key factor in maintaining the integrity of the genome. In contrast, tumor cells harboring a mutant p53 gene can no longer maintain genome integrity because the cells no longer receive the signal for cell-cycle arrest, resulting in marked genetic instability that allows the emergence of clones with increased malignant potential. With this model it is easy to understand the phenotype of mice harboring deletions of the p53 gene: these mice are viable but have a high incidence of cancer.

p53 regulation by the mdm2 protein. By binding specifically to the amino-terminus of p53, mdm2 blocks the p53 transactivation domain and inhibits p53 transcriptional activity. This binding also induces degradation of p53. This is crucial, since the biological activation of p53 is known to occur *via* stabilization/destabilization of the protein and not at the level of transcription. For example, the 20 to 50-fold increase in the levels of cellular p53 protein observed after treatment of cells with a genotoxic agent is due to an increase in the half-life of the protein and not to an effect on gene transcription or mRNA stability. Specific phosphorylation of the p53 amino-terminus occurs in response to DNA damage. This blocks the p53-mdm2 interaction, resulting in accumulation of the p53 protein through a reduction of mdm2-dependent degradation. While the kinases involved in this phenomenon have not been formally identified, → Chk2 and the ATM [→ ATM protein] gene product appear to be good candidates. It is highly likely that these post-translational modifications differ according to the type of DNA lesion.

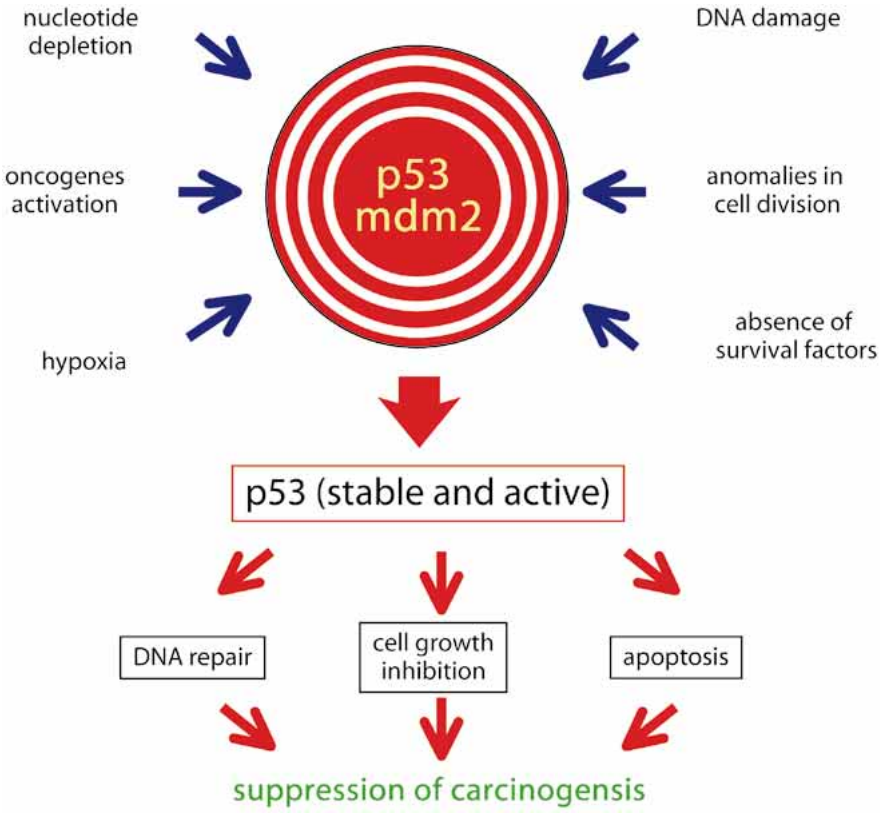
The mdm2 gene is one of the genes whose transcription is activated by p53. This activation results in feedback inhibition which lowers the intranuclear pool of p53, suggesting that mdm2 acts as a p53 'guardian'. Activation of cellular oncogenes such as c-myc or ras can also induce p53-dependent cell-cycle arrest, which is accompanied by nuclear accumulation of wild type p53 protein. This p53 stabilization is due to dissociation of p53/mdm2 complexes by the $p14^{ARF}$ protein that induces mdm2 translocation into the nucleoplasm. The $p14^{ARF}$ protein is encoded by the INK4a locus that also codes for the CDKI p16 protein in another reading frame. Thus, the p53 protein can be activated by multiple metabolic pathways that generally interfere with the p53-mdm2 complex.

p53 in cancer

p53 mutations are present in 40 – 45% of cancers, all sites combined. It is the most frequent genetic event demonstrated to date. By way of comparison, ras gene mutations are found in only 10 – 20% of cancers. These mutations are usually accompanied by a loss of heterozygosity in the short arm of chromosome 17. In most cases, the mutations are point mutations located in four of the five evolutionarily conserved domains (II to V) between amino acids 120 and 300. These transforming mutations inactivate the negative regulatory function of p53 on cell growth. However, in some cases the mutant gene also confers a dominant phenotype involved in maintenance and/or induction of transformation. p53 mutations have been associated with a poor prognosis in various types of cancer (breast, colon or bladder), but it is not routinely used as a clinical marker as its discriminative power is not high.

p53 mutations: a model for molecular epidemiology

Analysis of type of mutation in different types of tumors defines the mutational spectra according to the cancer under study. Generally speaking, there are two types of genetic alteration, those derived from endogenous processes resulting from errors occurring during the various biological processes linked to DNA metabolism, and those of exogenous origin involving environmental factors. The location and type of substitution occurring as a result of



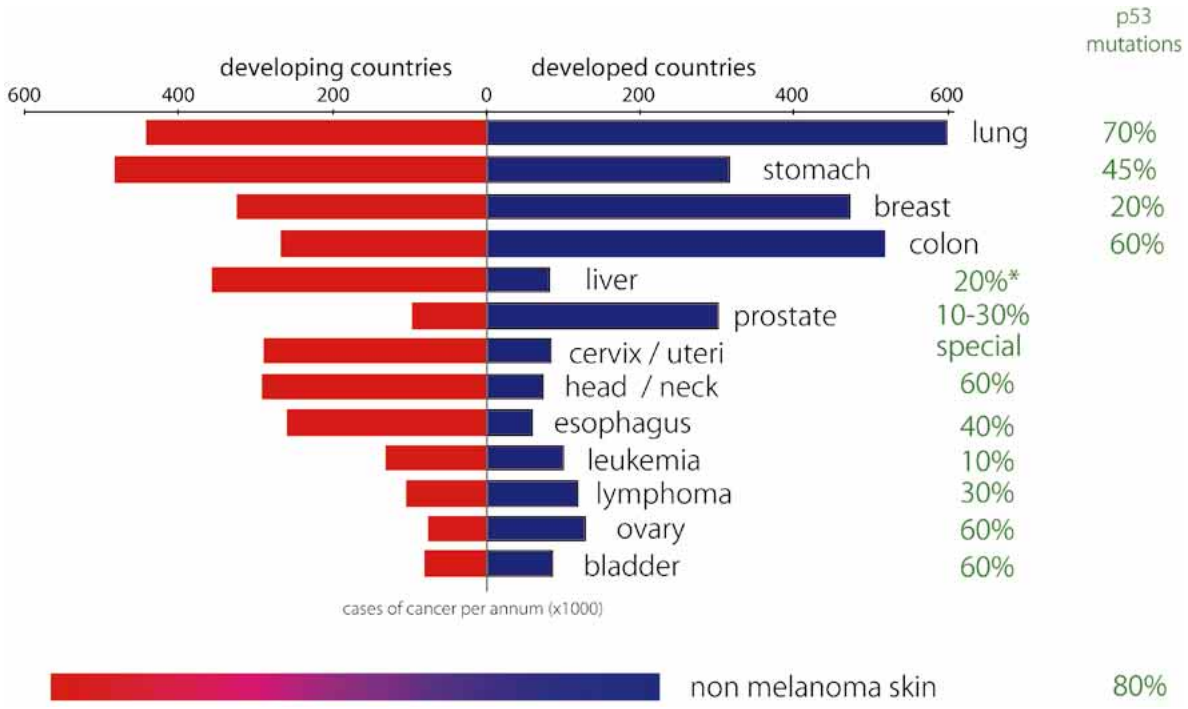
p53 Protein, biological and clinical aspects. Fig. 1 – Upstream and downstream signalling via the p53 protein.

these two types of alterations are different. It is therefore possible to use the spectra of these mutations to study the etiology of a cancer.

Endogenous mutations. Analysis of all mutational events affecting the p53 gene shows that 42% are G:C → A:T transitions, 60% of which affect a CpG dinucleotide. It is well known that spontaneous deamination of 5-methylcytosine at these nucleotides may be an important cause of this type of transition. Analysis of the mutational events in cancers such as colon cancer, malignant hemopathies or brain cancer (cancers known to be unrelated to exogenous carcinogens) shows that the mutation rate at the CpG dinucleotide is very high, thus suggesting that most of the mutations that alter the p53 gene in these cancers are due to endogenous processes related to the deamination of 5-methylcytosine.

Hepatocarcinoma and aflatoxin B1. In 1990, there were two reports of mutations in the p53 gene in hepatocarcinomas (HCC), with a predominance of the GC → TA transversion at the third base of codon 249 (Arg → Ser). In one case, the patient series was from Mozambique, while the second was from the Qidong province in China. These two regions are known for their consumption of food contaminated by the fungus Aspergillus flavis, a producer of aflatoxin B1, which is a very potent hepatic carcinogen implicated in the development of HCC and which is known to interact synergistically with the hepatitis B virus. A worldwide epidemiological study showed that the mutation in codon 249 is strictly specific to countries in which the food is contaminated by aflatoxin B1.

Skin cancer and ultraviolet radiation. In skin spinocellular cancer, the frequency of p53 mutation is high (80%) and C → T mutations predominate in pyrimidine dimers. It is well known that ultraviolet radiation, an etiological agent of most skin cancers, acts directly on these dimers. A particular characteristic of the action of UV radiation is the change in



p53 Protein, biological and clinical aspects. Fig. 2 – Frequency of the p53 mutation in human cancer. *The frequency of p53 mutations in liver cancer is higher in countries in which food is contaminated by aflatoxin B1. In cervical cancer, p53 is inactivated by the E6 protein expressed by human papilloma viruses.

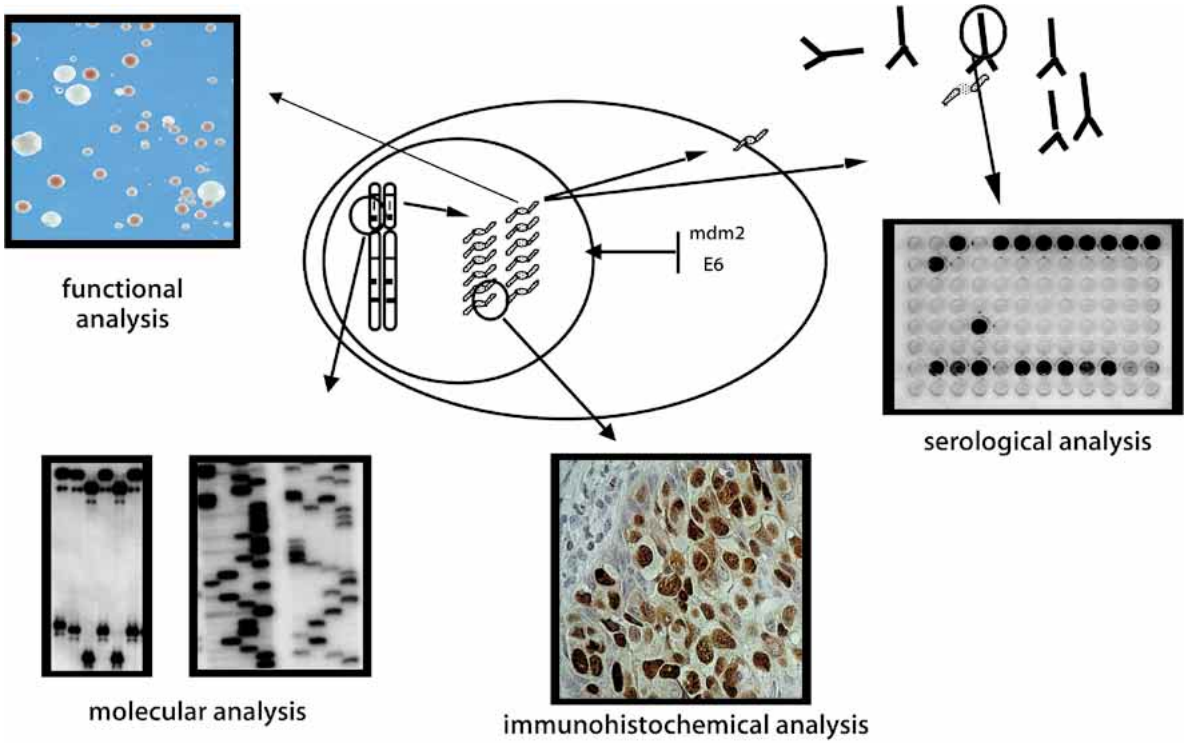
the bases CC → TT, observed in skin cancer such as basocellular cancers. Such p53 mutations seem to be very early events as they can be found both in precancerous lesions such as actinic keratosis and in normal skin exposed to UV. These results taken together (predominance of CC → TT lesions on the non coding strand) were experimentally confirmed in animals carrying UV-induced tumors.

Bronchopulmonary cancers and smoking. The p53 mutation profile in lung cancer shows a predominance of G:C>T:A transversions which are not present in other types of cancer. There is also a hot spot at codon 157, not found in other types of cancer. There are many lines of evidence indicating that the transversions in the p53 gene are due to the carcinogens in tobacco smoke (→ tobacco carcinogenesis):

- only lung cancer (and, to a lesser extent, head and neck and esophageal cancers which are also tobacco-related) has such a high rate of G:C>T:A transversions;
- there is a linear correlation between this transversion rate and the number of cigarettes smoked;
- non-smokers with lung cancer do not display this high transversion frequency;
- treatment of human bronchial cells with BPD (a major component of cigarette smoke) induces the appearance of adducts in codon 157, a guanine-rich region which is clearly a hot spot for formation of these adducts.

p53: a target for therapy?

p53 gene therapy. Mouse and human cells expressing a p53 mutation are resistant to apoptosis induced by certain antineoplastic agents such as fluorouracil and ionising radiation, in contrast to cells expressing wild type p53 which are sensitive to their action. A majority of studies tend to show that there is a relationship between p53 mutations and resistance to chemo- or radio induced DNA damage. These stu-



p53 Protein, biological and clinical aspects. Fig. 3 – Multifactorial analysis of p53 alterations in human cancer. Molecular analysis: PCR followed by sequence analysis enables direct study of the type of mutational event that alteres the gene. In over 90% of cases, this event is a point mutation that alters a single nucleotide among the 23,000 in the gene. Unlike the ras gene, for which only 3 of the 189 codons are targets of oncogenic mutations, in the p53 gene, mutations may occur in 300 of the 393 codons required for the synthesis of the protein. This high degree of heterogeneity makes diagnosis more difficult because the region to be analyzed extends over almost the entire gene. **Immunocytochemical approach:** In normal cells, p53 is undetectable because it has an extremely short half-life (15 to 20 min). In transformed cells, the mutant protein is much more stable, with a half-life of 4 to 12 hours, and it accumulates in the nucleus. It is therefore possible to perform an immunocytochemical diagnosis (coupled with a histological analysis) in tumor tissue to directly visualize this nuclear accumulation. Such an approach has been used in many different types of cancer with a generally good correlation between molecular analysis (presence of a mutation) and immunohistochemical analysis (overexpression of the mutant protein). The advantage of this approach is that it can be routinely used in histology laboratories. However there are also a number of disadvantages, including the fact that any mutations that abolish p53 expression (splicing signal mutations, nonsense mutations, insertions or deletions) do not produce the protein and therefore give a negative result. These types of mutations are found in 5 to 10% of cases. **Serologic analysis:** Anti-p53 antibodies have been found in most human cancers. There is generally a good correlation between their frequency and that of p53 gene alterations. Several multifactorial studies show a very good correlation between the presence of anti-p53 antibodies, overexpression of the mutant protein in the tumor and the presence of a mutation in the gene. It has been shown that overexpression of the p53 protein in tumor cells is responsible for the appearance of auto-antibodies. Serum p53 antibodies can be found several months before the clinical manifestation of the disease. **Functional assay:** A functional assay for p53 mutations has been described. Mutations are detected by assaying the transactivational activity of p53 protein in yeast. Because a specific mutant yeast strain is used, colonies containing mutant p53 are red, whereas colonies containing wild-type p53 are white. This test was used for detection of germline p53 mutations, but recent improvement in the test has led to its use in analysis of surgical specimens of tumor tissue.

dies are of major importance. First, if the role of p53 mutations in chemosensitivity is clearly established, knowledge of p53 status of a tumor might help to orient the treatment strategy accordingly. It is possible that reintroduction of wild type p53 into tumor cells, or conversion of mutant p53 to the wild type conformation, might restore apoptosis in these cells. Many studies on cell lines have shown that reintroduction of wild type p53 to tumor cells expressing a mutant p53 restores sensitivity to agents as diverse as irradiation, cisplatin and 5-FU Several phase I and II protocols are currently underway in order to check the efficacy of p53 gene therapy.

*Restoring normal function to mutant p53.*The specific DNA binding activity of p53 is crucial for its tumor suppression function. Naturally occurring mutant forms of p53 are deficient for specific DNA binding. However, several studies have indicated that their specific DNA binding can be reactivated. Short peptides derived from the p53 C-terminus can reactivate at least some mutant p53 proteins and trigger a p53-dependent biological response. These results may provide the basis for the design of p53-reactivating anti-cancer drugs.

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p70S6 Kinase

Definition

p70S6 kinase is a serine/threonine kinase that can be activated by mitogen activated MAP kinase or the → TOR pathway. p70S6 kinase phosphorylates the ribosomal protein S6 and thereby stimulates the translation of mRNAs with a 5′-oligopyrimidine tract, which typically encode components of the protein synthesis apparatus.

p73

Definition

Tumor protein 73 (p73); → p53 gene family.

p107

Definition

p107 is a protein of molecular mass 107 kD that has similarity to the → retinoblastoma protein (pRB).

p130CAS

Definition

p130CAS is an adaptor protein of 130 kD that is found in focal adhesions. It contains many protein/protein interaction domains that mediate binding to multiple proteins within this structure, including → FAK, → Src and several phosphotyrosine phosphatases. It becomes tyrosine phosphorylated in response to a variety of growth, differentiation and migratory stimuli and is thought to participate in many different cellular processes that require actin cytoskeleton rearrangements and cell movement.

p185NEU

Definition

→ HER-2/neu.

p300

Definition

→ CBP/p300; → EP300.

PACE Family

Definition

PACE is a family of pro-protein processing endoproteinases homologous to the yeast processing proteinase Kex 2 and the bacterial serine proteinase subtilisin. The serine proteinase furin (SPC1) is a major pro-protein processing enzyme localized in the trans Golgi network and functions in the processing of substrates as they transit through the secretory pathway towards the cell surface.

Packaging Signal

Definition

The packaging signal is a nucleotide sequence in a viral genome that directs the packaging of the viral genome into preformed capsids during the infectious cycle.

PAH

Definition

→ Polycyclic aromatic hydrocarbons.

Palindrome

Definition

A palindrome is adjacent → inverted repeats.

Pancreas

Definition

The pancreas is an abdominal organ with both endocrine and exocrine function; → gastrinoma.

Pancreas Cancer, basic and clinical parameters

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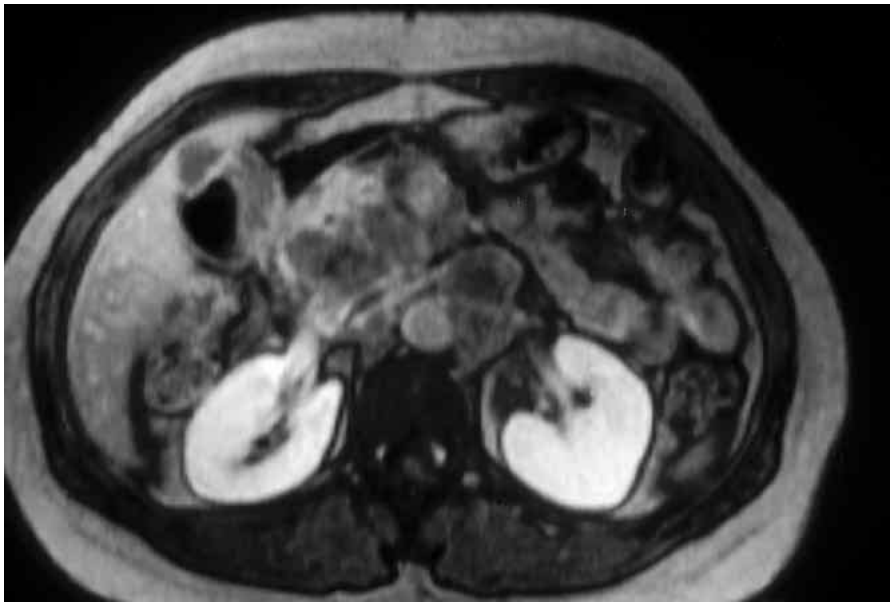
Definition

Cancer of the pancreas is an epithelial malignancy of the pancreatic gland that originates in more than 85% of the cases from ductal cells. In rare cases, tumors may also arise from acinar or endocrine cells.

Characteristics

General Clinical Features

Pancreatic adenocarcinoma is one of the most aggressive human malignancies, reflected by a mortality rate which closely follows that of its incidence. Its incidence is steadily increasing and today it is the fifth leading cause of cancer related deaths in the western hemisphere. Most patients are diagnosed with pancreatic cancer in the late course of the disease with unspecific symptoms such as fatigue, weight loss, jaundice and upper abdominal pain. At this stage pancreatic cancer has frequently invaded surrounding organs such as the duodenum and stomach, and the retroperitoneal tissue and



Pancreas Cancer, basic and clinical parameters. Fig. 1 – Abdominal magnetic resonance imaging. The MRI depicts a huge mass in the upper abdomen that originates from the corpus of the pancreas.

blood vessels (Fig. 1). Lymph node metastases occur in most cases and reflect a high metastatic potential.

Most patients present in the seventh or eighth decade of life with a male/female ratio of approximately 1.5:1. The etiology of pancreatic cancer remains unclear. However, environmental factors such as cigarette smoking and a high fat diet may predispose patients to the development of pancreatic malignancies. Inflammatory states such as chronic pancreatitis with calcification can have estimated risks of tumorigenesis that are up to 14 times higher than normal. Genetic factors, such as p16 germline mutations and mutations in the mismatch-repair system may also increase the probability of developing pancreatic adenocarcinoma.

Pathology

The most frequent site of pancreatic cancer is the head of the pancreatic gland (approximately 60%). The remainder of cases arise in the body (15%), the tail (5%) or are disseminated throughout the pancreas (20%). The vast majority of pancreatic cancers are of ductal origin. Rarely, they originate from acinar or endocrine cells. Ductal adenocarcinoma of the pancreas are desmoplastic malignancies composed of mucin-producing glandular cells infiltrating a non-neoplastic stroma that accounts for more than 50% of the tumor tissue. In addition to the fibrocytic stroma, cancer cells are also mixed with inflammatory cells, including lymphocytes. The histological progression from benign to malignant pancreatic disease starts with flat mucinous lesions that develop to papillary lesions without atypia, followed by lesions with atypia. The latter are followed by *in situ* carcinoma, finally reaching the state of infiltrating adenocarcinoma.

At the time of diagnosis, pancreatic cancer has generally invaded the peripancreatic fat tissue and lymph nodes or adjacent organs, such as the duodenum, stomach, peritoneum and vessels. Cancers restricted to the pancreatic gland are very rarely found. In particular, carcinomas located in the body and tail are diagnosed at a more advanced stage due to delayed diagnosis. Sites of hematogenous metastases formation are, in order of frequency, liver, lungs, adrenal glands and kidneys. However, local tumor recurrence due to remaining microscopic tumor foci seems, even in smaller tumors, to be the determining factor for patients survival (4).

Staging

The → UICC staging system is based on the size of the primary tumor (T), the extent of regional lymph node involvement (N) and the presence of metastases (M).

Genetics

An array of technologies has been applied to investigate relevant chromosomal and genetic changes in pancreatic cancer over the last few of years. The detection of specific chromosomal changes and altered tumor suppressor genes and oncogenes has significantly improved our understanding of the development and progression of this disease (3).

Cytogenetics.

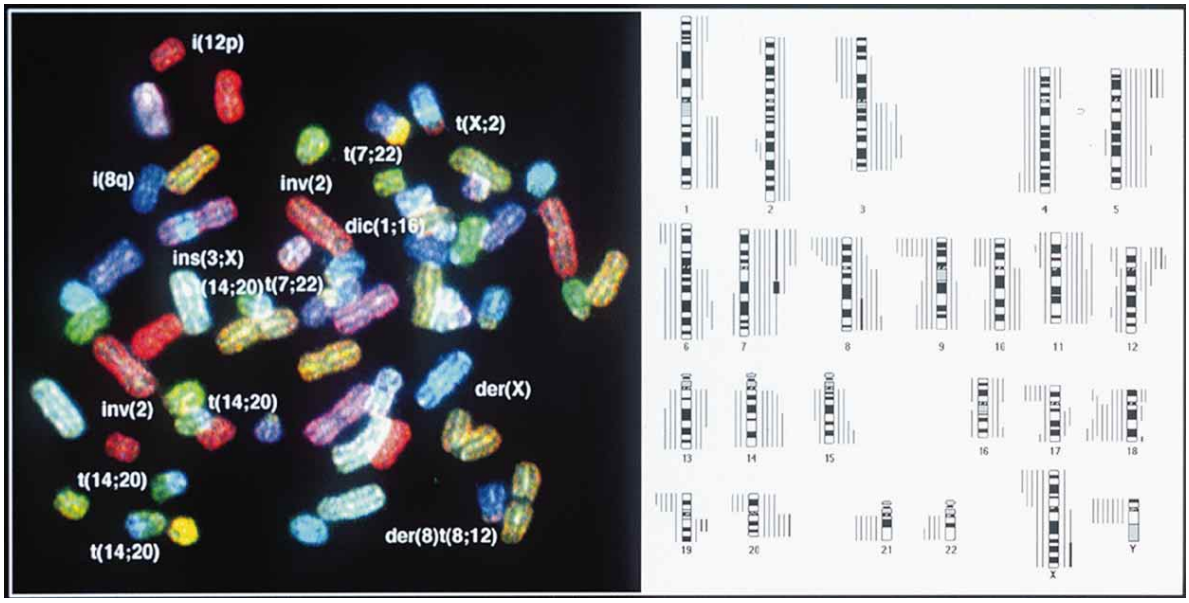
The application of modern fluorescence-in-situ technologies (FISH), such as comparative genomic hybridization (CGH) or spectral karyotyping (SKY) for the study of numerical and structural chromosomal aberrations, have revealed new insights into pancreatic tumorigenesis. Pancreatic carcinoma cells have a surprisingly high degree of chromosomal instability but also recurrent chromosomal gains at 3q, 5p, 7p, 8q, 12p and 20q and losses at 8p, 9p, 17p, 18q, 19p and 21. Most of these loci correlate well with known oncogenes or tumor suppressor genes. The high recurrence rate of these loci are the basis for the development for FISH-probes to enable the development of interphase cytogenetics for diagnostics and prognostics (2). As with other epithelial malignancies, a recurrent translocation has not been found in pancreatic cancer. Therefore, a translocation-produced gene fusion that forms chimeric oncoproteins does not seem to be an important mechanism in the development of pancreatic cancer (Fig. 2).

Oncogenes.

The *K-ras* oncogenes codes for a GTP-binding protein and plays a major role in pancreatic cancer. Activating point mutations of the K-ras oncogene occur in 70 – 90% of all ductal adenocarcinomas and are largely restricted to exon 12 and 13 of the *K-ras* gene at 12p12. Due to these mutations, the K-ras is constitu-

Pancreas Cancer, basic and clinical parameters. Table – UICC staging system.

primary tumor (T)	
TX	primary tumor cannot be assessed;
T0	no evidence of primary tumor;
T*is*	carcinoma *in situ*;
T1	tumor <2cm;
T2	tumor >2cm;
T3	tumor extends directly to the duodenum, bile duct or peripancreatic tissues;
T4	tumor extends directly to the stomach, spleen, colon, or adjacent vessels
regional lymph nodes (N)	
NX	regional lymph nodes cannot be assessed;
N0	no regional lymph node metastasis;
N1	regional lymph node metastasis;
distant metastasis (M)	
MX.	presence of distant metastasis cannot be assessed;
M0	no distant metastasis;
M1	distant metastasis



Pancreas Cancer, basic and clinical parameters. Fig. 2 – Unbalanced translocations reflected by gains and losses. Unbalanced chromosomal translocation lead to a highly recurrent and specific pattern of chromosomal alterations in pancreatic cancer.

tively active; therefore the signal transduction pathway remains active, stimulating proliferation and cellular transformation. *K-ras* mutations have been found in proliferative non-invasive ductal lesions, indicating that *K-ras* might play a role during early carcinogenesis. However, *K-ras* mutations have also been found in inflammatory and normal pancreatic tissue without neoplastic potential. This finding might limit the development of a gene based test system using *K-ras* mutations as an indicator for neoplastic or malignant cells in pancreatic juice, blood or stool of patients with pancreatic disease.

Tumor suppressor genes.

The relevant tumor suppressor genes in pancreatic cancer are → p53, → p16 and → DPC4. p53 is a nuclear binding protein that arrests cells at the G1/S checkpoint and also plays an important role in the induction of apoptosis after DNA damage. The *p53* gene is on chromosome 17p13. In about 50% of pancreatic cancers, it is inactivated by allelic loss and inactivating mutations. Loss of p53 function, therefore, results in a disturbed cell cycle and the loss of programmed cell death.

p16 has been mapped to 9p21 and is inactivated in about 90% of pancreatic cancers via allelic loss, inactivating mutations and/or hypermethylation of the promoter. p16 inhibits the promotion of the cell cycle by binding to the cyclin-CDK4 complex and preventing CDK4 activation of RB protein. Therefore, inactivation of p16 in pancreatic cancer disregulates another relevant cell-cycle checkpoint.

The tumor suppressor gene DPC4 is biallelically inactivated in about 50% of pancreatic cancers. Located on chromosome 18q, DPC4 codes for a peptide that is closely related to the MAD familiy of proteins (SMAD). These molecules play an integral part in the signal transduction from TGF-β superfamily cell surface receptors. Since it is known that TGF-β inhibits cell growth and proliferation, inactivation of DPC4 and loss of its inhibitory function may bestow a growth advantage upon cancer cells.

Hereditary forms

Several family studies have suggested that between 5 and 10% of pancreatic cancer may have a hereditary basis. A predisposition to

the development of pancreatic cancer has been shown for several genetic syndromes including hereditary pancreatitis, hereditary non-polyposis colorectal cancer (HNPCC) and the familial atypical mole-multiple melanoma (FAMMM) syndrome.

Hereditary pancreatitis is an autosomal dominant disorder that is characterized by an early age of onset. Patients suffer from recurrent acute pancreatitis that subsequently leads to chronic pancreatitis and carrying a significant risk factor for the development of pancreatic cancer. In the disorder, a mutation in the trypsinogen gene at 7q35 results in the inability to deactivate trypsin that results in autodigestion of pancreatic tissue.

Hereditary non-polyposis colorectal cancer (→ HNPCC) syndrome is another syndrome that predisposes individuals to pancreatic cancer. It is an autosomal transmitted disease caused by germline mutations in the mismatch repair system. Besides pancreatic cancer, patients also inherit a predisposition to other cancers, including colonic, breast, ovarian and endometrial carcinomas.

Patients with the → FAMMM syndrome have an elevated risk for the development of multiple atypical nevi, malignant melanomas and pancreatic cancer. In a subset of patients, a germline mutation in the p16 tumor suppressor gene is implicated.

Diagnosis

Unfortunately, there are no early signs or symptoms to identify pancreatic cancer, as gastrointestinal obstruction and other compromising sequellae occur. Patients that are worked up for abdominal pain or jaundice suspicious for pancreatic cancer typically undergo the following diagnostic procedures.

Abdominal Ultrasound.

Transabdominal pancreatic ultrasonography (US) is performed using high resolution real-time linear array or sector scanners combined with Doppler examination. The sensitivity of US in pancreatic cancer is as high as 85%. Lesions in the head are more visible than lesions in the body or tail of the gland due to intestinal gas. Computer tomography (CT) is clearly superior in terms of sensitivity and specificity compared to ultrasonography. In summary, US is a reliable screening method for the detection of pancreatic masses and liver metastases but is not recommended for examining patients if a malignant disease is strongly suspected.

Computed Tomography (CT).

Current techniques, including high resolution spiral CT, provide detailed images of the pancreas, the pancreatic and biliary duct system, the peripancreatic vessels and surrounding organs. Several studies have reported a sensitivity of 92% and a specificity of approximately 100% with spiral CT. However, only 65% of all tumors that were staged as resectable with spiral CT scans were actually candidates for resection. Therefore, laparotomy or surgical laparoscopy remain the only specific approaches to determine resectability.

Magnetic Resonance Imaging (MRI).

The importance of MRI in pancreatic cancer diagnostics, despite the advantage of avoiding ionizing radiation, is still to be determined. In most centers MRI is used to differentiate between malignant and benign disease when US or CT are equivocal or the administration of intravenous contrast agents is contraindicated.

Magnetic Resonance Cholangiopancreatography (MRCP).

Although the exact role of MRCP has not been determined it has demonstrated its potential to display changes in the pancreatic duct pathology. MRCP and ERCP findings correlate with pathology in 80 – 90% of the cases.

Endoscopic Retrograde Cholangiopancreatography (ERCP).

Endoscopic techniques, such as ERCP, can be applied for diagnosis and interventional management for patients with pancreatic disorders with a sensitivity and specificity of 90%. Endoscopic pancreatographic features of malignant disease include stenosis or displacement of the pancreatic and bile duct (e.g. double duct sign), alteration of secondary branches

and extravasation of contrast dyes due to necrosis. An additional major role of ERCP is the opportunity to provide drainage of an obstructed common bile duct by facilitating the insertion of stents into the biliary system. Also tissue sampling can be performed during ERCP using brush cytology, direct biopsy, endoscopic needle aspiration and aspiration of pancreatic and bile fluid. The opportunity to obtain tumor material during endoscopic procedures might become an important step, since molecular markers can be applied to these samples and might assist in the differential diagnosis of pancreatic disease.

Therapy

Although there has been considerable progress in the biological understanding and diagnostic tools of pancreatic cancer, the neoplasm continues to have one of the poorest prognoses of all human cancers. Surgical resection of small tumors is the only option for curative treatment. The five-year survival is below 5% and due to advanced tumor stage at time of diagnosis, therapies remain palliative for the vast majority of patients. Five-year survival rates increase to 30% percent when tumors smaller than 2cm are resected.

The surgical technique is dependent on location of the tumor and includes local excision in papillary tumors and left hemipancreatectomy in tumors of the pancreatic tail. However, the majority of pancreatic cancers are located in the pancreatic head on which two methods of resection are most commonly employed. The classical method, the Whipple-Kausch pancreatectomy, involves en-bloc removal of 1) the distal third of the stomach and the right half of the greater omentum, 2) the gall bladder including the distal bile duct system, 3) the duodenum and the proximal 10cm of the jejunum, 4) the head and parts of the body of the pancreas and 5) the peripancreatic and hepatoduodenal lymph nodes (5). A second method has been developed and successfully performed in the recent years called the pylorus-preserving pancreatectomy (PPPD). Instead of resection of the distal stomach, the pylorus is attached directly to a jejunal loop in PPPD (6). It remains unclear if either method is superior, but advocates of PPPD claim better nutritional outcomes and shorter operative times compared to standard Whipple-Kausch pancreatectomy.

In patients with advanced disease, multidisciplinary approaches are needed, involving the surgeon, endoscopist and radiologist to optimize palliative therapy in the setting of a limited life expectancy. Operative bypasses including choledochojejunostomies and gastroenterostomies have many advantages and remain as highly acceptable choices in the management of terminal disease. Non-operative stenting should be reserved for elderly patients or patients with very advanced disease who are poor operative candidates.

The resistance of pancreatic cancer to adjuvant, neoadjuvant and palliative chemo- and/or radiotherapy has remained a consistent disappointment over the last decades. Recent trials with gemcitabine, a cytidin analogue, have shown a decrease of disease-related symptoms, thus benefiting the quality of life of the patient. Overall, there are no convincing data that any drugs significantly increase the survival of a patient. Such results emphasize the importance of developing new diagnostic methods of pancreatic cancer to allow surgical resection at an earlier tumor stage.

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Pancreas Cancer, clinical oncology

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Definition

Pancreatic cancer is an adenocarcinoma of the pancreas (carcinoma of the pancreas). Cancer of the pancreas is usually lethal. The pancreas is a gland that secretes both digestive enzymes and insulin (from special cells called islet cells).

Characteristics

Anatomy

The pancreas is located on the upper abdomen located in the midst of many vital organs, including the liver, spleen, stomach, small bowel and large bowel. Because of its central location, it is very problematic when cancer spreads from the pancreas directly into the adjacent organs. In addition, the head of the pancreas (the portion on the patients' right side) covers the common bile duct. The common bile duct is the duct through which bile runs from the liver and gall bladder, is mixed with pancreatic juices and then emptied into the small bowel. Blockage of this common bile duct can lead to one of the early symptoms of pancreatic cancer.

In addition, the location of the pancreas near the back (in an area called the retroperitoneum) is problematic as that is the area which contains important vessels, such as the superior mesenteric artery and veins and the celiac plexus. This means that a cancer in the pancreas will frequently (and very early in the course of the disease) invade these vessels, which enables the tumor to spread to distant sites by these vessels (such as to the liver or lung). Vessel invasion also makes the pancreas cancer inoperable (see below). There are also many nerves that are located behind the pancreas and those nerves frequently affected by cancer of the pancreas.

Etiology (cause of the disease)

The cause of pancreatic cancer is unknown. In about 8% of patients, the disease appears to be inherited (familial pancreatic cancer - see below). The primary risk factor for pancreatic cancer that is not inherited is smoking. Other risk factors include alcohol, a history of surgical procedures for peptic ulcer disease and a history of inflammation of the pancreas (pancreatitis). Diabetes is frequently associated with cancer of the pancreas. However, it is unclear if diabetes is a risk factor for the development of pancreatic cancer or whether it is just a result of the pancreatic cancer damaging the insulin-producing (islet cells) cells of the pancreas.

Pathology

It is very important to determine what type of cancer of the pancreas one is talking about. The types of cancer involving the pancreas are as follows;.

- ***Ductal adenocarcinoma of the pancreas***: This is the most common (accounts for more than 90%) and the most lethal form of the disease. It is believed that this type of cancer arises in the cells that line the ducts of the pancreas. Ductal adenocarcinoma rapidly invades blood vessels, nerves and other organs. It is frequently at an advanced stage when it is diagnosed and has a bad prognosis.
- ***Mucinous cystadenocarcinoma:*** This pathologic type is an uncommon form of pancreas cancer. It tends to be less invasive and mainly causes problems because of its size. The usual treatment is surgical removal, unless other vital organs, blood vessels or nerves are involved. It is not a benign condition.
- ***Islet cell tumor:*** These are cancers that appear to arise from the small clusters of cells

called islets, which are scattered throughout the normal pancreas. Islets have cells capable of making many different hormones including insulin and glucagon. Islet cell tumors can cause symptoms as the excessive hormones that they make (such as insulin) can cause severe physiologic problems (such as hypoglycemia or low blood sugar). In general, islet cell tumors have a far better prognosis than other types of cancer of the pancreas.

The sections below will only deal with the most common type of pancreas cancers - ductal adenocarcinoma of the pancreas

Symptoms

Unfortunately, the symptoms associated with ductal adenocarcinoma of the pancreas are fairly non-specific and appear late. They include pain in the midepigastric (stomach area) or the back (usually due to nerve invasion by the tumor), nausea and/or vomiting, fatigue, loss of appetite and weight loss. A change in bowel habits with light colored stools is also a sign of the disease, as is a severe darkening of the urine. One rather drastic symptom is the appearance of jaundice (a yellowness of the whites of the eyes and skin), which is caused by the tumor closing off the bile drainage such that the bile (containing the pigment bilirubin) cannot be secreted, builds up in the blood and is deposited in the skin.

Making the diagnosis

The most effective way to determine whether or not there is a mass in the pancreas is *via* a special X-ray known as a spiral CT scan. Another, perhaps more sensitive method, is called an endoscopic ultrasound (or EUS) in which a tube (endoscope) is passed through the mouth into the stomach and an ultrasound device at the end of the tube sends out signals that are used to detect a mass in the pancreas. If a mass is detected, it is critical to obtain a histologic diagnosis to determine if the mass is cancer (or just a benign inflammation). A histologic diagnosis is obtained by inserting a needle into the mass to look for tumor cells, or the histologic diagnosis is obtained by performing an open surgical procedure and biopsy of the pancreas.

Staging of the disease

Pancreatic cancer is staged as being localized, locally advanced or metastatic (with distant spread).

- ***Localized*:** This stage means the tumor is confined within the pancreas with no major blood vessel involvement or involvement of areas outside of the pancreas.
- ***Locally advanced:*** Tumor involves major blood vessels or regional lymph nodes, but no cancer in other organs. In general, the tumor can be encompassed by a radiotherapy port. There can be no spread to distant organs.
- ***Metastatic*:** The pancreatic cancer has spread beyond the pancreas (usually into the liver, other surrounding organs or lung).

Prognosis

Pancreatic cancer has the worst survival of any cancer. The overall one-year survival for all patients is about 18% with fewer than 2% of patients living 5 years. For patients with localized pancreatic cancer who have surgical resections of their disease and no evidence of tumor spread beyond the pancreas in their pathology resections there may be as many as 20% who survive 2 years. However, it is rare to find that the pancreatic cancer is truly localized. For patients with locally advanced pancreatic cancer, the average survival is about 10 months if the patient is treated with radiation to the area in addition to chemotherapy (see below). For patients with advanced metastatic pancreatic cancer, the average survival (with treatment) is about 6 months.

Thus, the diagnosis of pancreatic cancer comes with a terrible prognosis. In order to make progress against the disease, it is important that new therapies be developed.

Treatment

- For patients with localized disease, the treatment is surgical resection. This gives the patient their only chance for prolonged survival (if the patient is fortunate to have truly localized disease). The surgical resection of the pancreas with surrounding organs and bowel reconstruction is frequently referred to as the Whipple procedure. As noted above, even with localized disease with no invasion noted on scans at the time of surgery, the pancreatic cancer frequently is found to have spread beyond the pancreas. Therefore, an important area of research is to use a neoadjuvant therapy approach. The idea with neoadjuvant therapy is to give therapy, such as radiation therapy, chemotherapy, or both, before surgery to try to 'down stage' the disease. Hopefully, this approach will help make more patients truly operable with complete removal of their tumor.
- For patients with locally advanced disease, there is controversy in terms of what constitutes the best treatment. The standard treatment is considered to be radiation plus chemotherapy (usually the drug, 5-fluorouracil) to sensitize the tumor to the radiation. This treatment has been reported to increase the average survival for a patient with locally advanced disease from 5 months up to 10 months. However, the radiation plus chemotherapy regimen is associated with substantial side effects. Therefore, a better approach (perhaps chemotherapy alone) for patients with locally advanced pancreatic cancer is being investigated.
- For patients with metastatic pancreatic cancer, treatment is usually chemotherapy or supportive care (pain control and treatment of other medical problems) only. Until recently there has been no chemotherapy that has improved patient survival. Recently, the new anticancer agent, gemcitabine, has been shown to improve the survival of patients with advanced pancreatic cancer, plus improve the quality of life of the patient (decreased pain, improvement of performance status). When patients with advanced pancreatic cancer were treated with gemcitabine, their survival was improved from 2% (in the control arm treated with 5-fluorouracil) up to 18% for patients receiving gemcitabine. Currently, investigators are building on this modest advance against advanced disease by combining gemcitabine with other new cancer therapy approaches.

Familial pancreatic cancer

Only about 3-8% of pancreatic cancers is thought to be familial. It is an area of intense study to determine if you have a parent or brother and/or sister with the disease, what type of monitoring and/or treatment is necessary. It is clear that some type of monitoring, such as endoscopic ultrasound, should be performed at special centers.

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Pap Smear

Definition

A Pap, or Papanicolaou smear, is a test where cells shed from the cervix, vagina and endometrium are collected as smears, prepared by special staining and then microscopically examined; → Papanicolaou test.

Papanicolaou Test

Definition

A Papanicolaou test is the microscopic examination of exfoliated cells obtained by swabs from the uterine cervix (cytology). Abnormal cells showing morphologic signs of papillomavirus infection and/or malignant changes can be identified, in which case usually a direct inspection of the cervix (colposcopy) is performed, eventually followed by biopsy and histological diagnosis. Cytology is currently used as the method in cervical cancer screening programs. Because of the relatively high false negative rate of cytology, it is being evaluated if detection of papillomavirus infection may become a supplementary method in cancer screening.

Papillomas

Definition

Papillomas are benign wart-like tumors seen in epithelial tissues such as skin and formed as a result of tumor promotion.

PAR-4

Definition

Prostate apoptosis response protein 4 (Par-4) is a transcriptional repressor.

Paracrine

Definition

Paracrine is a factor acting on the immediate neighbours of the cells that produce it. Following interaction with the related receptor(s), a paracrine activation is obtained (→ autocrine; → receptor tyrosine kinases). Refers to a short-range biologic action mediated by a soluble factor produced by one cell type that acts on another cell type. For example, it was initially thought that → scatter factor was produced only by mesenchymal cells such as fibroblasts and acted solely in a paracrine fashion on nearby epithelial cells through their c-Met receptors. However, scatter factor may promote tumorigenesis by → autocrine as well as paracrine loops. An example of the latter is the ability of scatter factor produced by primary tumor cells (eg., glioma or breast cancer cells) to stimulate tumor growth by inducing → angiogenesis in the host stroma in experimental mouse and rat tumor models.

Paracrine Stimulation

Definition

Paracrine stimulation is the stimulation of neighbouring cell types; → receptor tyrosine kinases.

Parafollicular Cells

Definition

→ Thyroid C cells.

Parathyroid Hormone-related Protein

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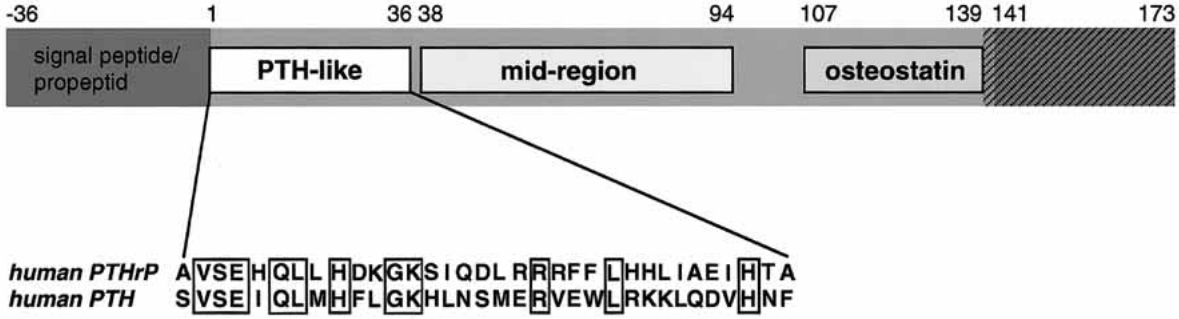
Synonyms

- PTHrP (parathyroid hormone-related protein)

Definition

PTHrP was identified as a tumor-derived humoral agent whose activities resembled those



Parathyroid Hormone-related Protein. Fig. – Initial translational products of human PTHrP. Translation of different PTHrP mRNA species results in the production of three PTHrP precursor isoforms (-36/139, -36/141 and -36/173). All three isoforms carry the PTH-like, the mid-region, and the osteostatin activities. As a secreted protein, PTHrP contains a signal sequence that is located within the first 36 N-terminal amino acids and mediates the translocation of PTHrP into the lumen of the endoplasmic reticulum.

of parathyroid hormone (PTH), the main regulator of calcium homeostasis. PTH and PTHrP bind to the same receptor, the PTH1R or PTH/PTHrP receptor. This interaction leads to bone resorption and calcium reabsorption in the kidney. PTHrP is responsible for the humoral hypercalcemia of malignancy (HHM), a common metabolic disorder in patients with neoplastic disease.

Characteristics

PTHrP is a secretory protein that primarily acts locally in an autocrine or paracrine fashion. Only when overproduced, for example by the mammary gland in nursing women, by the placenta in pregnant women or by the tumor tissue in cancer patients, does PTHrP enter the blood stream in amounts sufficient to unleash its calcium-mobilizing PTH-like activities. PTHrP undergoes extensive post-translational processing giving rise to peptides of different biological activities. N-terminal peptides are capable of interacting with the PTH/PTHrP receptor resulting in an increased cAMP level in target cells. Fragments containing the mid-region of PTHrP have the potential to prevent cells from undergoing apoptosis, requiring this peptide to translocate into the nucleolus. C-terminal PTHrP peptides show 'osteostatin' activity, counteracting bone resorption.

PTHrP is a multifunctional protein produced by almost every tissue. In development, PTHrP plays a crucial role in proliferation, differentiation and apoptosis. Disruption of the *pthrp* gene is lethal in mice due to abnormalities in cartilage development, resulting from increased apoptosis of chondrocytes. In the developing mammary gland, PTHrP is an essential paracrine factor for the epithelial/mesenchymal signal circuit required for branching morphogenesis and sexual dimorphism. PTHrP is needed for tooth eruption due to its stimulating effects on the bone resorption of the alveolar bone. PTHrP is important for the normal development of the skin as it regulates the differentiation of keratinocytes. Also, PTHrP is likely to play a role in pancreatic and renal development and may even have a function in early embryogenesis, in particular in the formation of parietal endoderm. In adults, proliferative or antiproliferative effects on regeneration processes, such as wound healing, have been attributed to PTHrP. An important role of PTHrP in controlling the vascular tone is emerging. As a vasorelaxant PTHrP regulates the contractility of many different vascular beds, including kidney microvessels and the arteries and venes of the mammary gland. During pregnancy, PTHrP is essential for the fetal-placental calcium transport, an activity that seems to be dependent upon the mid-regional part of PTHrP. Furthermore, a role of PTHrP in the survival of neurons is discussed.

PTHrP production is primarily regulated at the transcriptional level. In humans, *pthrp* gene

activity is under the control of three different promoters (P1-P3). The human P3, and the corresponding murine promoter, contain a conserved Ets/Sp1 composite enhancer element conferring the responsiveness of these promoters to Ets1, Sp1, retinoic acid, HTLV-I Tax and the → adenovirus E1A oncogene. PTHrP expression can be further regulated by growth factors such as TGFβ, or by steroids such as vitamin D3. Signaling pathways involving → PKC, Ras or cAMP can also affect PTHrP transcription.

Clinical Relevance

PTHrP is expressed by many tumors including carcinomas of the breast, kidney, prostate, colon, stomach and lung, as well as by some haematopoietic malignancies such as the → HTLV-I induced adult T-cell leukemia. PTHrP complicates cancer by inducing hypercalcemia. PTHrP production by tumors is often increased in later stages, suggesting that PTHrP may be beneficial to tumor progression. This notion is supported by the observation that PTHrP promotes metastasis of breast and lung carcinoma cells to bone due to its stimulatory activity on bone resorption. Furthermore, PTHrP protects prostate cancer cells from apoptosis and stimulates the growth of a number of cancer cell lines.

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PARP

Definition

→ Poly(ADP-ribose) polymerase (PARP) modifies nuclear proteins in a DNA-dependent manner. It has important function in a wide spectrum of cellular processes such as cellular differentiation, proliferation, cell transformation and in the recovery of cells after DNA-damage involved in → apoptosis. It is a protein of 1013 aa and 112 kD, and the gene locus maps to 1q42; → poly(ADP-ribosyl)ation.

PARP Inhibitors

Definition

→ Poly(ADP-ribose) polymerase inhibitors are a large number of low molecular weight compounds (NAD^+ analogs) that competitively inhibit PARP activity. Amongst the first to be described were nicotinamide, benzamide and 3-aminobenzamide. However, their potency and specificity is rather low. Subsequently, more sophisticated new compounds have been developed such as 4-amino-1,8-naphthalimide, 3,4-dihydro-5-methoxyisoquinolin-1(2H)-one (PD 128763), 8-hydroxy-2-methylquinazolin-4(3H)-one (NU1025) or 2-methylbenzimidazole-4-carboxamide (NU1064), to name but a few. These are much more potent than the first generation inhibitors and possess an improved pharmacokinetic profile.

With respect to specificity, it should be noted that while all inhibitors inhibit PARP-1 (by definition), at least some of the first generation inhibitors also interfered with other ADP-ribosyl transfer reactions, such as mono-ADP-ribosylation of proteins or NAD^+ glycohydrolases, albeit at different IC50 levels. Furthermore, the novel poly(ADP-ribose) polymerases recently identified are likely to be inhibited as well, perhaps at similar IC50 levels as PARP-1; → poly(ADP-ribosyl)ation.

Parvovirus

Definition

The term parvovirus describes a family of small, single-stranded DNA-containing viruses.

Patched

Definition

The patched gene encodes a putative membrane protein that acts as the ligand-binding component of the receptor complex for secreted → hedgehog proteins. There are two vertebrate patched genes, patched 1 and patched 2. The abbreviations used for patched are *ptc* in *Drosophila, Ptc* in the mouse and *PTCH* in humans.

PCNA

Definition

→ Proliferating cell nuclear antigen.

PCR

Definition

Polymerase chain reaction (PCR) is a method to amplify specific fragments of DNA or RNA.

PDD

Definition

→ Photodynamic diagnosis.

PD-ECGF

Definition

Platelet-derived endothelial cell growth factor (PD-ECGF) is an angiogenic factor for various organs and cancers and has been identified as thymidine phosphorylase (TP).

PDGF

Definition

→ Platelet-derived growth factor.

PDGFR

Definition

Platelet-derived growth factor receptor.

PDK1

Definition

Phosphoinositide dependent kinase 1

PDT

Definition

→ Photodynamic therapy.

PECAM

Definition

Platelet endothelial cell adhesion molecule-1 (PECAM-1) is a 130 kD glycoprotein of the immunoglobulin gene superfamily that localizes

to points of contact between confluent endothelial cells. It is phosphorylated by c-→ Src, and this phosphorylation is though to transduce cell migration signals to the cell, pointing to a role for both PECAM-1 and c-Src in → angiogenesis and → metastasis.

Pegylated Interferons

Definition

Pegylated interferons are interferons that are chemically modified by the covalent attachment of poly-ethylene glycol (PEG), which are used in antiviral therapies; → hepatitis C virus.

Pelvic Exenteration

Definition

Pelvic exenteration is the resection of the uterus, ovaries and fallopian tubes with the bladder and/or rectum in addition to regional lymphadenectomy.

Penetrance

Definition

Penetrance is a genetic term that describes the proportion of individuals with a specific genotype who express that character in the phenotype; the frequency with which a genetic trait is expressed phenotypically. For example, if by age 70, a disease had occurred in 60% of carriers of a particular mutation in gene, the penetrance would be 60% at age 70.

Pentagastrin

Definition

Pentagastrin is a hormone that has the property, among others, of stimulating thyroid C cells to release their stored calcitonin into the blood; → MEN 2.

Peptide Library

Definition

A peptide library is a collection of peptides with as many as several billion individual sequence permutations; → homing peptides.

Performance Status

Definition

Performance status is a scale used by clinicians to judge how patients are doing in terms of every day performance of their activities of daily living. There are two major performance scales utilized, including the Karnofsky Performance Status (KPS) that ranges from 0 to 100 in increments of 10s, with 100 being asymptomatic patients and 0 being dead. The other scale is the World Health Organisation (WHO) scale that ranges from 0 to 5 (in increments of ones), with 0 being in perfect condition and 5 being dead.

Pertussis Toxin

Definition

Pertussis toxin is produced by *Bordetella pertussis* and is a toxin that catalyzes the ADP-ribosylation of some → G-proteins at a cysteine residue near the C-terminus, resulting in uncoupling of receptors and G-protein.

PEST

Definition

PEST is a sequence in proteins consisting of proline (P), glutamate (E), serine (S) and threonine (T) that expedites the degradation of the protein. It has been shown experimentally that the PEST sequence can act as a modular domain to promote the physical association with and subsequent degradation by a protease such as calpain.

Peutz-Jeghers-Syndrome

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Synonyms

- Peutz-Touraine-syndrome
- hereditary hamartosis Peutz-Jeghers
- Hutchinson-Weber-Peutz-syndrome
- Lentiginosis polyposa Peutz
- PJS

Definition

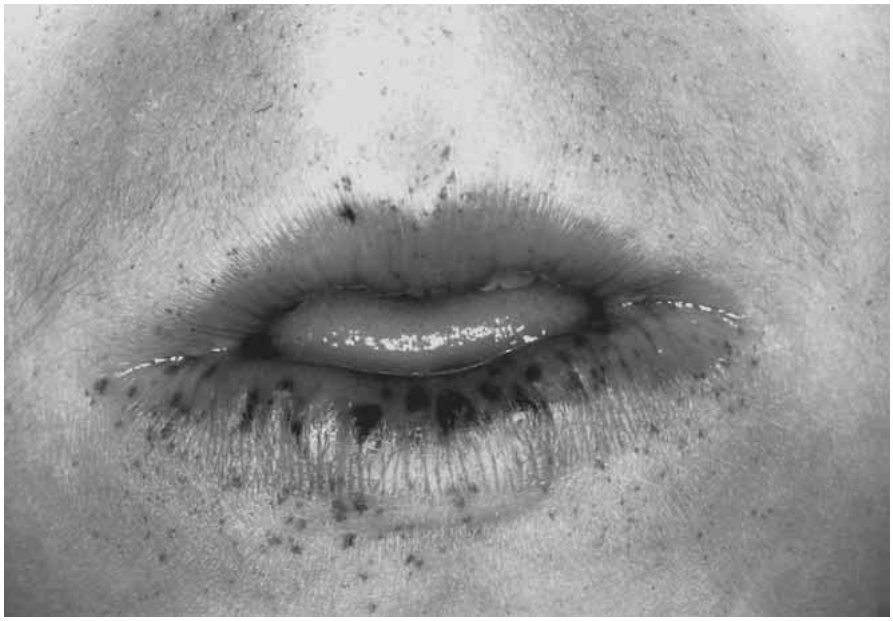
Peutz-Jeghers-syndrome is an autosomal dominantly inherited disorder that is characterized by the combination of:

- Lentiginosis, i.e. typical pigmented lesions.
- Hamartomatous polyposis that occurs mainly in the small intestine but also in the colon and the stomach. Extraintestinal hamartomas are rare; possible localizations include the gallbladder, the urinary bladder, the heart, and the respiratory tract.
- Increased risk for various types of cancer (e.g. Pancreas, gastrointestinal tract, bilateral breast cancer, rare gynecological tumors).

The clinical diagnosis is considered established when either two or more hamartomas or at least one hamartoma together with pigment spots or one hamartoma and positive familial history are found.

Characteristics

Although Peutz-Jeghers syndrome is autosomal dominantly inherited, a large number of sporadic cases (possibly new mutations) occur. The



Peutz-Jeghers-Syndrome. Fig. – Typical pigmented lesions perioral and on the lips.

disease is equally distributed between the sexes and the races. The clinical problems are bleeding, pain and intussuception caused by the intestinal hamartosis and the increased risk for various tumors. The majority of cases is caused by mutations in a gene called *STK11* or *LKB1* on chromosome 19p13.3, however, in some cases this gene was found not to be mutated. It seems therefore probable that mutations in a second (yet unknown) gene may also cause PJS. Since the syndrome is extremely rare, the overall rate of occurrence is not known.

Lentiginosis (pigment spots)

The typical pigmented spots occur mainly around the mouth, on the lips and in the buccal mucosa but may also be found on the hands, the fingers, the feet and around nose and eyes. In size the spots range from few millimeters up to up to approximately 1 cm in diameter. They are not elevated above skin level. Their color may vary from light brown to nearly black. They may be present at birth or can emerge during the first years of life, although in some cases first spots appear in higher age. In most patients the spots change during life, loosing intensity with increasing age. Histopathologically they show an increase of melanin and melanocytes in the basal layer of the epidermal epithelium. No malignant transformation has ever been reported. If they represent a cosmetical problem laser treatment is possible. Five to fifty percent of the PJS patients have no pigmented spots.

Hamartomatous polyposis

The hamartomatous polyps are mainly localized in the jejunum followed by the duodenum, the ileum, the colon and the stomach. Other localizations like the esophagus, the gall bladder, the urinary bladder, the heart and the respiratory tract have been described but remain extremely rare.

The main problems caused by the intestinal polyps are intussuception, bleeding and pain. Intussuception leading to bowel obstruction and necrosis is the main cause for emergency surgery in those patients. The age range where symptoms are initiated, reaches from newborn babies to old age. Initial clinical symptoms first occur in 33% of patients in the first decade of life, further 33% in the second.

The polyps in the intestine show a typical histopathological picture: intact intestinal goblet cell rich mucosa covers branching bundles of smooth muscle from the lamina muscularis mucosa. These polyps are usually benign but in some cases adenomatous and carcinomatous changes have been reported, leading to the suggestion of a hamartoma-adenoma-carcinoma sequence.

Increased risk for malignant tumors

An increased risk for a wide variety of malignant tumors in PJS-patients has been reported by several authors. The risk to develop of cancer is increased 9 to 18-fold.

The most frequent tumor sites include the gastrointestinal tract (stomach, small intestine, colon, the pancreas (here, according to one study the relative risk for cancer development is 100), the lung and the female breast (often bilateral). Furthermore, many patients develop tumors of the urogenital tract. In males Sertoli-cell tumors are found. In females rare tumors such as the benign bilateral form of the SCTAT (sex cord tumor with annular tubes) and the adenoma malignum of the uterine cervix is found next to the normal carcinomata of the ovaries, the cervix and the myometrium.

Furthermore, malignant tumors in PJS-patients have been found in almost all organ systems. It is therefore impossible to exclude any tumor from the spectrum found in PJ-syndrome.

Pathogenesis

Most cases of PJS are caused by a mutation in the *STK11* or *LKB1* gene. The gene product has a strong homology to the serine-threonine protein kinase XEEK1 from Xenopus. Recent results showed that the STK11/LKB1 protein is nuclear as well as cytoplasmic. It has a growth-suppressing activity which is mediated by a G(1) cell-cycle arrest.

The gene has been investigated in many different sporadic tumors like colorectal, gastric,

testicular, pancreatic, ovarian, and breast cancer, in malignant melanoma as well as in different tumor cell lines. Mutations of the *STK11/LKB1* gene were rare in these tumors, suggesting a minor role in the evolvement of sporadic tumors.

Loss of heterozygosity of the wild-type allele has been demonstrated in the hamartomatous polyps and in 70% of the malignant tumors investigated from PJS patients.

In a minority of PJS cases no *STK11/LKB1* mutation has been found, suggesting a second gene, responsible for the cases of PJS.

Screening recommendations

Regular examinations of PJS-patients have two different aims: 1) to avoid complications caused by intestinal polyps such as bleeding, pain, intussuception and bowel obstruction, 2) the early diagnosis of cancer or precursor lesions.

For the first aim, regular screening of the gastrointestinal tract is recommended. The second one is more difficult to achieve since malignant tumors have been reported in virtually all organ systems of PJS-patients. To date, there is no consensus what is the most adequate screening program for people at risk.

The 'Deutsche Krebshilfe' (German Cancer Aid Society) recommends anual physical examinations, starting at age 12. Endoscopy of colon and stomach and MR-Sellink procedure of the small bowel should start at age 12 and be repeated every two years.

However, in a case report, first symptoms of PJS have been described as early as within the first days of life. It has also been shown that one third of patients become symptomatic during the first decade of life. The 12-year of age threshold as a starting point for regular examinations is therefore debatable.

Should symptoms occur in people at a younger age that carry the gene or that are, with an otherwise unknown genetic status, at risk, immediate clarification is warranted.

In female patients, regular gynecological examination, including vaginal ultrasound, should start at the age of 18 and be repeated on an annual basis. In male patients, regular examination of the genital organs is required in order to detect Sertoli-cell-tumors.

At the age of 25, bilateral mammography in females should commence on a annual basis and pancreatico-biliary MRT (MRCP) is recommended to be carried out every other year.

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Peutz-Touraine-Syndrome

Definition

→ Peutz-Jeghers-syndrome.

P-glycoprotein

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Synonyms

- multidrug transporter
- Mdr1 protein
- gp170

Definition

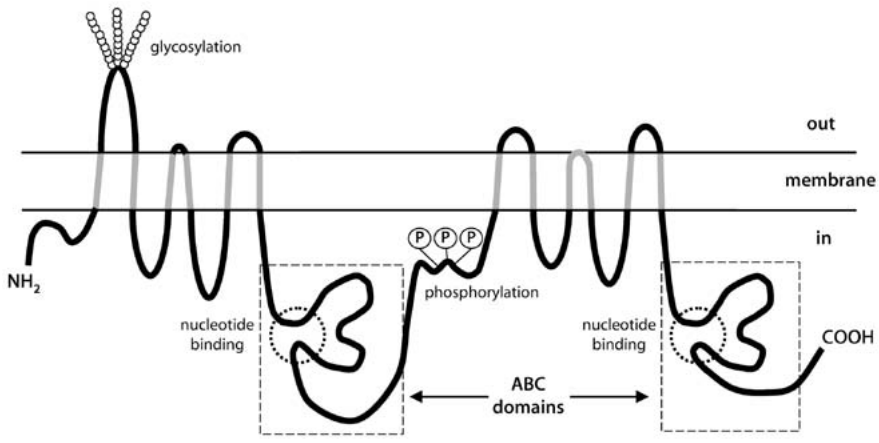
P-glycoprotein (Pgp), the product of the human *MDR1* gene responsible for a major form of → multidrug resistance in tumor cells, is a trans-

membrane protein that carries out ATP-dependent efflux of various lipophilic compounds, including many anticancer drugs. Pgp renders tumor cells resistant to such drugs, and Pgp expression has been shown to correlate with clinical drug resistance or negative prognosis in several types of cancer.

Characteristics

The best known form of multidrug resistance in mammalian cells involves cross-resistance to a large group of lipophilic drugs with different structures and mechanisms of action. Cellular targets of such drugs include microtubules (vinblastine, vincristine, taxol, colchicine), topoisomerase II (doxorubicin, etoposide), RNA polymerase (actinomycin D), ribosomes (puromycin), plasma membrane (gramicidin D) and mitochondria (rhodamine 6G). This multidrug resistant phenotype is due to increased expression of the gene termed *MDR1* (multidrug resistance 1). Expression of the *MDR1* gene in drug-sensitive cells is sufficient to produce the full pattern of multidrug resistance. Rodent cells carry two homologs of the human *MDR1* gene, both of which confer multidrug resistance. Another member of the *MDR* gene family, which is alternatively called *MDR2* or *MDR3* in humans, is closely related to *MDR1* but does not confer detectable multidrug resistance (the product of the gene functions as a phosphatidylcholine → translocase).

The resistance of *MDR1*-expressing cells was shown to result from decreased intracellular accumulation of the drugs, associated with an increased rate of drug efflux. Many lipophilic fluorescent dyes (e.g. Rhodamine 123, Hoechst 33342, calcein AM) also show decreased accumulation in *MDR1*-expressing cells. The exclusion of such dyes provides a convenient approach to the detection of multidrug resistant cells. The *MDR1* gene encodes a glycoprotein with a mobility of approximately 170 kD. This protein was originally found to be elevated in the membranes of multidrug resistant cell lines and was termed P- (for permeability) glycoprotein (Pgp). Pgp, encoded by the human *MDR1* gene, contains 1280 amino acids and consists of two similar halves separated by a short linker region containing multiple charged residues and phosphorylation sites (Fig. 1). Each of the two halves of Pgp contains a N-terminal hydrophobic and a C-terminal hydrophilic region. Each hydrophobic region includes six membrane spanning α-helices. The transmembrane segments were originally deduced through hydrophobicity analysis and subsequently confirmed by epitope mapping studies. Some studies suggest, however, the existence of one or more alternative transmembrane orientations for Pgp, which may coexist with the orientation shown in Fig. 1. Pgp conformation can be altered through the binding of different ligands, as indicated by differential immunoreactivity, altered patterns of proteoly-



P-glycoprotein. Fig. 1 – Scheme of the two-dimensional structure and transmembrane orientation of Pgp.

tic digestion and some other assays. However, it is unknown whether these conformational transitions involve changes in the transmembrane orientation of Pgp.

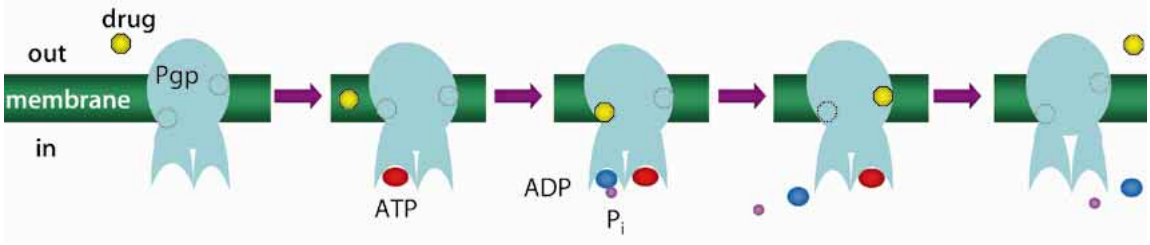
The hydrophilic regions of Pgp contain nucleotide binding sites and display the characteristic sequences of the ATP-binding cassette (ABC), a conserved domain shared by a large superfamily of proteins known as → ABC transporters. Some other ABC transporters are also involved in multidrug resistance, including the → multidrug-resistance protein (MRP) family and the MXR/BCRP/ABCP1 protein. The ABC domains of Pgp are responsible for its ATPase activity, which enables the pumping of Pgp transport substrates against the concentration gradient (Fig. 2). The ATPase activity of Pgp is strongly stimulated by many of its transport substrates. The presence of functional nucleotide binding sites in both ABC domains is required for both the ATPase activity and the drug efflux by Pgp.

All the documented Pgp transport substrates are lipophilic or amphiphilic, but not all the lipophilic compounds are transported by Pgp. A common functional feature of Pgp substrates is their entry into a cell by passive diffusion through the lipid bilayer and not through a specialized carrier. In agreement with this mode of uptake, there is substantial evidence that the initial binding of many substrates to Pgp occurs within the lipid bilayer rather than on the cytoplasmic surface of the plasma membrane. Photoaffinity labeling assays have implicated the transmembrane domains of Pgp as the primary sites for substrate binding. The ability of Pgp to transport different substrates can be increased or decreased by point mutations of specific amino acid residues. Most (but not all) of such residues localize to transmembrane domains. Mutations that affect the specificity of Pgp-mediated transport were found to affect substrate binding or release and the ability of individual substrates to stimulate the ATPase activity of Pgp.

In addition to its function as an efflux pump for different lipophilic compounds, Pgp was shown to act as a short chain lipid translocase in the plasma membrane. Pgp also confers resistance to → apoptosis induced by different agents (which need not be transported by Pgp), through an unknown mechanism. Other changes associated with Pgp expression in some cell lines include stimulation of volume regulated chloride channels, alteration of intracellular pH and increased ATP release. However, the putative role of Pgp in these phenomena requires further documentation.

Expression

Pgp is normally expressed in a variety of human tissues, including the adrenal cortex, lumenal surfaces of the kidney, liver, colon and jejunum, and endothelial capillaries of the brain, testes and papillary derma. It is also expressed in placental trophoblasts, in certain types of lymphocytes (particularly CD56-positive natural killer cells and CD8-positive T-cells) and in early hematopoietic progenitor cells of the bone marrow, where Pgp expression is positively correlated with the stem cell marker CD34. Analysis of mice with a knockout of one or both of their MDR1 homologs revealed some of the physiological functions of Pgp. Pgp knockout mice show a large increase in the accumulation of different drugs in the brain, indicating that Pgp in brain endothelial capillaries plays an im-



P-glycoprotein. Fig. 2 – A simplified hypothetical scheme of drug efflux by Pgp.

portant role in the blood-brain barrier. The knockout mice also show an increase in drug accumulation in the intestine and cardiac tissue, indicating that Pgp plays a physiological barrier role in several types of tissues.

Cellular and molecular regulation

Overexpression of Pgp in multidrug resistant cell lines, isolated after multistep selection with chemotherapeutic drugs, is frequently associated with the → amplification of the *MDR1* gene. Such amplification, however, appears to be specific for *in vitro* selection, since it occurs rarely if at all in Pgp expressing tumors. Similarly, point mutations of the *MDR1* gene that increase its ability to transport specific drugs have been found *in vitro* but not *in vivo*. Another mechanism of *MDR1* gene activation *in vitro* involves genetic rearrangements that bring the previously silent *MDR1* gene into proximity with a strongly transcribed gene, sometimes providing *MDR1* with an alternative transcription initiation site. There is evidence that such rearrangements may also occur in certain lymphoid malignancies. In most cases, however, increased *MDR1* mRNA expression does not involve any detectable genetic changes. Several *in vitro* studies and *in vivo* observations with tumors undergoing chemotherapy indicate that the *MDR1* gene can be activated epigenetically in response to different forms of cytotoxic stress or after treatment with protein kinase C-inducing agents. This activation may occur both at the level of transcription and of RNA stability. In particular, two CCAAT box-binding proteins, NF-Y and YB-1, were reported to mediate the induction of the *MDR1* promoter by genotoxic stress, but it is still unknown to what extent these transcription factors are responsible for *MDR1* activation during cancer chemotherapy.

At the protein level, Pgp undergoes post-translational modification through N-linked glycosylation in the first extracellular loop and phosphorylation by protein kinase C and protein kinase A in the linker region (Fig. 1). Neither of these Pgp modifications appear to be essential for the drug efflux function, as indicated by the activity of glycosylation- or phosphorylation-deficient mutants of Pgp in drug resistance assays. The phosphorylation mutants, however, show some quantitative changes in the stimulation of their ATPase activity by Pgp transport substrates. Another potential level of Pgp regulation is suggested by the observed variability in the percentage of Pgp that localizes to the plasma membrane (as opposed to Golgi or endoplasmic reticulum) in different cells. Modulation of the Pgp transport to the plasma membrane may represent another mechanism of its regulation.

Clinical Relevance

Pgp expression in clinical cancers has been extensively analyzed both at the RNA and protein level. These studies showed that, with regard to Pgp expression, most tumors can be divided into two classes. The first class is represented by those tumors that arise from Pgp expressing normal tissues, such as renal cell carcinomas, hepatomas, adrenocortical tumors or colorectal cancers. Pgp is commonly expressed in such tumors whether treated or untreated, and Pgp levels in some of these cancers show a positive correlation with morphological differentiation. Acute myelogenous leukemias (AML) may also belong to the same category. As stated above, Pgp expression in normal hematopoietic progenitor cells correlates with the expression of the stem cell marker CD34, and similar correlations for CD34 and Pgp were also reported in AML. Higher Pgp expression in more primitive hematopoietic cells might also account for the high levels of Pgp observed in the blast crisis of chronic myelogenous leukemia, in contrast to low or undetectable expression in the chronic phase.

The second class of tumors arise from normal tissues with low Pgp expression (carcinomas of the lung, ovary or breast, sarcomas, some types of leukemias). Pgp expression in untreated tumors of this class is usually low and it is often undetectable even by the most sensitive assays. Pgp expression in such tumors is frequently increased after chemotherapeutic treatment. It is unknown, however, whether this increase represents the selection of drug-resistant cells by anticancer drugs, results

from epigenetic *MDR1* induction by cellular damage or perhaps even represents a treatment-unrelated parameter of tumor progression. A number of studies showed a strong correlation between *MDR1* expression, on one side, and clinical drug resistance or the overall negative prognosis, on the other side. The strongest correlations of this type were reported in pediatric solid tumors (neuroblastomas, sarcomas, retinoblastomas). There are also many reports that Pgp is a negative prognostic factor in various adult cancers, such as ovarian, breast, esophageal and small cell lung cancers, as well as myelomas, leukemias and lymphomas. Not all investigators, however, have observed such correlations for Pgp, a conundrum which probably reflects the variability and poor standardization of diagnostic methods for Pgp detection.

Given the clinical significance of Pgp expression, considerable efforts have been placed into developing and testing agents that reverse Pgp-mediated multidrug resistance, as non-toxic supplements to chemotherapeutic drugs. Many agents (some of which are themselves transported by Pgp) were found to inhibit drug binding or transport by Pgp and to sensitize Pgp-expressing cells to anticancer drugs *in vitro* and in animal models. The first generation of agents to undergo clinical trials included Pgp inhibitors that had already been used in clinical practice, such as verapamil or cyclosporin A. The second generation comprises much more potent compounds developed specifically for Pgp inhibition, such as Valspodar (PSC-833), a non-immunosuppressive cyclosporin analog. Clinical trials of Pgp inhibitors showed a moderate but significant improvement in the treatment response of several hematologic malignancies, but solid tumor trials have not yet shown unambiguous benefits. Pgp inhibitors in clinical trials were found to increase the plasma drug concentrations, apparently through the inhibition of Pgp function in secretory organs. There are also reports of increased systemic toxicity in the presence of Pgp inhibitors, but such side effects appear to be clinically manageable. It remains to be determined whether the relatively disappointing results of the Pgp inhibitor trials may be improved through the use of inhibitors with different pharmacokinetic properties (such as inhibitory anti-Pgp antibodies) or different clinical protocols. It is also possible that the inhibition of only one of several mechanisms of drug resistance that exist in tumor cells may be insufficient to achieve clinical benefit.

Another Pgp-based therapeutic approach that has entered phase I clinical trials involves the use of the *MDR1* gene for gene therapy aimed at chemoprotection of hematopoietic stem cells. Several animal studies with *MDR1*-containing retroviral vectors have shown that this approach can provide chemoprotection against Pgp-transported drugs (principally taxol). It is conceivable, however, that some of the observed effects may not only be due to the effects of Pgp on taxol but to a general effect of *MDR1* on stem cell maintenance, possibly due to the antiapoptotic effect of Pgp. While the animal studies have been promising, substantial technical improvements in the methodology of gene transfer into human hematopoietic stem cells are needed to elucidate the clinical potential of this approach.

Other Pgp-oriented therapeutic approaches are being explored in preclinical studies. One such strategy is aiming Pgp inhibitors not at the multidrug-resistant tumor cells but rather at the blood-brain barrier, which may be permeabilized by inhibiting Pgp in brain endothelial cells. Such permeabilization would allow Pgp-transported drugs to be delivered into the brain, a known pharmacological 'sanctuary' of tumor metastases. Another potential strategy aims to prevent the conversion of Pgp-negative tumors to Pgp-positive. As described above, *MDR1* expression is induced epigenetically in response to cellular damage with chemotherapeutic drugs. This induction may be prevented *in vitro* by combining cytotoxic drugs with the inhibitors of protein kinase C and several other signal transduction pathways. The potential *in vivo* utility of these novel approaches remains to be explored.

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PGP

Definition

→ P-glycoprotein.

Phagocytosis

Definition

Phagocytosis is the intake of large particles, such as bacteria or parts of broken cells, and should not be considered an authentic endocytic process. It is used by many protozoans to ingest food particles and by blood cells (macrophages) to take in and destroy bacteria. After the binding of the target particle to the cell surface, the plasma membrane expands along the surface of the particle and eventually engulfs it. Vesicles formed by this process are much larger (1 to 2 μm) than those formed by → endocytosis.

Pharmacogenetics

Definition

Pharmacogenetics is the clinically important hereditary variation in drug response; → metabolic polymorphisms and cancer susceptibility.

Phenotype

Definition

Phenotype is the appearance or other characteristics of an organism, resulting from the interaction of its genetic constitution with the environment.

Pheromonal

Definition

Pheromones, as related to pheromonal, are substances that are secreted to the outside of an individual organism and sensed by a second individual of the same species.

Philadelphia Chromosome

Definition

The Philadelphia chromosome was the first cytogenetic abnormality associated with a malignancy. This chromosomal lesion results from a reciprocal translocation between the long arms of chromosomes 9 and 22 - t(9;22) (q34;q11). It was originally described in patients with chronic myeloid leukemia and subsequently in patients with acute leukemia. The term Philadelphia chromosome refers to the chromosome 22 lacking approximately half of its long arm. The translocation disrupts the normal ABL and BCR genes on chromosomes 9 and 22, resulting in a chimeric → BCR-ABL gene that encodes a fusion product with transforming ability.

Phorbol Ester

Definition

A phorbol ester is a diterpene ester isolated from croton oil produced by the plant croton

tiglium. 12-O-tetradecanoyl-13-acetate or TPA is a prototype phorbol ester that shows high activity as a tumor promoter and high activity for activating its receptor; → protein kinase C.

Phosphatase

Definition

A phosphatase is a specialized protein possessing enzymatic activity that catalyzes the removal of phosphate groups from amino acids, fatty acids, sugars or nucleic acids. It has the opposite effect to a kinase.

Phosphatidylinositol-3-kinase

Definition

Phosphatidylinositol-3-kinase (PI3K) is a lipid kinase enzyme that functions as a key intermediary in a variety of signal transduction pathways, including several distinct pathways originating from the → scatter factor-activated c-→ Met receptor. PI3K plays pivotal roles in scatter factor-mediated pathways for cell motility, morphogenesis and cell survival. It consists of an 85 kD regulatory subunit and a 110 kD catalytic (enzymatic) subunit. The regulatory subunit binds to and is phosphorylated by activated c-Met receptor, resulting in stimulation of PI3K-lipid kinase catalytic activity. PI3K is also activated indirectly through an interaction with the → Gab1 adaptor protein, and is an effector protein of → Ras, interacting with GTP-bound Ras to transmit the signal (Ref.: Marshall C(1998) Ras effectors. Curr Opinion Cell Biol 8: 197-204).

Phosphodiesterase

Definition

Phosphodiesterase is a group of enzymes that hydrolyze the phosphodiester bond in cyclic nucleotides, like cAMP and cGMP, to form AMP or GMP. This is the main inactivation mechanism for cyclic nucleotides involved in signal transduction.

Phospholipase C

Definition

Phospholipase C is a ubiquitous enzyme that catalyses the cleavage of phosphatidyl inositol 4,5-bisphosphate into inositol 1,4,5 trisphosphate (IP3) and diacyl glycerol (DAG). IP3 releases intracellularly stored Ca^{2+} resulting in an increase in the free cytosolic Ca^{2+} concentration, while DAG regulates various effectors like some isoforms of → protein kinase C. While β-isoforms of phospholipase C are regulated by → G-proteins, γ-isoforms are primarily regulated through → receptor tyrosine kinases.

Phospholipase D

Definition

Phospholipase D (PLD) represents a family of enzymes responsible for the hydrolysis of phosphatidylcholine (PC) into phosphatidic acid (PA) and choline. It is regulated by growth factors and cytokines receptors, as well as by GTPases of the → Ras superfamily, mostly → Rho family proteins, Ral and Arf proteins. At least two mammalian members have been idientified, which are involved in secretion and cell growth control. PLD1 has been shown to have oncogenic potential.

Phosphorylation of Proteins

Definition

Phosphorylation is an important mechanism by which the activity of cellular proteins can be reversibly altered by covalent modification. It involves the addition of a phosphate group to a specific serine, threonine or tyrosine residue, with the phosphate coming from adenosinetriphosphate (ATP). Phosphorylation is carried out by → protein kinases, the removal of the phosphate group is carried out by phosphoprotein phosphatases, reversing the biological effect of the phosphorylation. A chance of normal phosphorylation processes, e.g. by mutation of corresponding genes, is an important factor in cancer development; → RAS activation.

Photodynamic Diagnosis

Definition

Photodynamic diagnosis is performed with photodiagnostic agents; artificial fluorophores selectively accumulate in neoplastic tissues and allow the detection of neoplasia on the basis of fluorescence. The photodiagnostic agent → 5-aminolevulinic acid is not a fluorophore by itself, but induces the formation of the fluorophore protoporphyrin IX by the neoplastic cell; → fluorescence diagnostics.

Photodynamic Therapy

Definition

Photodynamic therapy is the use of light to destroy malignant cells. After the administration of a radiosensitizing agent, preferentially taken up by neoplastic cells, light of a specific wavelength is delivered to a tumor. Excitation of this agent results in the formation of cytotoxic radicals that kill the tumor cells directly or kill recruited vascular cells, inducing thrombosis and ischemic necrosis of the tumor.

Photosensitivity

Definition

Photosensitivity is the increased sensitivity to the development of edema and/or erythema by ultraviolet (UV) light; → xeroderma pigmentosum.

PI3K

Definition

→ phosphatidylinositol-3-kinase.

PIBIDS

Definition

PIBIDS is a genetic syndrome, a photosensitive form of trichothiodystrophy, which is associated with a defect in a protein complex that plays a role in transcription and DNA repair; → xeroderma pigmentosum.

PIM-1

Definition

The *PIM-1* proto-oncogene, which is a potent collaborator of → *MYC* in inducing lymphomagenesis in the mouse, encodes a serine/threonine protein kinase. While expression of *PIM-1* is restricted primarily to the hematopoietic lineage, inducible *PIM-1* expression has been observed in response to a wide variety of mitogens and cytokines, including PMA, IL-2, GM-CSF, G-CSF, IL-6, IL-3 and interferon γ.

PIN

Definition

→ Prostatic intraepithelial neoplasia.

Pinocytosis

Definition

→ Endocytosis.

PJS

Definition

→ Peutz-Jeghers-syndrome.

PKC

Definition

→ Protein kinase C.

Plasma Cell

Definition

Plasma cells are terminally differentiated B cells that are responsible for immunoglobulin secretion and that mainly localize within bone marrow and lymph nodes.

Plasma Cell Granuloma

Definition

→ Inflammatory myofibroblastic tumor.

Plasma Membrane

Definition

The plasma membrane is the continuous membrane defining the boundary of every cell.

Plasmid

Definition

A plasmid is bacterial, autonomous self-replicating extrachromosomal circular DNA.

Plasmin System

Definition

The plasmin system is composed of the cell membrane-bound serine protease u-PA (urokinase-type plasminogen activator), which targets plasminogen, an inactive serine protease precursor. Plasminogen is produced by liver cells and is found abundantly in the blood. u-PA cleaves plasminogen to yield the active protease plasmin, which degrades a variety of extracellular matrix components; → progression of tumors.

Plasminogen Activator

Definition

Plasminogen activator is an enzyme that acts in coagulation to convert plaminogen to its active form plasmin, but also cleaves other latent enzymes, resulting in active forms of some → matrix metalloproteinases (for example).

Plasminogen-related

Definition

Plasminogen-related protein is a protein that shares structural similarity and evolutionary relationship with plasminogen.

Platelet-derived Growth Factor

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Definition

Platelet-derived growth factor (PDGF) is a family of growth-factors with mitogenic activity for connective tissue cells, such as fibroblasts and smooth muscle cells, as well as for certain other cell types.

Characteristics

PDGF isoforms are disulfide-bonded dimers of homo- or heterodimers of A- and B-polypeptide chains. The constituent chains are proteolytically processed after synthesis to yield mature subunits of about 100 amino acid residues. The A- and B-chains are 60% identical in their mature sequences, with a perfect conservation of 8 cystine residues. The two subunits in the dimers are arranged antiparallel. In addition to the two interchain disulfide bonds, each subunit contains six additional cysteine residues that are arranged in a characteristic cysteine knot structure. The amino acid sequences of PDGF chains are homologous to those of vascular endothelial cell growth factors.

PDGF isoforms exert their cellular effects via binding to two structurally related protein tyrosine kinase receptors, denoted α- and β-receptors. The dimeric ligand binds two receptor molecules simultaneously; receptor dimerization leads to juxtaposition of the kinase domains of the receptors, whereby they phosphorylate each other in *trans*. The 'autophosphorylation' activates the receptor kinases and initiates intracellular signal transduction by recruitment of SH2-domain containing signal transduction molecules. Thereby several signal transduction pathways are initiated, ultimately leading to cell proliferation, cell migration, changes in cell morphology and inhibition of cell death (see figure). Intracellular cell signaling is characterized by extensive cross-talk between different signaling pathways. Moreover, stimulatory and inhibitory pathways are often induced in parallel.

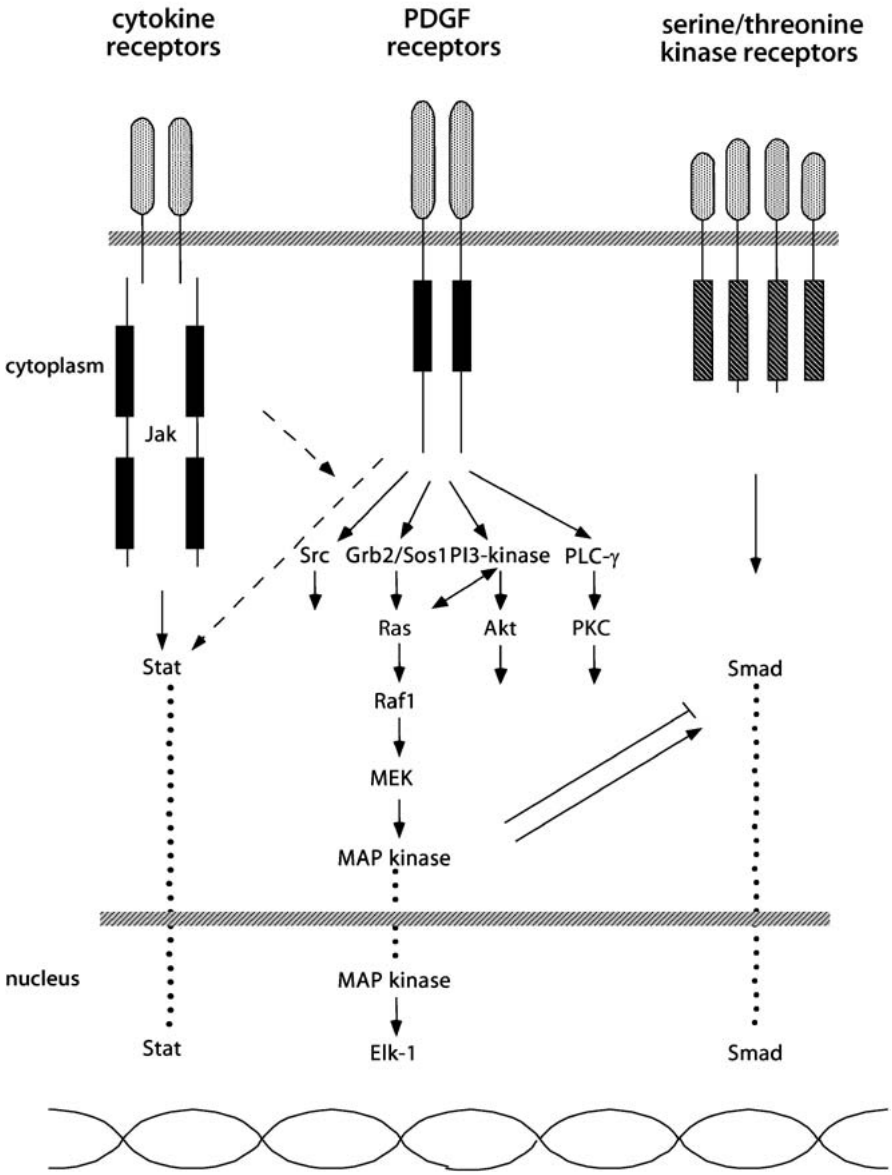
Gene

The PDGF A-chain gene is localized on chromosome 7 and the B-chain gene on chromosome 22. Both PDGF genes contain seven exons. The first exon encodes untranslated sequences and the signal sequence, the second and third exons encodes N-terminal sequences that are removed during proteolytic processing, the fourth and fifth exons encode most of the mature parts of the proteins, and exon 7 is mainly noncoding. Exon 6 encodes C-terminal sequences that in the case of the B-chain may be removed during processing. The A-chain occurs as two different splice forms, with and without the exon 6 sequence. Since the exon 6 sequence contains a stretch of basic amino acid residues that bind to extracellular matrix molecules, the absence or presence of the exon 6 sequence affects the localization of PDGF.

Bioactivity

Sequencing of PDGF revealed that the B-chain is almost identical to the v-Sis → oncogene product, the transforming protein of simian sarcoma virus. Subsequent studies have shown that transformation of cells by the simian sarcoma virus occurs via → autocrine stimulation involving a PDGF-like growth factor.

The demonstration that overactivity of PDGF had transforming effects prompted studies on the expression of PDGF in human tumors. PDGF was found to be commonly expressed in glioblastomas and sarcomas, tumors



Platelet-derived Growth Factor. Fig. – Signal transduction via receptors for growth regulation factors. Ligand binding induces dimerization of PDGF receptors as well as of other tyrosine kinase receptors, cytokine receptors or receptors with serine/threonine kinase activity. Within receptor oligomers autophosphorylation of specific residues occurs, which initiates different signaling pathways. Extensive cross-talk takes place between different signal transduction pathways.

derived from cell types normally expressing receptors, suggesting autocrine stimulation of growth.

PDGF is expressed also by several cancers that are derived from cell types that do not express PDGF receptors. Such tumor-derived PDGF has been shown to be involved in a paracrine manner in the formation of supporting connective tissue cells that surrounds the tumor cells. PDGF stimulation of connective tissue cells in the stroma also contributes to the increased interstitial fluid pressure of tumors, which causes a decreased uptake of drugs into the tumor and precludes efficient chemotherapy of tumors. PDGF has also been shown to have an angiogenic activity, through

direct effects on capillary endothelial cells as well as indirectly through the recruitment of supporting pericytes that reinforce the walls of the newly formed vessels.

Clinical Relevance

The normal function of PDGF is to promote the development of different kinds of connective tissue cells during embryonal development. Often PDGF is produced by different epithelial cell types and acts on neighbouring connective tissue cells expressing PDGF receptors. In the adult PDGF stimulates wound healing, and topically applied PDGF-BB (becaplermin) has been shown in clinical trials to increase the rate of healing of different types of wounds. PDGF also regulates the interstitial fluid pressure in connective tissue. PDGF released from platelets may also exert a negative feed back control of platelet aggregation; binding of PDGF to α-receptors on platelets inhibits platelet aggregation.

The important function of PDGF in the development and homeostasis of connective tissue suggests that overactivity of PDGF may result in fibrotic reactions. In support of this notion, overexpression of PDGF has been shown to be involved in glomerulonephritis, liver cirrhosis, myelofibrosis and lung fibrosis. The stimulating effect of PDGF, released from platelets or secreted from macrophages, on smooth muscle cells at sites of injury to the endothelial cell layer of arteries, also contributes to the intimal hyperplasia seen in atherosclerotic reactions. In malignancies, PDGF may be involved in → autocrine stimulation of tumor cell growth as well as in → paracrine stimulation of cells in the stromal compartment.

The involvement of PDGF in several serious disorders including malignancies makes PDGF antagonists highly warranted. Several types of such antagonists have been developed, including molecules that bind PDGF and prevent it from binding to its receptors, e.g. antibodies, soluble extracellular domains of receptors or Selex DNA aptamers, as well as low molecular weight inhibitors of the receptor kinase.

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Platyfish

Definition

→ Xiphophorus.

Pleckstrin Homology Domain

Definition

The pleckstrin homology domain is a domain found on functionally diverse proteins which is involved in signal transduction and cytoskeletal organization (e.g., Dbl family proteins). This domain, of approximately 100 amino acids, may function as a protein-protein or protein-lipid interaction domain.

Pleiotropic

Definition

A pleiotropic gene is one that affects more than one (apparently unrelated) characteristic of a phenotype.

Ploidy

Definition

Ploidy refers to the number of copies of the chromosome set present in a cell; a haploid cell has one copy and a diploid has two copies.

PLZF

Definition

PLZF is a smooth muscle myosin heavy chain disrupted by the → inv(16).

PML Nuclear Bodies

Definition

PML nuclear bodies are nuclear structures that are disrupted in acute promyelocytic leukemia and by certain viruses, which are the repository for several proteins.

PMS1

Definition

Postmeiotic segregation increased (*S.cerevisiae*) 1 (PMS1), also known as PMSL1, is a protein of 932 aa and 105 kD that is involved in mismatch repair of DNA. Mutations in PMS1 are associated with familial nonpolyposis colon cancer (→ HNPCC) (→ Lynch syndrome), and the gene locus maps to 2q31-33.

PMS2

Definition

Postmeiotic segregation increased (*S. cerevisiae*) 2 (PMS2), also known as PMSL2, is a protein of 862 aa and 95 kD that is involved in mismatch repair of DNA, and interacts with → MLH1. Mutations in PMS2 are associated with familial hereditary nonpolyposis colon cancer (→ HNPCC) (→ Lynch syndrome), and the gene locus maps to 7p22.

PMSL1

Definition

→ PMS1.

PMSL2

Definition

→ PMS2.

PNET

Definition

Primitive neuroectodermal tumor (PNET) are tumors composed of small densely packed hyperchromatic cells found in the CNS (central PNET) or originating from soft tissue and bone (peripheral PNET). Peripheral PNET are genetically characterized by a translocation t(11;22)(q24;q12), are of neural crest origin and grouped with the → Ewing sarcoma family of tumors. Central PNET do not show a t(11;22) translocation and exhibit divergent differentiation patterns along neuronal (e.g. cerebral neuroblastoma) or ependymal (e.g. ependymoblastoma) lines, or are found in locations above the cerebellar tentorium (connective tissue covering the dorsal surface of the cerebellum) (e.g. pineoblastoma). Medulloblastomas are infratentorial (infra = below) central PNETs.

POC

Definition

→ POMC.

Point Mutations

Definition

Point mutations are changes involving single base pairs.

Pointed

Definition

Pointed is a helix-loop-helix protein domain found in a certain subset of Ets proteins, named after the *Drosophila* Ets protein, Pointed. The pointed domain is suggested to be a protein binding interface.

Poly(ADP-ribose) Polymerase

Definition

Poly(ADP-ribose) polymerase (PARP) is also known, less frequently, as NAD^+ ADP-ribosyltransferase (polymerizing) [pADPRT], ADP-ribosyltransferase [ADPRT] and poly(ADP-ribose) synthetase [PARS]; → poly(ADP-ribosyl)ation.

Poly(ADP-ribosyl)ation

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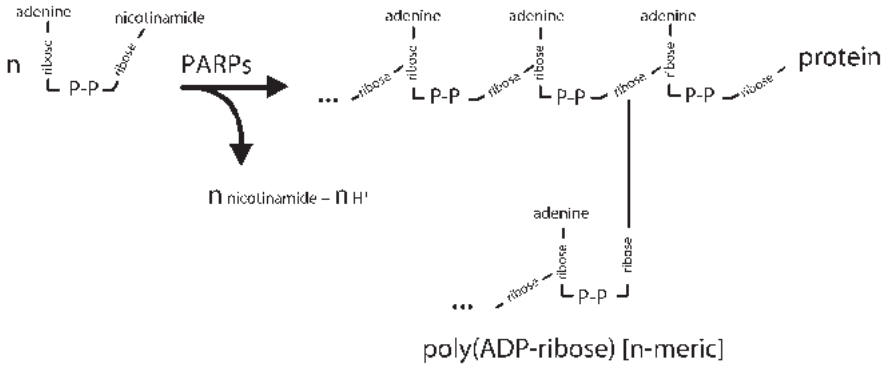
Definition

Poly(ADP-ribosyl)ation is a post-translational modification of glutamate or aspartate residues of various nuclear proteins, with NAD^+ serving as precursor (Fig. 1), and represents an immediate eukaryotic cellular response to DNA damage as induced, e.g. by ionizing radiation, alkylating agents and oxidants (1-5). Poly(ADP-ribosyl)ation is catalyzed mostly by the 113-kD enzyme → poly(ADP-ribose) polymerase (PARP), the gene of which (ADPRT) has been mapped to human chromosome 1q42. For clarity, the classical 113-kD-enzyme should now be termed PARP-1 since a number of additional polypeptides able to catalyze poly(ADP-ribosyl)ation have recently been identified. These novel members of the PARP family include PARP-2, PARP-3, vPARP and Tankyrase and they collectively seem to account for up to 25% of the total cellular poly(ADP-ribose) production. The major enzyme, catalyzing the catabolism of poly(ADP-ribose) by hydrolyzing the ribose-ribose linkages in the polymer, is poly(ADP-ribose) glycohydrolase (PARG), the gene of which has been mapped to human chromosome 10q11.23.

Characteristics

Catalytic function of PARP-1 and life cycle of poly(ADP-ribose)

While PARP-1 is constitutively and abundantly expressed in proliferative tissues, its catalytic activity is stimulated dramatically by double-stranded DNA with interruptions in the sugar-phosphate backbone (i.e., single-strand or double-strand breaks). PARP-1 has been detected in most eukaryotes and displays a characteristic and highly conserved domain structure (which can be further broken down into

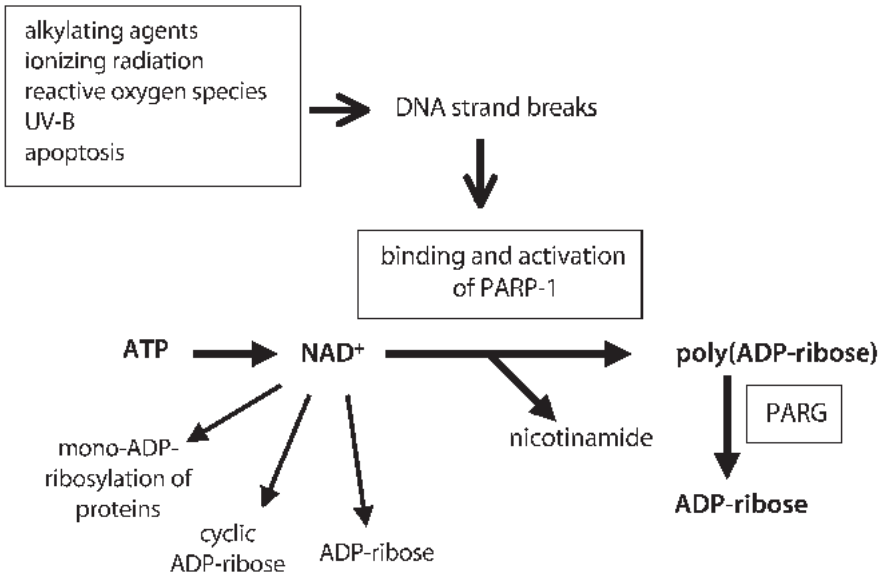


Poly(ADP-ribosyl)ation. Fig. 1 – Schematic representation of the structure of poly(ADP-ribose).

subdomains): Its DNA-binding domain, which is located at the amino-terminus, binds to single- or double-strand breaks in DNA via two zinc fingers.

Binding of PARP-1 to broken DNA induces an immediate and dramatic activation of the catalytic center residing in the carboxy-terminal NAD^+-binding domain of the enzyme. PARP-1 is catalytically active as a dimer. In intact cells the enzyme itself is the major target protein ('acceptor') for covalent modification with poly(ADP-ribose). This automodification reaction is thought to occur mostly on a specific domain located between the DNA-binding and the NAD^+-binding domain, respectively. However, in living cells a number of additional acceptor proteins have been identified, such as histones and topoisomerases, and *in vitro* many more proteins can be modified with this polymer. The half-life of poly(ADP-ribose) is very short under conditions of DNA breakage due to its rapid degradation by PARG and other catabolic enzymes. Hence, the existence of poly(ADP-ribose) in intact cells is transient, and depends on the existence of DNA strand breaks. The life cycle of poly(ADP-ribose) in the context of living cells is depicted schematically in Fig. 2.

A number of molecular functions for PARP-1 and/or poly(ADP-ribose) have been proposed



Poly(ADP-ribosyl)ation. Fig. 2 – Life cycle of poly(ADP-ribose). PARP-1, poly(ADP-ribose) polymerase-1; PARG, poly(APD-ribose) glycohydrolase.

and the following list is by no means exhaustive. It must be emphasized that many of the claims are based on work on subcellular systems, and that the relevance for the *in vivo* situation is not known.

1. Molecular functions related with regulation of DNA strand breaks and DNA repair:
- Direct control of the activity of DNA-processing enzymes (DNA polymerases, ligases, topoisomerases etc) by their covalent modification with poly(ADP-ribose).
- Poly(ADP-ribosyl)ation of histones as a mechanism to cause localized chromatin relaxation, thus allowing access of DNA repair enzymes to the damaged site.
- Poly(ADP-ribose) as a 'histone shuttle'; high-affinity non-covalent binding of (unmodified) histones to PARP-1-automodifying poly(ADP-ribose) should lead to localized chromatin relaxation, thus allowing access of DNA repair enzymes to the damaged site.
- PARP-1 as a component of a multi-protein complex carrying out DNA base-excision repair (short-patch or long-patch type); such a protein complex would also comprise the 'adaptor protein' XRCC1, DNA polymerase-β and DNA ligase III. PARP-1 would detect DNA strand breaks near the original lesion and could recruit the other partners. PARP-1 automodification might serve to regulate formation or function of such a complex.
- Locally restricted generation of ATP from poly(ADP-ribose) by pyrophosphorylytic cleavage, to be used for the DNA ligation step in DNA base excision repair.
- Signalling of DNA damage through non-covalent binding of poly(ADP-ribose) with p53, $p21^{WAF}$, myristoylated alanine-rich C kinase substrate (MARCKS) proteins etc.

2. Molecular functions related with the maintenance of genomic stability:
- Protection of 'open' DNA strand breaks by PARP-1 binding, to prevent non-specific DNA degradation and/or undesirable recombination. PARP-1 automodification might then serve to weaken the interaction with DNA, thus allowing repair activities to proceed.
- *[N.B. A primary role of PARP-1 and/or poly (ADP-ribose) in DNA repair (see above) could of course indirectly contribute to the maintenance of genomic stability under conditions of genotoxic stress.]*

3. Molecular functions related with DNA replication:
- PARP-1 as a component of a multi-protein DNA replication complex comprising enzymes for leading- and lagging-strand DNA synthesis, some of which are potential targets for poly(ADP-ribosyl)ation (DNA polymerase-α, RPA, → topoisomerase I, PCNA, DNA ligase I).
- Direct interaction between PARP-1 DNA-binding domain and the catalytic subunit (p180) DNA polymerase-α-primase tetramer, independent of DNA. Thus PARP-1 might participate in a DNA damage monitoring system, with its nick-sensor function perhaps playing a regulatory role when the progressing replication fork encounters breaks in the template.

4. Molecular functions related with gene expression:
- Participation of PARP-1 in the activation of → E2F-1 gene promoter after mitogenic stimulation of cells, leading to increased expression of both E2F-1 and DNA polymerase-α and entry into S-phase (see 'Molecular functions related with DNA replication').
- PARP-1 (independent of its catalytic function) as a transcriptional co-factor for B-MYB (a member of the MYB family of nuclear transcription factors) and for → AP-2.
- PARP-1 as a cofactor for th NFkB-mediated transactivation; in PARP-1 deficient mice, the typical lipopolysaccharide-induced increases in serum levels of TNF-α, in inducible nitric oxide synthase (iNOS) expression and in nitric oxide production are lacking, and endotoxic shock does not develop.
- Silencing of RNA polymerase II-dependent transcription by PARP-1 activity in a cell-free system. Inhibition of TATA-binding

protein (TBP) and the transcription factor YY1 by poly(ADP-ribosyl)ation (possible only prior to their binding to DNA).
- PARP-1 as interaction partner with retinoid X receptors (RXR) and poly(ADP-ribosyl) ation as a negative regulator of transcription through this member of the nuclear receptor superfamily.
- PARP-1 as interaction partner of other transcription factors such as p53, Oct-1, PC1, E47, TEF-1 and DF1-4.
- Poly(ADP-ribosyl)ation as a regulator of chromatin compaction and DNA methylation status.

5. Molecular functions related with energy metabolism:
- Massive poly(ADP-ribose) synthesis leading to NAD^+ depletion and consequent necrotic / apoptotic cell death.

Cellular functions

Depending on the intensity of DNA damage inflicted to cells and the cellular proliferation / differentiation status, PARP-1 has two contrasting functions, both of which are firmly established:

Cytoprotection and maintenance of genomic stability.

To study the cellular function of poly(ADP-ribosyl)ation various strategies have been employed to abrogate poly(ADP-ribosyl)ation. These include competitive low-molecular weight → PARP inhibitors, expression of a dominant negative PARP-1 version, PARP-1 antisense RNA expression, or disruption of the PARP-1 encoding gene (*Adprt1*)gene disruption in the mouse germ line. The results have consistently revealed that poly(ADP-ribosyl) ation significantly contributes, both *in vivo* and in cell culture, to the recovery of proliferating normal and malignant cells from low-level DNA damage as induced, e.g. by alkylating agents or ionizing radiation. This effect has been linked mechanistically with an involvement of PARP-1 in DNA base-excision repair. Furthermore, there is clear evidence that poly(ADP-ribosyl)ation counteracts the induction of genomic instability by DNA damage, as assessed by several biological markers such as chromosomal aberrations, sister-chromatid exchange, gene amplification or mutagenesis. In addition, fibroblasts from *Adprt1*-null mice have been reported to display morphological abnormalities and a slower growth rate than wild-type cells. These protective functions of PARP-1, as well as its recently demonstrated role in maintaining telomere length in mice, are in line with correlative data showing an association of mammalian and human longevity with high cellular poly(ADP-ribosyl)ation capacity [→ telomerase].

Cell death by energy depletion.

In stark contrast to the cytoprotective function mentioned above, PARP-1 overactivation may lead to cell suicide due to severe and irreversible depletion of NAD^+ and consequently of ATP pools (Fig. 2). Whether the type of cell death induced is always necrotic or may be of the apoptotic type has not yet been clearly resolved. By comparing *Adprt1*-null with wild-type mice and derived cells and by using competitive PARP inhibitors, this cytotoxicity mechanism has been identified to be operative in several nonproliferative cell types. These include (i) pancreatic islet cells exposed to relevant DNA-damaging compounds (reactive oxygen species, nitric oxide, streptozotocin), (ii) neurons after regional ischemia-reperfusion damage of the brain (known to provoke widespread release of reactive oxygen species and nitric oxide in the affected area, thus leading to the loss of many neurons and to brain infarct), (iii) dopaminergic neurons exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; a drug known to provoke release of reactive oxygen species only in the dopaminergic neurons of the substantia nigra, thus leading to selective neuronal death and Parkinsons syndrome). In each case it has been shown that the cells from *Adprt1*-null mice were highly protected from cell death and the animals displayed increased resistance to clinical disease. Likewise, administration of PARP inhibitors *in vivo* has led to prevention of cell death in post-ischemic heart and skeletal muscle and renal tubular cells.

In addition to the above, roles of PARP-1 have been described in other cellular phenomena including in apoptosis, but here the picture is much less clear at the present state of knowledge.

Apoptosis.

Apoptosis is associated with dramatic changes concerning the poly(ADP-ribosyl)ation system. There is a well documented proteolytic cleavage of PARP-1 into two fragments by activated caspase-3 during the execution phase of apoptosis, which represents one of the most frequently used biochemical markers for apoptosis. This cleavage is thought to abrogate the responsiveness of PARP-1 to DNA strand breaks. On the other hand, despite the very robust occurrence of PARP-1 cleavage, there is evidence from studies in several different cell systems for a substantial accumulation of poly(ADP-ribose) (i.e., the product of PARP activity) in apoptotic cells.

How these somewhat contradictory findings are to be reconciled is not yet clear, although there are number of possible explanations: There might be differences in timing (activation first, followed by cleavage) or in the population of polymerases (not all PARP-1 molecules may be cleaved; or polymerases other than PARP-1 produce apoptosis-associated poly[ADP-ribose]). More generally, it is still very controversial whether in the various phases of apoptosis there are positive or negative roles [i] for intact PARP-1 protein, [ii] for its cleavage products, [iii] for poly(ADP-ribose) synthesis (or its possible prevention by PARP-1 cleavage) and/or [iv] for the associated consumption or even depletion of NAD^+ (or their possible prevention by PARP-1 cleavage).

Clinical Relevance

Genetic Cancer Risk Assessment

Gene polymorphisms, and any resulting functional polymorphisms, in cellular factors involved in recognition and processing of DNA damage may well be expected to contribute to the genetic risk profile of an individual with regard to the development of organ-specific cancers or cancer in general. While the active human *Adprt1* gene locus on chromosome 1q42 encoding PARP-1 does display several polymorphisms, such as a polymorphic dinucleotide repeat located in the promoter region and base substitutions in the coding region, genetic association studies of these polymorphisms with human cancer have not been reported.

By contrast, a two-allele polymorphism in a *Adprt1*-related DNA sequence (very likely a processed pseudogene) at 13q33-qter, which arises from a 193-bp conserved duplicated region within the more frequent A allele, has been studied in several ethnic subpopulations and the association of each allele with different types of cancer has been investigated. In non-cancer control populations, the frequency of the B allele was 3-fold higher among Blacks as compared to Caucasians.

In endemic Burkitt lymphoma there was a strong association with the presence of at least one copy of a rare B allele. An increased frequency of the B allele was also found in cases of multiple myeloma and prostate and colon cancer, but only among black patients. By contrast, comparisons of lung cancer cases (large cell carcinoma and adenocarcinoma only) with controls revealed significant enrichment of the B allele in Mexican-American patients only (an ethnic group with very low B allele frequency in general) and not in African Americans.

Taken together this 13q33-qter polymorphism may prove useful as a predictive factor for the risk for specific cancer types in persons from specific ethnic backgrounds. Whether or not this polymorphism has any impact on the cellular poly(ADP-ribosyl)ation status or represents just a marker for a relevant neighboring gene remains to be seen.

Therapy

For several decades cytotoxic tumor therapy, as delivered by the application of cytostatic drugs or ionising radiation, has been the most frequently administered forms of cancer therapy. The sensitizing effect of PARP inhibitors in proliferating cells undergoing sub-lethal DNA da-

mage has long been considered as a potentially useful mechanism to render conventional cytotoxic treatment regimens more effective at eradicating tumour cells in the body. Available data from cell culture experiments suggest that the most suitable cytotoxic agents to be combined with PARP inhibitors would be methylating agents, bleomycin and ionizing radiation, but not anti-metabolites. However, the first-generation inhibitors (benzamide and its derivatives) had significant drawbacks, particularly for the *in vivo* application, such as poor solubility, lack of potency and limited specificity of the compounds. Therefore, in recent years large numbers of novel PARP-inhibitory compounds have been synthesized and characterized, some of which proved to be at least 50-fold more effective as chemopotentiators than the reference inhibitor 3-aminobenzamide, and act in sub-micromolar (rather than millimolar) concentrations. It will be interesting to see the effects of these novel compounds in animal models and clinical studies of cytotoxic tumor therapy.

Apart from therapy of cancer, the significant potential of PARP-inhibitory substances with appropriate pharmacokinetic profile as drugs for a 'cell rescue therapy' is obvious, given the prominent role of NAD^+-depleting and suicidal effect of PARP-1 overactivation in various pathophysiological conditions mentioned above ('Cellular Functions'/ 'Cell death by energy depletion'). In this context, PARP inhibitors, which should target pancreatic islet cells, neurons, myocardial or skeletal muscle, or renal tubular cells (corresponding to the respective pathophysiological condition to be treated), could prove useful to prevent manifestation of highly prevalent, disabling and even lethal diseases like type-I diabetes mellitus, ischemic stroke, myocardial infarction or renal failure.

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Polycyclic Aromatic Hydrocarbons

Definition

Polycyclic aromatic hydrocarbons (PAH) are a group of compounds consisting of more than two condensed benzene rings, generally formed in the incomplete combustion of organic matter; → tobacco carcinogenesis.

Polyadenylation

Definition

Polyadenylation is the addition of a sequence of polyadenylic acid to the 3′ end of a eukaryotic RNA after its transcription.

Polymorphism

Definition

→ Genetic polymorphism.

Polytene Chromosome

Definition

Polytene chromosomes are giant chromosomes found in flies. The large size is due to repeated rounds of replication without cell division (endoreplication). The chromosomes exhibit banding patterns, which have been extensively documented and are very useful for cytogenetic mapping.

POMC

Synonyms

- proopiomelanocortin
- MSH
- POC
- CLIP
- ACTH

Definition

POMC (proopiomelanocortin), a protein of 267 aa and 29 kD. The *POMC* gene maps to 2p23.3. The different active peptides

- adrenocorticotropin
- β-lipotropin
- α-melanocyte stimulating hormone
- β-melanocyte stimulating hormone
- β-endorphin

are generated by specific enzymatic cleavages at paired basic residues.

Effects

- ACTH stimulates the adrenal glands to release cortisol (produced by pituitary gland);
- MSH (melanocyte stimulating hormone, produced by pituitary gland);
- β-endorphin and met-enkephalin are endogenous opiates.

Positional Cloning

Definition

Positional cloning is the identification of a (disease) gene based on its suspected location (genetic map position) on a chromosome. In order to obtain proof of identity, several candidate genes in the critical region need to be screened for mutations in patients.

Postlabeling

Definition

^{32}P-postlabeling is a sensitive method for the detection and quantification of → DNA adducts. DNA is extracted in microgram quantities from tissue samples and enzymatically digested to mononucleotides. These are labelled with radioactive phosphorus (^{32}P) and the resulting ^{32}P-labelled adducts are separated from normal nucleotides by two-directional thin layer chromatography. Quantification can proceed by autoradiography, and identification is possible by co-chromatography with authentic samples; → biomarkers.

Post-replication Repair

Definition

Post-replication repair is a DNA repair process in which DNA lesions are bypassed during replication by specialized DNA polymerases.

pp60$^{c\text{-}Src}$

Definition

→ SRC.

pp60$^{v\text{-}Src}$

Definition

→ SRC.

PPNET

Definition

Peripheral primitive neuroectodermal tumor (pPNET), as opposed to the unrelated central primitive neurectodermal tumors (PNET), is mainly comprised by brain tumors. It is also known as Ewing sarcoma, with at least two neural markers expressed on the cell surface or the presence of Homer-Wright rossettes in the tumor.

PPOL

Definition

→ PARP.

PRAD1

Definition

Parathyroid adenomatosis 1 (PRAD1), also known as → *CCND1*, which encodes → cyclin D1. It is a disease involved in B-lymphocyte malignancy, particularly mantle cell lymphoma, by a chromosomal translocation t(11;14)(q13;q32) that involves *CCND1* and immunoglobulin regions at BCL1, and also in a subset of parathyroid adenomas by a t(11;11)(q13;q15) that involves *CCND1* and the parathyroid enhancer (*PTH*).

PRB

Definition

pRB is the product of a → tumor suppressor gene which is inactivated in retinoblastoma and various other tumor types. pRb inhibits G1/S cell cycle progression by interacting with transcription factors, such as → E2F, to block transcription of growth regulating genes; → retinoblastoma protein, biological and clinical functions and → retinoblastoma protein, cellular biochemistry.

Preprosomatostatin

Definition

Preprosomatostatin is a 116 amino acid long precursor molecule of somatostatin, containing a signal peptide that is cleaved to yield a 92 amino acid long prohormone. This prohormone contains the somatostatin moiety and is further processed to SST-28 and SST-14.

Pre-replicative Complex

Definition

This is a complex of proteins, originally identified by *in vivo* footprinting, present at replication origins only during late mitosis and G_1.

Primary Cells

Definition

Primary cells are eukaryotic cells taken into culture directly from the organism. They often survive for only a relatively short period of time before they go into senescence.

Primary Sclerosing Cholangitis

Definition

Primary sclerosing cholangitis (PSC) is an idiopathic cholestatic liver disease of presumed autoimmune etiology, striking (typically) young men and strongly associated with inflammatory bowel disease. It is characterized by chronic inflammation and progressive destruction of the intrahepatic and extrahepatic bile ducts; → cholangiocarcinoma.

Primer

Definition

A primer is a short sequence (RNA or DNA) that is paired with one strand of DNA (or RNA), providing a free 3′-OH end where a DNA polymerase starts the synthesis of a deoxyribonucleotide chain.

Primitive Neuroectodermal Tumor

Definition

Primitive neuroectodermal tumor (→ PNET) is a central nervous system tumor composed of small undifferentiated cells (→ brain tumors).

PRKDC

Definition

Protein kinase, DNA-activated, catalytic polypeptide.

PRKMK1

Definition

Mitogen-activated protein kinase kinase 1; → MAP kinase.

PRKMK2

Definition

Mitogen-activated protein kinase kinase 2; → MAP kinase.

PRKMK3

Definition

Mitogen-activated protein kinase kinase 3,; → MAP kinase.

Programmed Cell Death

Definition

Programmed cell death is a biological process, also termed → apoptosis, in which specific signals induce a cascade of biochemical events that result in cellular death.

Progression

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Definition

Tumor progression defines the process of invasiveness which leads to settlement and growth of a tumor cell in an organ that is distant from the site of the primary tumor. Such a 'secondary' tumor is called metastasis.

Characteristics

Benign tumors are defined by loss of growth control, loss of contact inhibition, a reduced requirement for growth factors or autocrine production of growth factors. Malignant tumors, i.e. metastasizing tumors, are defined by their capacity of invasiveness. This implies that the tumor invades the surrounding tissue, the vascular system and distant organs. The process is called the metastatic cascade and is composed of the following elements:

- loss of contact between the individual tumor cell and the tissue of the primary tumor
- penetration through the basal lamina of the primary tumor
- invasion of blood and/or lymphatic vessels
- adaptation to the blood pressure
- adhesion to vessel endothelia, extravasation, embedding and growth in organs different from the primary tumor, which includes the requirement for supply with nutrients by → neoangiogenesis.

Cellular & Molecular Regulation

Metastasis formation is independent of the process of oncogenesis, which is the consequence of genetic alterations within a single cell, whereas tumor progression relies on a concerted interaction with surrounding elements, like the extracellular matrix and host cells. It is important to note that no single step in the metastatic cascade is necessarily associated with the malignant phenotype. Corresponding processes will be seen during implantation of the fertilized egg, organogenesis, stem cell maturation and lymphocyte migration. It is therefore hypothesized, though not proven, that tumor progression can, but not necessarily must, be accompanied by additional genomic alterations of the transformed tumor cell, but instead may be initiated by inappropriate silencing or activation of (regulatory) genes.

The process of metastasis formation commences with the dissociation of the metastasizing cell from the surrounding tumor cell. The network elements involved in this dissociation are homotypic cell-cell adhesion molecules like → E-cadherin, whose expression becomes strongly reduced. Thereafter, the isolated tumor cell starts to interact with the surrounding tissue, composed of fibroblasts, endothelial cells, the extracellular matrix and the basal membrane as a specialized form of the extracellular matrix. Molecules that are involved in the interaction between the metastasizing tumor cell and host cells or elements of the extracellular matrix become upregulated or *de novo* expressed. These are adhesion molecules, motility factors, growth factors including angiogenic factors and matrix degrading enzymes. The invasive process itself is composed of three steps:

- binding
- local proteolysis
- migration.

Most elements of both the extracellular matrix and the basal lamina, like collagens, hyaluronic acid and proteoglycans, form networks that fulfill static functions by maintaining the tissue structure. The extracellular matrix has pores that allow cell migration, while the basal membrane is dense and hinders penetration of cells. The extracellular matrix also fulfills dynamic functions by binding to cell membrane anchored receptors that possess signal transducing capacity, and by serving as a depot for growth factors. The interaction between the tumor cell and the extracellular matrix/basal membrane is a central event in the process of metastasis formation, which will be repeatedly observed, i.e. during the dissociation from the primary tumor, during intravasation in hematogenously metastasizing cells and, depending on the structure of the organ, during settlement. Depending on the local environment, each of the three steps may be modified accordingly. There are several modes how these physiological processes can be altered to allow for invasiveness:

- quantitative or qualitative alterations in the components of the extracellular matrix or their cellular receptors
- enhanced delivery of cytokines, growth and motility factors; these factors are frequently bound to elements of the extracellular ma-

trix and are inactive in this form; delivery requires the degradation of the binding element, e.g by matrix degrading enzymes
- regulation of gene expression in the tumor cell by contact with elements of the extracellular matrix, a process important during organogenesis; the reverse process, namely stroma induction by the tumor cell, is of particular importance for neoangiogenesis.

To pass through the basal membrane the tumor cell requires matrix degrading enzymes. These are trypsin, cathepsin, metalloproteinases, the plasmin system and heparanases. Under physiological conditions these enzymes are important in tissue repair; they can be produced by the tumor cell or by surrounding cells after stimulation by the tumor cells. Most of these enzymes are produced as inactive pro-enzymes. By binding to a receptor they are converted into the active form. Notably, lytic activity is also regulated by the presence of natural inhibitors. Invasiveness requires either an excess of the enzyme or its receptor, or an imbalance between enzyme/receptor and inhibitor.

Adhesion molecules dominate the interaction between the metastasizing tumor cell, the vascular endothelial cell and the extracellular matrix. The metastasizing tumor cell does not produce genetically altered adhesion molecules. Instead, quantity and distribution as well as glycosylation patterns may become modulated.

In conclusion, all the phenomena associated with tumor progression have physiological counterparts in trophoblast implantation, embryonal morphogenesis and tissue repair. The distinction to these physiological processes relies on an imbalance of the individual components whereby, importantly, the tumor not only influences the surrounding tissue but also will be influenced by it.

Clinical Relevance

Conventional therapeutic regimen can in most instances cope with the primary tumor. Cancer mortality, therefore, is mainly due to the formation of metastasis. Tumor progression is probably not accompanied by additional genetic alterations of the transformed tumor cell, but rather by silencing or activation of unaltered genes. This implies that the tumor cell, in order to fulfill the process of metastasis formation, requires the expression of a set of gene products, which in this combination will not be found on non-transformed cells or on locally growing tumor cells. There is also evidence that the individual members of the set of molecules, which are required for tumor progression, interact and are of mutual influence. Although the identification of the individual members and their interactivity remains to be completed, therapeutic trials on modulation of adhesion molecules, on blockade of matrix degrading enzymes or on inhibitors of → angiogenesis have already been initiated. Although no definitive results are available, highly encouraging observations in individual patients have been reported that support the concept of intervention with metastasis formation by biological targeting.

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Progression of Tumors

Definition

Progression of tumors is a dynamic process of neoplastic evolution towards autonomy from the host. The process occurs because tumor cells, which are genetically unstable, generate

mutants that experience selective pressures (i.e., phenotypic advantage, host selection, therapy selection). Ultimately, the cells that comprise a tumor mass acquire increasing malignant characteristics; → multistep development.

Proliferating Cell Nuclear Antigen

Definition

Proliferating cell nuclear antigen (PCNA) is a cell cycle-regulated nuclear protein of 261 amino acids and 28 kD. It is an auxillary protein of DNA polymerase δ and is involved in the control of eukaryotic DNA replication by increasing the processibility of the polymerase during elongation of the leading strand. It is a subunit of DNA polymerase (the processivity factor of DNA polymerase δ and ε) and therefore is required for DNA replication. It is abundant in the nucleus of proliferating cells (hence its name) and is also involved in nucleotide → mismatch repair and nucleotide → excision repair. The gene maps to 20p12.

Promoter

Definition

A promoter is usually within the vicinity of the start site of transcription of a gene and directs RNA polymerase binding to initiate transcription (RNA synthesis). A constitutive promoter is permanently active in cells and a conditional or inducible promoter is only active when specific inducers (e.g. hormones) are present. Eukaryotic promoters are usually more complex that bacterial promoters. Specific (often very short) sequences within the promoter sequence provide specific binding sites for transcription factors. Upon binding these factors can mediate the desired pattern of gene transcription, depending e.g. on tissue type or stage of development.

Promotion

Definition

Promotion is the process of enhancing the development of a cancer by exogenous and endogenous promoting agents; are thought to act after → initiation of carcinogenic events.

Prosome

Definition

→ Proteasome.

Prostaglandins

Definition

Prostaglandins are bioactive lipids derived by oxygenation of the 20-carbon fatty acid, arachindonate, by cyclooxygenase; COX-2 [→ COX-2].

Prostate Cancer, basic characteristics and experimental models

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Definition

Prostate cancer is a male-specific and age-related genitourinary cancer. It is also classified among the hormone-related cancers, as androgens are necessary for the normal and neoplastic growth of the prostate. The prostate gland contributes to normal sexual function by producing a glandular secretion necessary for sperm viability and motility, and also plays a

role in normal bladder control. Wrapped circumferentially around the upper part of the urethra, the prostate can be subdivided histologically and anatomically into three zones; central, peripheral and transitional. The prostate epithelium comprises secretory/luminal, basal and endocrine/paracrine cells; the non-epithelial or stromal compartment of the gland consists of smooth muscle cells and fibroblasts. → Adenocarcinomas of the → acini are the most commonly observed types of prostate cancer, and hallmarks of these are slow growth rate and high degree of heterogeneity and multi-focality. The majority of prostate adenocarcinomas localize to the peripheral zone. The observations that → PIN (prostatic intraepithelial neoplasia) lesions also localize to this zone lend strong support to the hypothesis that PIN is most likely a precursor for prostate cancer. Additional support for the precancerous nature of PIN stems from the observations that PIN phenotypes and genotypes are intermediate between those of the normal and and those of the neoplastic prostate epithelium.

Characteristics

Incidence, risk factors and chemopreventive measures

In the United States and Europe, prostate cancer is the most frequently diagnosed non-cutaneous neoplasm and ranks second to lung cancer in cancer-related deaths. A recent surge in the number of patients diagnosed with prostate cancer has resulted from improved early detection methods as well as from the current trend towards an aging population. Although the etiology of prostate cancer remains speculative, several known risk factors for predisposition and promotion have been identified and include age, family history and race/ethnicity. Specifically, the average age for diagnosis is 72, and the incidence increases exponentially with age. The risk of developing prostate cancer doubles if one's father, brother or son has presented with the disease. Finally, in the United States, African Americans have the highest, Asians the lowest and Caucasians an intermediate rate of prostate cancer incidence/mortality. In addition to these risk factors, epidemiological studies have correlated prostate cancer susceptibility with environmental exposures to compounds capable of inducing oxidative stress (such as heterocyclic amines found in dietary saturated fats) as well as with deregulation of the → androgen-signaling pathway. Accordingly, antioxidants (such as those found in soy, tea, tomatoes, the mineral selenium and vitamin E) and anti-androgens are being promoted as prostate cancer chemopreventive agents.

Diagnosis, prognosis and treatments

Because localized prostate cancer is often amenable to therapy, annual screens for males over the age of 50 are recommended as a means for early detection. As an aside, there is a camp that argues against screening due to the fact that only a small percentage of diagnosed prostate cancers will progress and become clinically significant (see below). The most common and effective screens currently in use are blood tests to assess for elevated levels of the serum biochemical marker → PSA and the digital rectal examination (DRE) to assess for palpable aberrations in the prostate gland. Systematic biopsies are indicated if results from the PSA test or DRE are suspicious, both to confirm histologically the presence of malignancy and to assess the extent and burden of the tumor (grading systems utilized include → TNM and → Gleason). Apart from their diagnostic value, these biopsies generally are indicative of prognosis and determinant of the type of treatment to be administered.

Excluding 'watchful waiting', the first line treatment for early organ-confined disease is radical prostatectomy or external beam radiation therapy. Advanced prostate cancer, detected either initially in newly diagnosed patients or in treated patients who show signs of progression/recurrence (e.g., PSA relapse), is characterized by metastatic disease with primary deposit sites in regional lymph nodes and the skeletal bone. Metastatic prostate cancer is treated systemically by hormone therapy [→ hormone replacement] to effect androgen deprivation or ablation. This hormonal manipu-

lation is frequently palliative, but is rarely curative, since androgen independent disease ensues. As an aside, this androgen resistant disease may involve cells that have bypassed or sensitized the androgen-signaling pathway during their growth and differentiation. Alternatively, androgen-resistant disease may involve cell types, such as basal or neuroendocrine, that normally do not depend upon the androgen-signaling pathway. Hormone-resistant prostate cancer is refractory to current therapeutic modalities. Systemic chemotherapy or radiotherapy can be used for palliation of discomfort in this end-stage disease.

With respect to future prospects in prostate cancer therapy, alternative treatments for organ confined disease (e.g., interstitial → brachytherapy; three-dimensional conformal → radiation therapy) and improved methods of androgen ablation (e.g., → adjuvant/→ neoadjuvant to surgery or radiation; maximal androgen blockade) are currently being evaluated for efficacy. Pharmacological agents are being rationally designed to inhibit the structure, cell cycling, metastasis or angiogenesis of tumor cells as well as to promote their differentiation or programmed cell death. In the near future, vaccine strategies including those that involve immunization with tumor-specific antigens (e.g., PSA) may be included in the therapeutic armamentarium. Finally, cytoreductive or even corrective gene therapeutic strategies may become realities with improved gene delivery systems and a better understanding of the molecular pathogenesis of prostate cancer initiation and progression.

Molecular mechanism of prostate cancer initiation and progression

Aside from providing new targets for therapeutic intervention, the definition of molecular and genetic markers of prostate cancer etiology and progression is invaluable for screening, diagnostic and prognostic purposes. For instance, while histologic prostate cancers occur in roughly 40% of men over the age of 50, only 10% of these cancers become clinically significant and only 3% contribute to cause of death. Our ability to use molecular genetic markers to distinguish between those prostate cancers that will remain indolent and those that will become aggressive should allow us to assign priorities to therapeutic efforts and to circumvent over-treatment and the associated risks of incontinence and sexual impotence.

Recent studies of other cancer types (e.g., colorectal) have provided significant insight into the molecular basis of disease initiation and progression. In contrast, efforts to hone in on master regulators of growth control in the normal prostate whose deregulation is causal to prostate cancer pathogenesis have not yet met their just rewards. This could relate in part to the marked heterogeneity of prostate cancer, both inter- and intra-tumorally as well as on the molecular genetic level. In addition, changes on the → epigenetic level, as opposed to merely the mutational level, may be particularly consequential in this cancer type and have yet to be explored fully. Notwithstanding, the assessment of the chromosomal, genetic and gene expression changes that correlate with prostate neoplasia have provided several molecular clues. For instance, linkage analyses of familial prostate cancers have mapped susceptibility loci to Chromosome 1q24-31 and Chromosome Xq27-28. Classical and more recent molecular cytogenetic studies have identified chromosomal regions exhibiting recurrent prostate cancer-associated gains (for examples, whole chromosome gains of 7, 8 and X; regional amplification of 8q24) or losses (for examples, whole chromosome loss of Y and others; interstitial deletion of 10q23-26, 8p21-22, 16q22-24) in sporadic cases. Known regulators mapping to these 'hotspots', as well as novel candidates uncovered within these regions, have been evaluated for their effects in prostate cancer cell lines or animal models and for mutational status in human tumor samples. These studies, as well as similar assessments of more general oncogenes/tumor suppressor genes, have implicated a plethora of factors/pathways as pathogenetic to prostate cancer development when deregulated. Among the most convincing candidates implicated to date are the androgen receptor, insulin growth factor axis, epidermal growth factor axis, the cell cycle dependent kinase inhibitors p16ink4a and p27kip1, Pten dual spe-

cificity phosphatase, E-cadherin, telomerase, KAI1, p53 and bcl-2. Multistep prostate carcinogenesis schemas are now emerging wherein the changes in chromosomal regions and/or specific regulators such as these are correlated with morphologic transitions from benign prostatic epithelium to PIN to invasive carcinoma to metastatic and ultimately androgen independent disease.

Experimental model systems

Several experimental models of prostate cancer exist and have proven valuable for studying disease progression at the molecular, cellular, physiological and pathological levels as well as for testing therapeutic or preventive regimens. These models include the rat, dog, human prostate cancer cell lines and → xenografts, and the genetically manipulated mouse.

Rat models of prostate cancer include those that are spontaneous and those that are induced by carcinogens or hormonal treatment. These in turn have given rise to transplantable models and cultured cell lines. The preeminent rat model is the Dunning R-3327 model that originated as a spontaneous prostate cancer in a Copenhagen rat that was then serially passaged to generate a variety of sublines with distinct characteristics. The dog is among the few animals that develop prostate cancer spontaneously after a long latency, as is the case in man. Additional features of canine prostate cancer that recapitulate the human disease are that it is preceded by high grade PIN and routinely metastasizes to the bone. The establishment of prostate cancer cell lines and xenografts from primary human prostate tumors has proven quite difficult; more success has been achieved with metastatic lesions. Among the most extensively used and best-characterized human prostate cancer cell lines are PC-3, LNCaP and DU-145. The advantage of the xenografts (including CWR-22 and LuCaP23) over these cell lines is that they permit the study of the influence of the microenvironment, of progression to androgen independence and to metastatic disease, etc. Limitations of xenografts include the immunocompromised nature of the host mouse and the difference between the species of the tumor and that of the host.

The murine prostate appears to be highly resistant to the development of spontaneous prostate cancers, but these adenocarcinomas can be elicited through the introduction of potent transforming agents. In the mouse prostate reconstitution (MPR) model, the fetal urogenital sinus (prostate precursor tissue) is infected with retroviruses of the Myc and Ras oncogenes and then implanted under the renal capsule of a host mouse. These mice carrying transgenic prostates develop focal prostate adenocarcinomas with high frequency. More conventional transgenic mouse models for prostate cancer have resulted from the targeting of SV40 T antigen to the prostate using a variety of promoters. Among these is the TRAMP (transgenic adenocarcinoma of the mouse prostate) model in which T antigen expression is driven by the rat probasin promoter in a hormonally- and developmentally-regulated manner. A recent mouse prostate cancer model has emerged through the transgenic expression of insulin growth factor 1 (IGF-1) in basal epithelial cells of the prostate. This model confirms a role for the IGF-1 signaling pathway in prostate cancer development, a theory that was founded on correlations between serum IGF-1 levels and prostate cancer risk. The near future likely foresees a blossoming of genetically engineered mouse prostate cancer models that are developed based on newly-recognized molecular targets and on second generation transgenic and gene targeting strategies (e.g., inducible transgenes, conditional knockouts, etc.).

Because of the heterogeneous nature of human prostate cancer with respect to genetic and chromosomal profile, histologic appearance and biologic behavior it is recognized that multiple, distinct laboratory and pre-clinical models are necessary to bring us closer to understanding and defeating prostate cancer. Immediate priorities along these lines involve using existing and emerging models to:

- identify novel molecular genetic markers,
- elucidate the mechanisms dictating progression to androgen independence and metastatic disease,

- better characterize the pre-neoplastic stages of the disease, and
- evaluate chemopreventive and therapeutic modalities.

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Prostate Cancer, clinical oncology

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Definition

Prostate cancer is a malignant neoplasm that arises in the male prostate gland. Most prostate cancers are adenocarcinomas (95%). About 4% have transitional cell morphology and are thought to arise from the lining of the prostatic urethra. Few have neuroendocrine morphology and are believed to arise from the neuroendocrine stem cells normally present in the prostate or from aberrant differentiation programs during cell transformation. Of the adenocarcinomas, 70% arise in the peripheral zone, 15–20% in the central zone and 10–15% in the transition zone. These zones can often be identified by transrectal ultrasonography. Most adenocarcinomas are multifocal with synchronous involvement of multiple zones of the prostate and this may be due to clonal and non clonal tumors. In addition, multifocality may indicate a more aggressive tumor biology. The most commonly used system of classifying histiologic characteristics of prostate adenocarcinomas is the → Gleason score, which is determined by the glandular architecture within the tumor.

The predominant pattern, as well as the second most common pattern is given a grade of from 1 to 5. Grades are based on the extent to which the epithelium assumes a normal glandular structure. Grade 1 indicates a near normal pattern and grade 5 the absence of any glandular pattern (less malignant to more malignant). The sum of these two grades is referred to as the Gleason score. This scoring method was found to be superior in predicting disease outcomes when compared to using the individual grades alone. Score 2-4 is considered low grade, 5-7 is considered moderate grade and 8-10 is considered high grade. High grade prostatic intraepithelial neoplasia (PIN) represents the putative precancerous end of the morphologic continum of cellular proliferations within prostatic ducts, ductules and acini. Two grades of PIN are identified (low grade and high grade), and high grade PIN is considered to be a precursor to invasive adenocarcinoma. The continum which culminates in high grade PIN and early invasive cancer is characterized by basal cell layer disruption, basement membrane disruption, progressive loss of secretory differentiation markers, increasing nuclear and nucleolar abnormalities, increasing proliferative potential and increasing variation in DNA content (aneuploidy). Clinical studies suggest that PIN may predate carcinoma by up to ten years or more. The clinical importance of recognizing PIN is based on its strong association with carcinoma and hence its identification in prostate biopsy specimens war-

rants further search for concurrent invasive carcinoma.

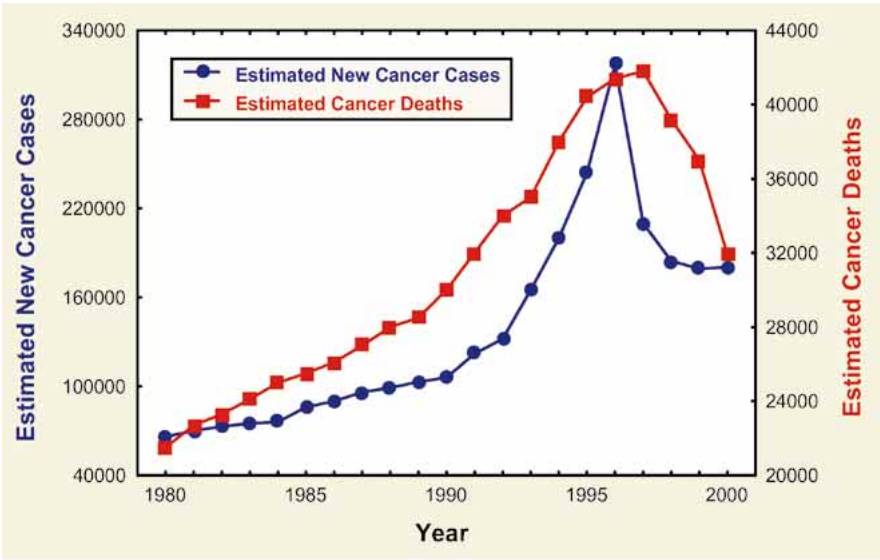
Characteristics

Clinical epidemiology and risk factors

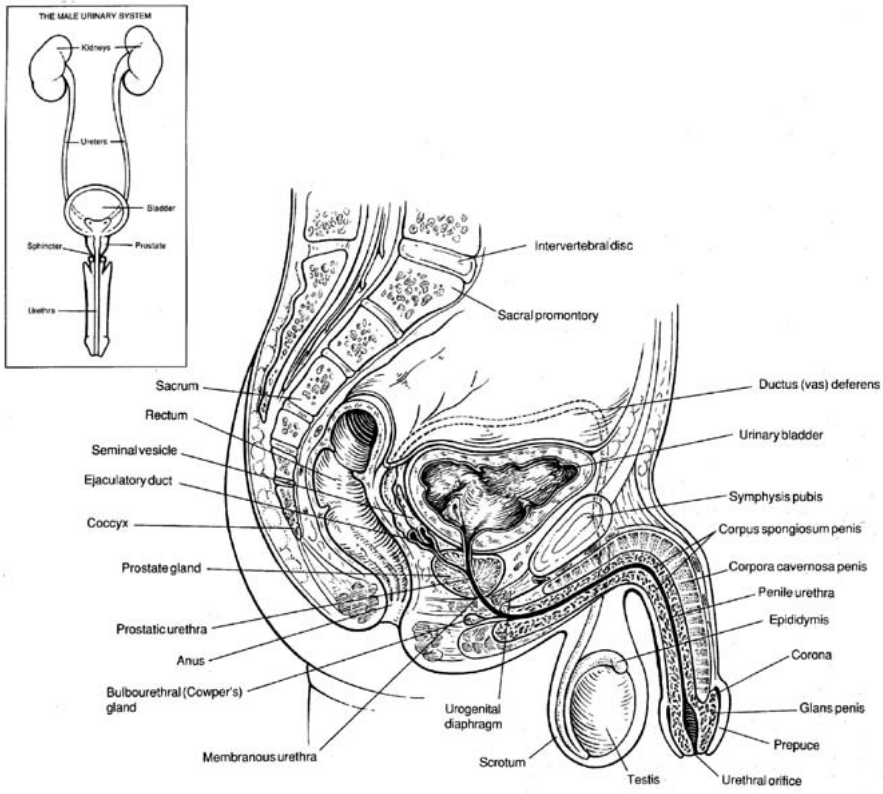
In the United States, prostate cancer incidence rates remain significantly higher in black men than in white men. Between 1989 and 1992 prostate cancer incidence rates increased dramatically. This is probably due to earlier diagnosis in men without any symptoms through the increased use of the → prostate-specific antigen (PSA) blood test. Prostate cancer incidence rates are currently declining, with peak rates in 1992 among white men and in 1993 among black men. During 1992-1996, prostate cancer mortality rates declined significantly (less 2.5% per year). Although mortality rates are continuing to decline among white and black men, mortality rates in black men remain more than twice as high as rates in white men. Prostate cancer is also found during autopsies carried out following other causes of death. The incidence of latent or autopsy cancer is much greater than cases of clinical cancer. It may reach up to 80% by age 80. Interestingly, incidence of the latent form of the disease is similar worldwide, while the incidence of clinical cancer is high in northern Europe and North America, intermediate in southern Europe, Central and South America and low in eastern Europe and Asia.

Etiology

Genetics, diet and hormones have been implicated as etiologic factors. Alteration of genes on chromosome 1 and on the X chromosome have been found in many patients with a family history of prostate cancer. In addition, genetic studies suggest that strong familial predisposition may be responsible for up to 10% of prostate cancers. Recently, several reports have suggest a shared familial risk (inherited or environmental) for prostate and breast cancer. In the US, blacks have a higher incidence and aggressiveness of the cancer when compared to Caucasian men, which in turn have a higher incidence than men of Asian origin. A high fat diet may lead to increased risks while a diet rich in soy protein may be protective. These observations have been proposed as some of reasons



Prostate Cancer, clinical oncology. Fig. 1 – New cancer cases and deaths in the United States of America. Data compiled from 'Cancer Statistics' issues 1980-2000 (published by the American Cancer Society).



Prostate Cancer, clinical oncology. Fig. 2 – Anatomy of the male genitourinary system.

for the low incidence of this cancer in Asia. Hormonal causes have also been postulated since androgen ablation causes regression of prostate cancers and eunuchs do not develop adenocarcinoma of the prostate.

Tumor biology and genetics

Several studies found p53 immunoreactivity to have a statistically significant correlation with a poor prognosis following radical prostatectomy, but others have failed to find such a correlation. Loss of heterozygosity (LOH) at chromosome 17p, where the p53 gene resides appears to be a poor prognostic marker in either primary or metastatic prostate cancer. The product of the retinoblastoma gene (Rb) is a tumor suppressor that regulates cell-cycle progression and its loss has been associated with prostate tumor progression and disease-related death due to prostate cancer. Two other genes, E-cadherin and the recently described PTEN, are known to be altered in prostate cancer. Dysfunction of E-cadherin *in vitro* and *in vivo* is associated with an invasive phenotype. In a recent study, abnormal expression of this gene was an independent prognostic factor in localized prostate cancer. Sufficient data for judging the prognostic value of PTEN is currently unavailable. Bcl-2 inhibits apoptosis in many cell types and overexpression has been associated with the hormonally independent phenotype in prostate cancer. Quantitation of tumor microvessel density (MVD) has revealed that tumors exhibiting extraprostatic extension have a higher microvessel density suggesting a relationship exists between MVD and tumor stage in localized prostate cancer.

Clinical presentation

Incidental findings:

- Elevated prostate specific antigen (PSA) or alterations in newer forms of PSA (percent free PSA); screening for prostate cancer is

controversial; recent evidence from Canada and Austria would suggest a beneficial effect on prostate cancer mortality.
- Tissue removed at the time of transurethral resection for benign hypertrophy (BPH).
- Abnormal digital rectal examination (DRE).

Local findings:

- Hematuria.
- Incontinence.
- Urinary retention due to bladder outlet obstruction with associated hydronephrosis and renal failure.

Metastatic findings:

- Weight loss and loss of appetite.
- Bone pain with or without pathologic fracture.
- Lower extremity pain and edema from nodal metastasis obstructing venous and lymphatic tributaries.
- Uremic symptoms from ureteral obstruction either due to local prostate growth or retroperitoneal adenopathy secondary to nodal metastasis.
- Neurological compromise from bony metastases to spine.

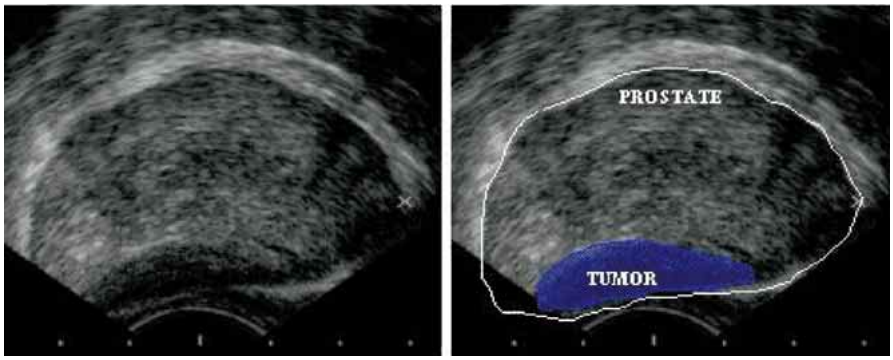
Diagnosis and staging

→ Transrectal ultrasound (TRUS) is used to examine the prostate (Fig. 3). Historically, it was believed that all cancers appear as hypoechoic (low echoes on the ultrasound, compared to normal prostate). However, more recent data indicates that such areas, even they are often found with together with cancers, are not specific enough for diagnostic purposes. Therefore, a systematic approach to prostate biopsy was developed in the early 1990s to sample most areas of the gland, irrespectivly of ultrasonographic abnormalities. In this approach, TRUS was used to guide the performance of 6 core needle biopsies of the prostate (3 on the left lobe of the gland and 3 on the right). More recently, several groups have shown that 10 or more systematic biopsies of the peripheral zone and occasionally the transitional zone carried out under TRUS, offer an increased rate of diagnosis over 6 core biopsies. The TRUS guided biopsy procedure is routinely carried out in the urologist's office.

Once a cancer is found a Staging Work-up is undertaken. It includes:

1. Laboratory work-up: determination of PSA (prostate specific antigen) level.
2. Imaging work-up: Work-up depends on the clinical staging.

Clinical stage of primary lesion, as determined by DRE (digital rectal examination), PSA level and Gleason score of the primary lesion, as determined by TRUS guided biopsy. For example, increased clinical stage of the primary, PSA level >10 and Gleason score >7 are correlated with increased risk of extraprostatic spread and are considered to be the key factors in determining the staging work-up and the predicted patient prognosis. PSA levels <10 with low or



Prostate Cancer, clinical oncology. Fig. 3 – Transrectal ultrasound of the prostate.

moderate grade histology with no or minimal findings on physical exam may proceed on to treatment. PSA levels >10 with high grade histology or physical findings suggesting stage T3 disease should in most cases undergo staging → CT scan and bone scan (Fig. 5). → MRI is superior to bone scan in evaluating bone metastasis, but is so far relatively impractical for routine total body surveys. Instead it is used to determine the etiology of questionable lesions found on bone scan. Use of MRI to stage the local lesion is controversial. Prostate specific membrane antigen (PMSA) immunoscintigraphy with the indium-labeled immune conjugate CYT-356 (ProstaScint™) has also been used for the detection of prostate cancer metastases, especially to the lymph nodes. The 1997 UICC/AJC TNM staging system is most commonly used (Table).

Management

The stratification of patients to particular treatments is based on the evaluation of the patients life expectancy and the biological aggressiveness of the tumor. The biopsy grade, clinical stage PSA level and, when available, the results of image studies can provide such prognostic information. Treatments such as watchful waiting, hormonal therapies and potentially curative therapies such as radiation and surgery are available. Treatment selection also requires a thorough analysis of the risks and benefits of each option by the patient, enabling him to have input on the therapy selection.

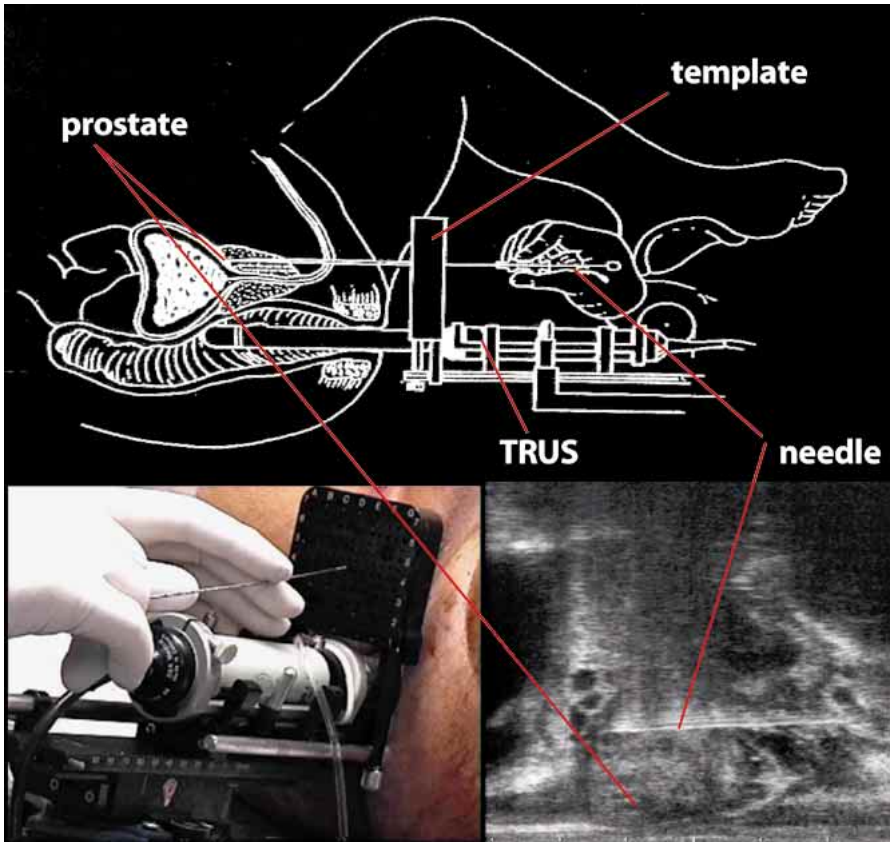
- Early localized disease (T1-2N0M0): Watchful waiting with a program of regular examinations and PSA and DRE monitoring. Advanced age, significant comorbidities are indicators. Excellent 10-15 year survival for well-differentiated tumors. External beam radiation treatment used with curative intent. Modalities include conventional external beam, conformal external beam and → brachytherapy (Fig. 4). Randomized trials have found total androgen ablation should be combined with radiation for improved disease specific survival and increased time to recurrence. Complications from radiation include cystitis, proctitis, enteritis, impotence and urinary incontinence. Radical prostatectomy is removal of the prostate and seminal vesicles and pelvic lymphadenectomy. For low stage, low PSA, well-differentiated tumors the lymph node dissection may be omitted. There are currently three approaches used to remove the prostate

Prostate Cancer, clinical oncology. Table – The 1997 UICC/AJC TNM staging system; [1]nodes of 'true pelvis' (ie: below bifurcation of common iliac vessels).

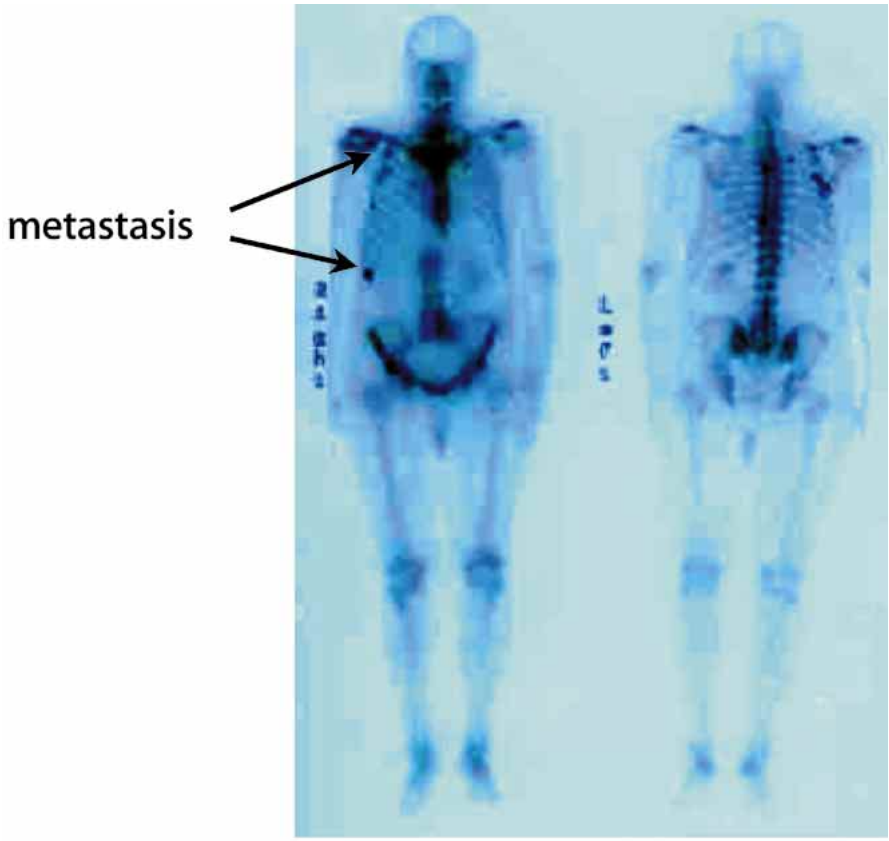
UICC/AJC 1997	description
T1a	tumor incidental histologic finding in 5% or less of tissue resected
T1b	tumor incidental histologic finding in more than 5% of tissue resected
T1c	tumor identified by needle biopsy (e.g., because of elevated PSA); tumors found in one or both lobes by needle biopsy, but not palpable or visible by imaging
T2a	tumor involves 1 lobe
T2b	tumor involves both lobes
T3a	extracapsular extension (unilateral or bilateral)
T3b	tumor invades seminal vesicle(s)
T4	tumor is fixed or invades adjacent structures other than seminal vesicles (bladder neck, external sphincter, rectum, levator muscles, and/or pelvic wall)
N1[1]	node metastases
M1	distant metastasis

gland including radical retropubic prostatectomy, radical perineal prostatectomy and laparoscopic retropubic prostatectomy. The following patient criteria are commonly used guidelines for patients to be candidates for radical prostatectomy regardless of the approach; age less than 70 years, few comorbidities, life expectancy >10 years, Gleason score ≤7 and PSA ≤10. Complications include impotence, incontinence and urethral stricture. Modifications to the classic technique to spare the neurovascular bundles has allowed improved potency and continence outcomes.

- Locally advanced disease (T3-4N0M0): Watchful waiting is an option in highly selected patients. Usually not an option due to relative aggressive nature of these tumors. Radiation options as for early disease. Most common approach is external beam radiotherapy combined with androgen deprivation. If brachytherapy is used, often combined with external beam and androgen deprivation. Surgery usually not an option outside clinical trials on special situations.
- Hormonally naïve metastatic disease: Radiation as above for patients with nodal only disease. When this is accompanied by long term androgen deprivation, increased cure rates may result. In patients with bony disease medical (→ LHRH agonists ± anti-androgens) or surgical (bilateral orchiectomy [→ orchiectomy, bilateral]) androgen ablation. Complications include decreased libido, impotence and hot flashes. Radiation therapy is used to palliate symptoms such as bone pain from metastatic deposits (Fig. 5). → Laminectomy or other orthopedic procedures can be used to palliate symptoms such as bone pain from metastatic deposits.
- Hormonally refractory metastatic disease: Androgen ablation therapy can induce a prolonged response (several years) in most men with metastatic disease. However, in



Prostate Cancer, clinical oncology. Fig. 4 – Prostate brachytherapy.



Prostate Cancer, clinical oncology. Fig. 5 – Staging bone scan.

most patients the disease becomes 'androgen independent' and begins to grow despite androgen withdrawal. At this time, hormone-refractory prostate cancer is not curable. All the available forms of therapy are palliative, which means that they can be used only to slow the progression of the disease and to relieve symptoms. Generally, for the patient who begins to fail hormonal therapy and has received combined hormonal therapy with an LHRH agonist and an antiandrogen or with an orchiectomy and an antiandrogen, antiandrogen withdrawal (stopping the antiandrogen) is undertaken. In many patients this will result in a short term decrease in PSA level. For patients who received LHRH agonist alone or orchiectomy alone, secondary forms of hormonal therapy may be considered, such as the addition of an antiandrogen or suppression of adrenal androgen synthesis. These manoeuvers are generally of limited benefit. Radiation therapy is used to help manage pain associated with the growth of bone metastases and this can be delivered via external beam therapy or osteomimetic radioisotopes (ie. strontium-89 radionuclides) which tend to be absorbed into areas of bone remodeling such as is found in bone metastases. Chemotherapy has been used but no regimen has yet provided long tern disease free intervals. However, newer regimens have shown less toxicity and improved outcomes with respect to partial tumor responses. Newer experimental approaches such as gene therapy, immunotherapeutics, differentiation therapies, induction of apoptosis (programmed cell death), inhibition of cell signaling and of angiogenesis are currently in clinical trials.

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Prostate Specific Antigen

Definition

Prostate specific antigen (PSA) is a serine protease secreted by prostate glandular tissue and found in small amounts in the blood stream. A rise in circulating levels of PSA could indicate malignancy, but is also seen with benign conditions such as benign prostatic hypertrophy (BPH) or prostatitis. Traditional PSA screens have considered a range of 2.5-4.0ng/ml to be the upper limit of normal PSA serum levels, and a value >4.0ng/ml to be suspect. To diminish the number of false positives and negatives seen with traditional PSA screens, these tests are being refined to take into account additional parameters such as age, PSA velocity, PSA density, ratio of free to bound PSA, etc. A relapse in PSA levels, or their failure to decrease, is indicative of progressive disease. PSA-based vaccines are currently being tested as prostate cancer-specific immunotherapeutic agents.

Prostatic Intraepithelial Neoplasia

Definition

Prostatic intraepithelial neoplasia (PIN) represents the putative precancerous end of the morphologic continuum of cellular proliferations within prostatic ducts, ductules and acini (→ prostate cancer). Two grades of PIN have been identified (low grade and high grade), and high grade PIN is considered to be a precursor to invasive carcinoma. Cellular atypia of the prostate epithelium is observed upon histological analysis of biopsy samples. The phenotypes (e.g., proliferative index, basal cell layer disruption) and genotypes of high grade PIN lesions are intermediate between those of the

normal and those of the neoplastic prostate epithelium.

Protease

Definition

A protease, or proteinase, is an enzyme that cleaves another protein.

Proteasome

Definition

A proteasome, also known as prosome or macropain, is the major intracellular protease in eukaryotes;. It is a complex containing 32 or more subunits, where the substrates are mainly ubiquitinated proteins. The proteasome is characterized by its ability to cleave peptides with arg, phe, tyr, leu and glu adjacent to the leaving group at neutral or slightly basic pH. It has an ATP-dependent proteolytic activity and may also catalyze basal processing of intracellular antigens. Proteasomes are found in the cytoplasm and in the nucleus; → ubiquitination.

Proteasome Degradation

Definition

Proteasome degradation is the degradation of proteins mediated by a multi-subunit complex of proteases within a cell; → ubiquitination.

Protein Kinase

Definition

Protein kinases are enzymes that move phosphate groups from ATP to serine, threonine or tyrosine residues in another protein.

Protein Kinase C

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Definition

Protein kinase C (PKC) represents a family of serine/threonine protein kinases comprising at least 10 structurally related enzymes. PKC isozymes have been grouped into three subfamilies. The conventional or classical PKCs (cPKCs) can be activated by Ca^{2+}, diacylglycerol (DAG) or phorbol esters and include the isotypes α, β I, β II and γ. Novel PKCs (nPKCs), comprising the isoforms ε, δ, η and θ are also activated by DAG or phorbol esters but are Ca $^{2+}$-independent. The more recently discovered 'atypical' PKCs (aPKCs) ι (the mouse homologue of ι has been termed λ) and ζ are Ca^{2+}- and DAG -independent and do not respond to phorbol esters. Each PKC isozyme is encoded by a separate gene with the exception of PKC β I and β II, which represent alternative spliced variants.

Characteristics

Each PKC isozyme consists of a single polypeptide containing an amino-terminal regulatory domain and a carboxy-terminal catalytic domain connected by a hinge region that is highly sensitive to proteolytic cleavage by cellular proteases (Fig.).The enzymes possess regions that are highly conserved between different PKC isoforms (termed C1 to C4) and five variable regions (V1 to V5).

- The C1 region, which is present in all PKC isozymes, contains an autoinhibitory pseudosubstrate domain. The amino acid sequence of the pseudosubstrate domain resembles the phosphorylation motifs in PKC substrates but contains an alanine instead of the serine or threonine, which act as phosphate acceptors in PKC substrates. The C1 region of cPKCs and nPKC contains

two cysteine-rich domains in tandem orientation, which serve as DAG- or phorbol ester binding sites. Only one copy of this cysteine-rich domain is found in DAG- and phorbol ester- non-responsive aPKCs.

- The C2 region of the cPKCs has been identified as the Ca^{2+} -binding site. A homologous region is also found in nPKCs but the absence of a critical aspartate residue renders this C2-domain incapable of Ca^{2+}-binding. Atypical PKCs lack a C2 domain. The C3- and C4-domains are parts of the catalytic region. ATP is bound to the C3 region, whereas the C4 domain contains binding sites for substrates (1).

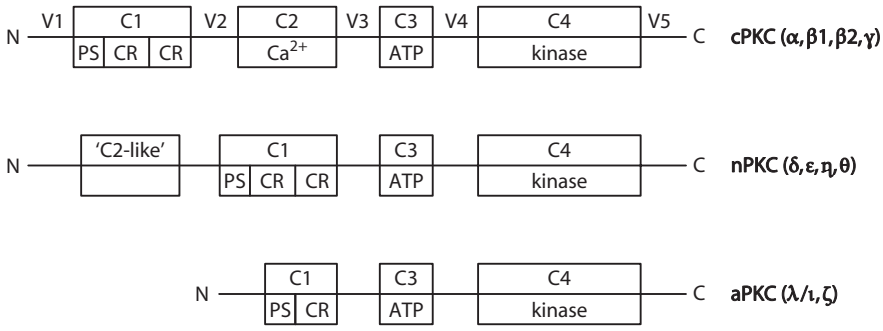
Mechanism of activation

Activation of PKC requires an unmasking of the catalytic domain by the removal of the pseudosubstrate. The necessary conformational change is mediated by the binding of DAG or phorbol ester in the presence of a lipid cofactor, especially phosphatidyl-serine. Other lipids, like free fatty acids, phosphatidyl-choline, lysophosphatidic acid and phosphatidyl inositol 3,4,5-trisphosphate, can also act as co-factors for some PKC isotypes. There is evidence that the pseudosubstrate sequence, once removed from its binding site, may contribute to membrane binding through its basic residues; a process that is also controlled by protein-protein interaction. Membrane association or translocation to membrane compartments is often considered as a hallmark of PKC activation. Ca^{2+} has been shown to increase the affinity of cPKCs to acidic phospholipids. In addition to allosteric regulation by Ca^{2+} and lipid co-factor, phosphorylation of PKC is essential for enzyme activation. The enzyme phosphoinositide dependent kinase 1 (PDK1) has been identified as a major upstream kinase catalysing phosphorylation probably of all PKC isotypes within the activation loop of the C4 domain of the catalytic region. In cPKCs, the PDK1-mediated phosphorylation is followed by phosphorylation on two additional sites within the V5-region of the carboxy-terminal sequence, probably due to autophosphorylation. Autophosphorylation of one site, Thr-641, is essential for the catalytic activity of nPKC δ. Novel PKC δ is the only PKC isoform that is subject to phosphorylation on tyrosine residues. However, the biological significance of the tyrosine phosphorylation of PKC δ remains controversial. Protein-protein interaction appears of particular relevance for the regulation of atypical PKCs. LIP (λ/ι interacting protein) has been identified as a PKC λ/ι specific activator. Binding of par-4 to aPKC λ/ι or a PKC ζ inactivates the enzyme.

PKC-binding proteins

PKC-interacting proteins can be classified into different subgroups according to their function:

- PKC substrates (also termed STICKS);
- regulatory proteins like LIP, par-4, or syndecan-4;



Protein Kinase C. Fig. – Structure of PKC isozymes: C1 - C4 constant regions; V1-V5 variable regions; PS pseudosubstrate domain; CR cystein- rich region; ATP : ATP-binding domain; Ca^{2+}-binding domain (note that the C2 region in nPKCs is uncapable of Ca^{2+}- binding as outlined in the text).

- docking proteins like RACKS, AKAPS or caveolin that may be involved in regulating the intracellular localisation of PKCs;
- scaffolding signalling proteins that cluster specific PKC isoforms together with other signalling elements in order to regulate a particular signal transduction pathway.

Putative PKC scaffolds include ZIP and proteins of the 14-3-3 family. This list of PKC-binding proteins is incomplete and grows continuously. The techniques employed to detect PKC-interacting proteins, e.g. the yeast two-hybrid system or overlay assays, do not always prove that these interactions occur *in vivo* and do not indicate the biological significance of this interaction. Therefore, these data should be interpreted with caution.

Bioactivity

Enzymes of the PKC family are important elements of intracellular signal transduction (2-5). They are differentially involved in the regulation of a broad variety of cellular functions: These include proliferation; differentiation; apoptosis; immune response; release of messengers from endocrine, exocrine and neuronal tissues; modulation of ion channels, receptors, transporters; organisation of the cytoskeleton; contraction and basal tone of smooth muscles. In many cases, however, the implication of PKC is based on effects obtained with phorbol esters or PKC-inhibitors. Since none of them are PKC-specific, the role of PKC in several systems needs to be re-examined. More specific techniques that are now available include depletion of defined PKC-isozymes by antisense techniques, expression of dominant negative or constitutively active mutants of particular isoforms, and generation of 'knock-out' mice lacking functional alleles for a defined PKC isoform.

PKC in cancer

PKC first attracted the attention of oncologists when it was found that PKC acts as a highly specific receptor for the classical tumour promoting phorbol esters like → TPA. The mechanism by which TPA exerts its tumour promoting activity is still unclear. Elucidation of the mechanism of action of phorbol esters is hampered by the fact that they act in a bimodal fashion; after an initial activation of phorbolester-responsive PKC isozymes, they cause a depletion of these proteins. Thus, it is unclear whether the phorbol ester effect is due to an activation or an inhibition of a PKC-mediated reaction. Furthermore, in addition to PKC, other highly specific non-kinase receptors for phorbol esters have been described, including the chimaerins, Munc13/1 and Ras-GRP. Binding to these receptors is activated by the same co-factors activating c- and nPKCs and inhibited by classical PKC inhibitors. Interestingly, chimaerins act as Rac-GAPs, thereby regulating the duration of the active state of the Ras-homology protein Rac. Ras-GRP functions as an guanylate nucleotide exchange factor for Ras and activates Ras by promoting the GTP-charged form of Ras. Thus, the chimaerins as well as Ras-GRP should be considered as perhaps equally important as PKC in tumour promotion.

The implication of a role of the enzymes of the PKC family in the regulation of cellular proliferation is documented by a vast number of studies (3, 5). However, when the effects are analysed with respect to individual PKC isotypes, the observations are frequently contradictory and generally confusing. For instance, PKC α has been described as activated and overexpressed in several tumours, like hepatomas and breast cancer, and to transform MCF7 cells following ectopic overexpression. However, in B16 melanoma cells or K562 cells ectopic expression of PKC α was found to inhibit cellular proliferation or to induce differentiation. Similar controversial findings have been reported for PKCs β, γ and ζ. Thus, the function of PKC isozymes is to a great extent cell type dependent. A more general picture has emerged with regard to nPKC ε, which was found to act as a growth-promoting enzyme in most systems, except in neuronal cells. Overexpression of PKC ε leads to transformation in fibroblasts and epithelial cells. Based on these findings, the PKC ε encoding gene is considered to represent an oncogene. Expression of dominant negative aPKC λ/ι reverses transformation by Ras. These and other findings (see

below) suggest that PKC λ/ι acts as a positive regulator of cell growth. More uniform effects have also been observed with PKC δ, which appears to act as a general suppressor of tumour growth. A better understanding of the biological role of PKC requires elucidation of their function at the molecular level. Information describing the biochemical function of individual PKC isoforms in mitogenic signal transduction is accumulating.

An implication for the role of PKC isoforms in the regulation of the Ras>Raf>MEK>ERK pathway is well documented. This pathway, also known as the MAP kinase cascade, is a major route for the transmission of mitogenic signals from growth factor receptors or oncogenic Ras. PKCs α and η activate cRaf and PKC ζ stimulates MEK. Evidence for a co-ordinate function of aPKC λ, nPKC ε and aPKC ζ for the Ras-mediated induction of c-Fos by the Ras>Raf>ERK pathway and the transcriptional activation of cyclin D, a critical step for the progression of cells through the G1-phase of the cell cycle, has been published. Other PKC isoforms including α, δ and η have been shown to arrest cells in G1 by enhancing the cell cycle inhibitory proteins p21waf/cip and p27kip. Several PKC isozymes, notably λ/ι and ζ, are implicated in cytoskeletal functions, thereby linking cell shape alterations to the cell cycle.

Activation of survival pathways by an inhibition of apoptosis is of utmost importance for malignant transformation and maintenance of the transformed phenotype. PKC isotypes α, δ and ζ are frequently associated with these mechanisms. The function of PKC α has been reported to act pro- and anti-apoptotic, dependent on cell type. The anti-apoptotic activity of α has been correlated to a PKC α-mediated activation of Akt, an inhibitor of programmed cell death. PKC δ is involved in the execution phase of → apoptosis; its function is at least in part explained by a caspase 3-mediated activation of this PKC isoform. A clear picture has emerged with regard to PKC ζ; atypical PKC ζ stimulates cell survival by activating NfκB either by mediating the release from the inhibitor I-κB or by phosphorylating the RelA subunit of NFκB. Similar effects have been reported for the structurally related aPKC λ/ι. Furthermore, par-4, a prominent mediator of apoptosis binds and inactivates PKC ζ.

Resistance to anti-tumour agents has been correlated to overexpression or activation of several PKC isotypes. This also applies to multidrug resistance mediated by an overexpression of the MDR1 gene encoded P-glycoprotein. However, the biochemical basis of this correlation remains obscure. A PKC-mediated phosphorylation of the P-glycoprotein could be excluded, as deletion of all PKC phosphorylation sites did not affect the pumping activity of the P-glycoprotein. The correlation is in part based on observations indicating a reversion of the resistant phenotype by PKC inhibitors. In some cases, however, these PKC inhibitors were found to interact directly with the P-glycoprotein and to inhibit its activity by a PKC-independent mechanism. It is intriguing to speculate that the observed correlation between intracellular PKC activity and drug resistance is due to a PKC-mediated activation of anti-apoptotic survival pathways described above. This may also explain the observed synergistic effects of combinations of PKC inhibitors with established anti-tumour agents.

Clinical Relevance

The implication of PKC isozymes in tumour cell proliferation, invasion and metastasis, apoptosis and drug resistance has led to the development of PKC inhibitors that can be used for the chemotherapy of cancer (2, 5). Several of these agents have entered clinical trials. Bryostatin-1, a macrocyclic lactone derived from *Bugula neritina*, occupies the same binding site as phorbol esters but is not a tumour promoter and leads to a depletion of c- and n-type PKCs. Phase II trials with bryostatin are ongoing. Phase II studies with an antisense construct targeted against PKC α (ISIS 3521) is in progress. These studies include patients with brain tumours, breast, colon, ovarian and prostate cancer. Safingol, a dihydrosphingosine analogue, has been employed in clinical trials as a sensitising agent in order to potentiate chemotherapy. Many other compounds are now in pre-clinical development. The majority

of these PKC inhibitors interact with the ATP binding site of the catalytic region. Most of them are bisindolylmaleimides or indolocarbazoles and are structurally related to the biological compound staurosporine, a product of *Streptomyces staurosporeus*. More recently, a series of very potent PKC inhibitors was described that represent derivatives of balanol, an azepine natural product. Balanol analogues also interact with the ATP binding site of c- and nPKCs.

However, PKC inhibitors are not only of interest as potential anti-tumour agents. Other areas in which PKC inhibitors are presently investigated include diabetes, cardiovascular diseases, inflammation and immunological disorders and diseases of the central nervous system (4).

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Protein Tyrosine Kinase Receptor

Definition

Protein tyrosine kinase receptors are receptors for growth regulatory factor that contains a protein tyrosine kinase domain in its cytoplasmic part. They are activated by ligand-induced oligomerization; → receptor tyrosine kinases.

Proteinase

Definition

A proteinase, or protease, is an enzyme that cleaves another protein.

14-3-3 Proteins

Definition

14-3-3 proteins are an abundant family of small proteins that bind to a wide variety of proteins involved in signal transduction, cell cycle and apoptosis. They bind to these proteins via specific serine phosphorylated sequence.

Proteoglycans

Definition

Proteoglycans are large glycoproteins, consisting of long polysaccharide chains (glycosaminoglycans) attached to a relatively small protein core. They are mainly found in the → extracellular matrix or attached to the cell surface by a membrane spanning region or a glycosylphosphotidylinositol anchor.

Proteome

Definition

The proteome is the complete profile of proteins expressed in a given tissue, cell or biological system at a given time.

Proteomics

Definition

Proteomics is the systematic analysis of the protein expression of healthy and diseased tissues.

Prothymosin α

Definition

Prothymosin α, also known as TMSA, PTA or ProT, is a protein of 110 amino acids and 12 kD. The human PTMA or TMSA gene locus maps on chromosome 2. Prothymosin α is a nuclear thymic polypeptide that may mediate immune function by conferring resistance to certain opportunistic infections. It may also play an intracellular role in cell proliferation and cell division.

Proto-oncogene

Definition

A proto-oncogene is a normal cellular gene that, when activated by mutations, acquires oncogenic function. The activating mutations can include intragenic point mutation, translocation (creating a fusion transcript or resulting in transcriptional up-regulation) or amplification with the consequence of enhanced expression; → oncogene.

Protoporphyrin IX

Definition

Protoporphyrin IX is a naturally occuring fluorophore. It is the immediate precursor of heme in the heme-synthetic pathway and may be used to detect neoplasia by fluorescence. Its concentration in neoplastic tissue may be enhanced by systemic or topical supply of → 5-aminolevulinic acid; → fluorescence diagnostics.

PSA

Definition

→ Prostate specific antigen.

PSC

Definition

→ Primary sclerosing cholangitis.

Pseudoautosomal

Definition

Pseudoautosomal refers to sex-chromosome linked genes that are inherited as if they were autosomes. Usually the human X- and Y-chromosomes differ in respect to their genes and as the consequence males have only one allele of an X- or a Y-linked gene. An exception is the → MIC2 gene that can be found on either, the X- and the Y-chromosome and females and males can have two copies of this sex-chrosome liked gene.

Pseudogene

Definition

A pseudogene is a DNA region that does not function as a gene, although it displays extensive homology to a known gene. This lack of function is mainly due to the loss of signals that are required for transcription (the promoter sequence) or due to mutations that prevent it from being translated into protein.

PTB

Definition

Phosphotyrosine binding (PTB) domains bind to proteins, such as growth factor receptors, in a phosphotyrosine-dependent fashion and plays an important role in signal transduction.

PTC

Definition

Mouse → *patched* gene.

PTCH

Definition

Human → *patched* gene.

PTCH1

Definition

PTCH1 is a gene involved in → basal cell nevus syndrome.

PTEN

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Synonyms

- MMAC1
- TEP1

Definition

PTEN (phosphatase and Tensin homolog deleted on chromosome Ten) is a tumor suppressor gene located in human chromosome band 10q23. The protein product of the *PTEN* gene (PTEN) is a phosphatase, a protein that removes phosphate groups from other molecules. Specifically, PTEN is a phosphatase for both phosphorylated lipids and phosphorylated proteins. The former activity is linked to the ability of PTEN to block cellular signals that promote growth and survival.

Characteristics

Somatic mutation of *PTEN*

All normal cells have two copies of the *PTEN* gene. However, loss of one or both copies of the *PTEN* gene in the tumor cells of a number of common cancer types has been increasingly recognized. These tumors types include cancers of the prostate, endometrium, brain (→ glioblastoma multiforme), thyroid, ovary, kidney (→ renal carcinoma) and skin (→ melanoma). In cancers of the prostate and in glioblastoma, *PTEN* mutation is associated with the more aggressive forms of the disease. For example, astrocytic brain tumors are classified by the histological grade of the tumor. While *PTEN* mutations are rare in anaplastic astrocytoma (less aggressive), mutations are common in glioblastoma multiforme (the most aggressive). Similarly, loss of the PTEN protein product is associated with higher grade prostate tumors, and *PTEN* mutations are found more frequently in metastatic prostate tumors when compared to those that remain confined to the prostate organ.

Germline mutation of *PTEN*

Mutation of *PTEN* in the germ line DNA is associated with the development of two inherited syndromes with overlapping clinical features. Patients with these syndromes, Cowden disease and Bannayan-Zonana syndrome, develop abnormal growths (hamartomas) of the skin, hair follicle (trichilemmoma), breast, thyroid and

intestinal tract, as well as neurological abnormalities. Such patients also have a higher incidence of cancers of the breast and thyroid. Thus both common cancers and certain rare inherited cancer predisposition syndromes can result from mutation of *PTEN* and the consequent loss of function of the gene product.

Biochemical functions

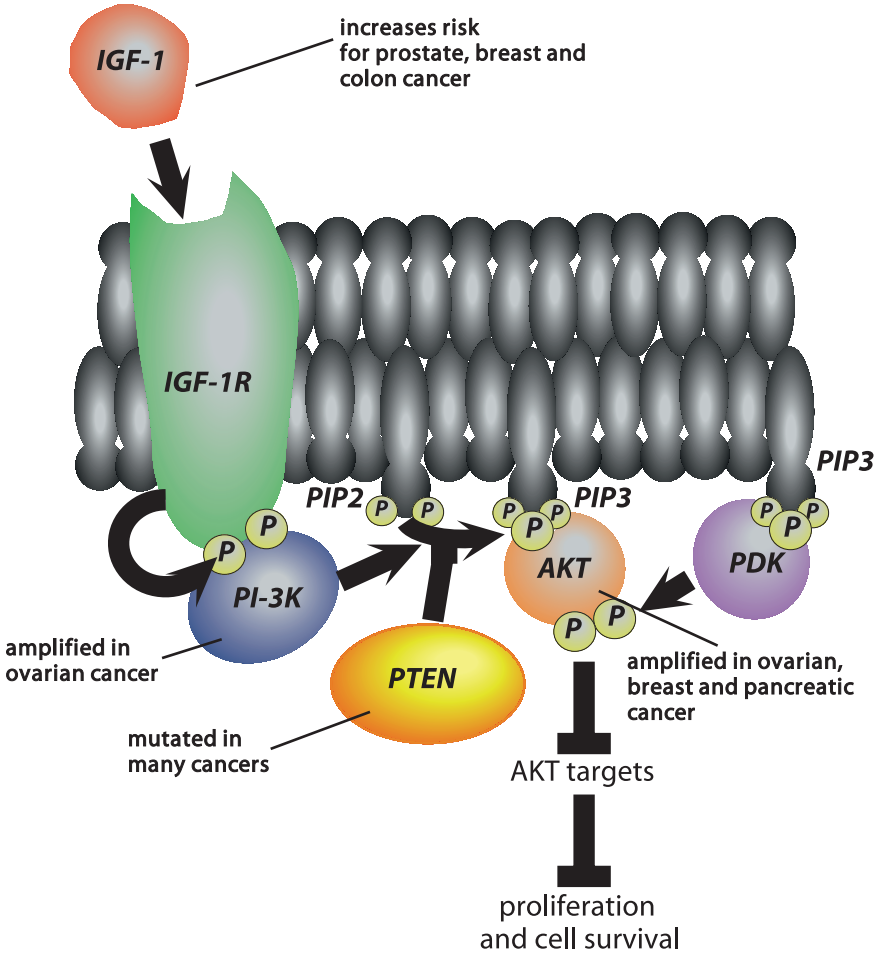
Biochemical and structural studies of the PTEN protein have revealed that it is a member of the phosphatase family of proteins. These proteins typically remove phosphate groups (often the elements transferred during cellular signaling) from other macromolecules including proteins, lipids and nucleic acids. When assayed *in vitro*, PTEN can dephosphorylate (removes the phosphate from) protein substrates and in particular has a strong preference for acidic protein substrates (such as poly-glutamic acid:tyrosine copolymers). In addition, and more importantly with respect to its *in vivo* function, PTEN dephosphorylates specific membrane associated lipids. Specifically, PTEN can dephosphorylate the phosphate on the third position of phosphoinositide-3,4,5-trisphosphate (PI3,4,5P3) and phosphoinositide-3,4-bisphosphate (PI3,4P2). These lipids are produced in cells by the action of a specific kinase known as phosphoinositide-3 kinase (PI3K) (discussed further below). Of note, the vast majority of mutations in PTEN, which are derived either from tumors or from the germ line of patients with Cowden disease, alter the PTEN protein such that is can not dephosphorylate these lipids, while in some case these mutations do not alter protein phosphatase activity. These data suggest that PTEN lipid phosphatase activity is essential for preventing tumor growth. These considerations suggest that PTEN functions as a tumor suppressor, primarily by acting as a damper on the transmission of signals through the pathway governed by phosphoinositide-3 kinase.

Phosphoinositide-3 kinase signaling

The signals carried by this pathway typically begin at the cell surface, where growth or survival factors in the extracellular environment bind to their cognate receptors at the cell surface (Fig.). For example, insulin-growth factor I (IGF-I) binds to the IGF-I receptor (IGF-IR). These receptors are typically tyrosine kinases (proteins that phosphorylate other proteins on tyrosine residues), and when triggered by growth factor binding there is an activation of the kinase activity and a resulting auto-phosphorylation on the part of the receptor that resides on the inner surface of the plasma membrane. This autophosphorylation creates a docking site for PI3K, which is recruited to the receptor and activated. PI3K then phosphorylates the inositol head group of the lipid creating PI3,4,5P3 or PI3,4P2. Once these new phosphoinositides are generated they in turn serve as docking sites for other protein kinases. In particular, a kinase known as AKT is recruited to the membrane where it in turn becomes activated. AKT is then free to phosphorylate a number of other proteins. AKT phosphorylation typically acts to inhibit downstream targets such as BAD, GSK3 and the forkhead transcription factors (AFX, FKHR, and FKHRL1). As a result AKT is a potent survival factor and can induce cellular proliferation.

How is the pathway PI3K/PTEN relevant to cancer?

The association between activation of the PI3K and the development of cancer is well established and can be achieved through multiple mechanisms: In animals, a number of tumor causing viruses produce proteins that activate this pathway. For example, the DNA tumor virus known as Polyoma produces an oncogenic protein known as Middle T antigen. Middle T can activate PI3K by recruiting it to membrane through a growth factor-independent mechanism. In chickens, a form of the avian sarcoma retrovirus (ASV 16) contains a copy of the chicken PI3K gene which has been modified so that it directly targets to membrane where it can activate signaling, again without growth factor induction. Finally, a murine retrovirus known as AKT8 contains a copy of the AKT gene that is activated by fusion to the retroviral gag protein.



PTEN. Fig. – The Phosphoinositide-3 kinase/PTEN signaling pathway. Growth factors such as IGF-I (red), bind to their cognate receptors (green). Binding triggers receptor autophosphorylation and recruitment of phosphoinositide-3 kinase (PI3K) (blue). Activated PI3K phosphorylates phosphoinositide-4,5-bisphosphate (PIP2) and converts it to phosphoinositide-3,4,5-trisphosphate (PIP3). PIP3 recruits AKT (orange) to the membrane where it is phosphorylated and activated by PDK1 (purple). Activated AKT can then phosphorylate a number of downstream targets and thereby typically inactivating them. Usually, these proteins exert an inhibitory effect on proliferation or survival. PTEN (yellow-orange) inhibits signaling through this pathway by dephosphorylating PIP3 back to PIP2.

In human cancers, activation of this pathway can be achieved through loss of PTEN, which results in constitutive phosphorylation and activation of AKT. In addition, the gene for the catalytic subunit of PI3K (*PI3KCA*) undergoes amplification in ovarian cancer. Finally, there are three AKT kinases in mammalian cells (AKT-1, -2, -3) and amplification of AKT-2 has been noted in breast, ovarian, and pancreatic cancers.

Conservation of the PI3K/PTEN pathway in Caenorhabditis elegans

The PI3K pathway is remarkably conserved through evolution. In *C.elegans* this pathway regulates life span and also regulates the induction of a so-called dauer state. The pathway includes homologs of the IGF-I receptor (daf-2), PI3K (AGEI), PTEN (daf-18), AKT 1 and AKT 2 (AKT1, AKT2), PDK1 an enzyme that activates AKT (PDK1) and a forkhead transcription factor (daf-16). The connection between AKT and

forkhead has proved illuminating with respect to this pathway in mammalian cells, where homologues of daf-16 appear to regulate both proliferative and apoptotic signals downstream of PTEN and AKT. In the future, additional relationships and genes discovered in *C.elegans* are likely to have relevance to human cancers.

Loss of PTEN deregulates PI3K signaling

As predicted from the biochemical considerations, loss of PTEN leads to constitutive deregulation of components of the PI3K pathway. Murine cells genetically engineered to lack PTEN have elevated levels of PI(3,4,5)P3, and AKT is found in a constitutively phosphorylated and hence constitutively activated state. Tumor cells that lack PTEN, likewise, are marked by a dramatic increase in AKT activation and in the phosphorylation state of AKT substrates, including downstream targets such as FKHRL1, GSK3 and 4EBP1. Tumor cell lines that lack PTEN can be used to ask about the cellular consequence of PTEN restoration. As described below PTEN, like p53, can act as a negative regulator of proliferation and as an enhancer of apoptosis. In addition, PTEN can alter cellular motility, invasion and potentially tumor angiogenesis.

PTEN as a cell cycle regulator

Reconstitution of PTEN to certain tumor cell lines (786-O renal carcinoma cells and U87MG glioblastoma cells) that lack PTEN results in the accumulation of these cells in the G1 phase of the cell cycle and a decrease in their proliferative rates in culture. When these cells are suspended in soft agar cultures, growth is arrested. The ability of PTEN to regulate cell-cycle progression requires lipid phosphatase activity and is antagonized by constitutive activation of AKT kinase. In addition, the connection to cell cycle regulation appears to be mediated through the induction of the cyclin dependent kinase inhibitor, p27, by PTEN. This latter step is probably mediated through the forkhead transcription factor family. These transcription factors, in the absence of a functional PTEN protein, are constitutively phosphorylated by AKT, and are consequently held in the cytoplasm through interactions with 14-3-3 proteins. When forkhead proteins are re-localized to the nucleus, they can induce p27 and arrest cells in G1.

The effects of PTEN reconstitution on cell cycle control are in keeping with the resulting proliferative lesions seen in tissues and cells genetically engineered to knock-out the *PTEN* gene. In cells that are heterozygous or homozygous null for *PTEN*, AKT is aberrantly phosphorylated, with evidence for a dose dependent effect of *PTEN* loss. In tissues, *PTEN* heterozygosity results in excessive proliferation (hyperplasia) in the thyroid and prostate. Labeling studies indicate that this results from an excessive number of cells entering the cell cycle. In addition, murine cells genetically engineered to lack both copies of *PTEN* have abnormal cell cycle kinetics. Thus, *PTEN* plays a necessary role in preventing unwanted cellular proliferation.

PTEN as a regulator of cell-death

The PI3K pathway and in particular AKT have been previously linked to the regulation of cell survival. In keeping with this idea, PTEN reconstitution to certain PTEN null cells, results in the induction of cell death. Again, loss of function studies have been revealing. Specifically, PTEN null murine fibroblasts have a defective response to apoptotic stimuli, and PTEN heterozygous mice develop a lymphoid hyperplasia syndrome that results from a failure of these cells to undergo cell death at the appropriate time.

Mechanistically there are a number of ways that PTEN could influence apoptotic signaling. AKT kinase is known to phosphorylate a number of substrates that are particularly relevant to apoptosis, including the pro-apoptotic proteins BAD and Caspase 9. For example, AKT phosphorylation of BAD results in BAD binding to and being sequestered by 14-3-3 proteins. In addition, the Forkhead transcription factors (FKHR, FKHRL1 and AFX) that are AKT substrates can all induce apoptosis in certain cells. Furthermore, FKHRL1 appears to be capable of regulating the transcription of Fas ligand, a po-

tent inducer of apoptosis. This is of particular interest because the lymphoid hyperplasia syndrome that develops in PTEN +/- mice phenocopies (mimics) a similar syndrome seen in mice bearing mutations in elements of the Fas signaling pathway. Thus, one possibility is that loss of PTEN leads to constitutive phosphorylation of forkhead factors, defects in Fas mediated signaling and a subsequent failure to undergo apoptosis in response to pro-apoptotic signals.

PTEN as a regulator of cell motility and adhesion

Cancer cells, especially those that metastasize, must acquire the ability to escape their local environment, and thus cancer cells are often more motile and invasive then normal cells. PTEN can regulate adhesion and motility in at least two ways: First, the PI3K pathway, in addition to regulating AKT, also regulates a set of small GTPase proteins (Rho/Rac/Cdc42) that regulate motility and invasion. Loss of PTEN can lead to deregulation of this arm of the PI3K pathway. In addition, PTEN protein phosphatase activity has been linked to the regulation of cell spreading through the dephosphorylation of focal adhesion kinase (FAK). With respect to FAK regulation, the protein phosphatase activity was sufficient, as the aforementioned tumor derived mutants lacking lipid phosphatase activity remain active in these assays.

PTEN as a regulator of angiogenesis

Lastly, AKT activation has been associated with the regulation of endothelial cell nitric oxide (eNOS), and PTEN loss has been associated with the loss of regulation of the hypoxia-inducible factor 1 (HIF-1) gene product. These connections suggest that PTEN null tumors may have an increased tendency to become vascularized, and in certain PTEN null prostate tumors there is evidence that this may be the case.

Clinical relevance and therapeutic implications

The demonstration that PTEN, a commonly mutated tumor suppressor, functions primarily as an antagonist of signaling through the PI3K pathway has triggered considerable enthusiasm for the development of small molecule inhibitors of the protein kinases activated by PTEN loss. Drugs that inhibit certain kinases have been developed previously and thus strategies and methods are in place for doing so again. In this pathway the therapeutic targets include the IGF-I receptor, PI-3 kinase, AKT kinases and PDK kinases. The hope for the future is that loss of PTEN will serve as a molecular marker or so-called predictive factor. That is, PTEN loss would lead to the development of a tumor that is particularly dependent upon constitutive signaling through the PI3K pathway. If so, then these tumors might be exquisitely sensitive to small molecule inhibitors of the kinases in this pathway.

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PTHrP

Definition

Parathyroid hormone related protein (PTHrP) is an autocrine/paracrine factor involved in a variety of cellular activities. It is often overexpressed in human tumors, can lead to hypercalcemia of malignancy (HHM) and is supposed to play a role in tumorigenesis.

PTP-BAS

Definition

→ PTPN13.

PTP1E

Definition

→ PTPN13.

PTPN13

Definition

Protein tyrosine phosphatase non-receptor type 13 (PTPN13) is also known as APO-1/CD95/Fas-associated phosphatase and the gene locus maps to 4q21.

PUM

Definition

Peanut-reactive urinary → mucin.

PUR-1

Definition

Purine binding transcription factor; → MAZ.

PVT-1

Definition

Pvt-1 (murine) oncogene homolog, is a MYC activator that maps to 8q24, approximately 300 kbp distal to MYC. PVT-1 is involved in rare translocations in Burkitt lymphoma and other B-cell disorders, such as promyelocytic leukemia and multiple myeloma. The juxtaposition of PVT-1 in the vicinity of MYC activates MYC expression.

Pyk2

Definition

Proline-rich tyrosine kinase 2 (Pyk2); → Ras activation.

Q

Quadriradial Chromosomes

Definition

Quadriradial chromosomes are 4-armed, aberrant chromosomes probably formed by unresolved recombination between homologous chromosomes.

Quasispecies

Definition

Quasispecies are viral variants resulting from mis-incorporation of nucleotides during replication and leading to diversification of the original strain.

Quenched Fluorescent Substrates

Definition

→ Matrix metalloproteinases are strict endopeptidases and do not cleave 'typical' synthetic substrates comprised of a peptide sequence coupled to a colorimetric or fluorimetric leaving group. To enable continuous monitoring of enzymatic activity, quenched fluorescent peptide substrates have been designed. These peptides incorporate two fluorophores into a larger (typically 7-9 amino acids) peptide sequence including an internal MMP-susceptible peptide bond (such as Gly-Leu). Based on the principles of fluorescence resonance energy transfer, the fluorescence of fluor 1 in the unhydrolyzed substrate is quenced by radiationless energy transfer to fluor 2. Following internal peptide bond cleavage, fluor 2 is no longer in the correct proximity and orientation to accept energy transfer from fluor 1, thereby resulting in a proportional increase in fluorescence emission from fluor 1 as substrate hydrolysis proceeds.

Quinone Reductase

Definition

→ NAD(P)H-quinone oxidoreductase.

R

RACE

Definition

5′-rapid amplification of cDNA ends (5′-RACE) allows the amplification of the 5′ end of a cDNA where there is incomplete knowledge of the mRNA of interest. → PCR primers are designed for the known sequence within the mRNA and for a homopolymer tail that has been added to the 3′ end of the first strand of cDNA. The amplified fragment, obtained by nested PCR, contains the 5′ remainder of the cDNA that is followed by the known cDNA sequence.

Rac-GAP

Definition

Rac-GAP is a GTPase-activating protein for Rac, which is a small GTP-binding protein and member of the family of Ras-homology proteins (→ Rho family proteins).

RACKS

Definition

Receptors for activated C kinase.

RAD50

Definition

Rad50 is a protein forming a multi-function complex with → Mre11 and Nbs1 [→ Nijmegen breakage syndrome], and is involved in the processing of DNA damage for DNA maintenance including → repair of DNA and recombination.

RAD51

Definition

Rad51 is a protein found in all eukaryotic organisms from yeast to man, with a central role in homologous recombination. It has homology to the bacterial → RecA protein, and similarly catalyses pairing and strand exchange between a damaged DNA molecule and an undamaged homologous molecule (→ homologous recombination repair).

RAD52

Definition

Rad52 is a recombination protein facilitating → Rad51-mediated homologous pairing of DNA.

RAD54

Definition

Rad54 is a member of protein family suggested to have DNA helicase activity, unwinding DNA to promote recombination.

Radiation Therapy

Definition

Radiation therapy, also known as radiotherapy, is a kind of therapy that uses radiation energy that comes from radioactive materials to fight neoplastic disease. It is typically performed with an external beam origin.

Radioresistant DNA Synthesis

Definition

Radioresistant DNA synthesis (RDS) is the continued replication of a cell's DNA despite an ionising radiation challenge. The majority of normal cells react to ionising radiation by halting replication of their (damaged) DNA.

RAF-1

Definition

Raf-1 is a dominant → oncogene coding for the serine/threonine kinase p74raf-1 that bears homology to → protein kinase C. p21ras recruits p74raf-1 on to the plasma membrane and provides a mechanism for signal transduction, leading to cell proliferation or differentiation; MAP kinase-kinase-kinase [→ MAPK kinase kinase].

Rapamycin

Definition

Rapamycin is a bacterial macrolide with antibiotic and immunosuppressive activity and structural similarity to FK506. In a complex with FK506 binding protein (FKBP), rapamycin inhibits → TOR (target of rapamycin). Rapamycin treatment of cells leads to dephosphorylation and inactivation of p70S6 kinase and also to dephosphorylation of 4E-BP1 (eukaryotic initiation factor 4E binding protein 1), thereby promoting its binding to and inactivation of eIF4E (eukaryotic initiation factor 4E), leading to a cell cycle arrest in G1 phase.

RAS

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Definition

The word Ras comes from a contraction of **Ra**t **s**arcoma, the tumor where the first gene of the family was identified, as part of the genome of a → retrovirus isolated from a carcinogenesis protocol (→ oncogenes).

Characteristics

The *Ras* genes, strictly speaking, are only a small group within a large family of related genes (the *Ras* superfamily). They encode proteins that perform a range of important cellular functions including signal transduction (Ras, Ral, Rho), cytoskeletal regulation (Rho), vesicle transport (Rab) and nuclear-cytoplasmic transport (Ran). The *Ras* genes relevant for human cancer are *H-RAS*, *K-RAS* and *N-RAS*.

- *H-ras* was initially isolated from the Harvey sarcoma virus;
- *K-ras* from the Kirsten sarcoma virus;
- *N-ras* was isolated by DNA-mediated gene transfer from a human neuroblastoma.

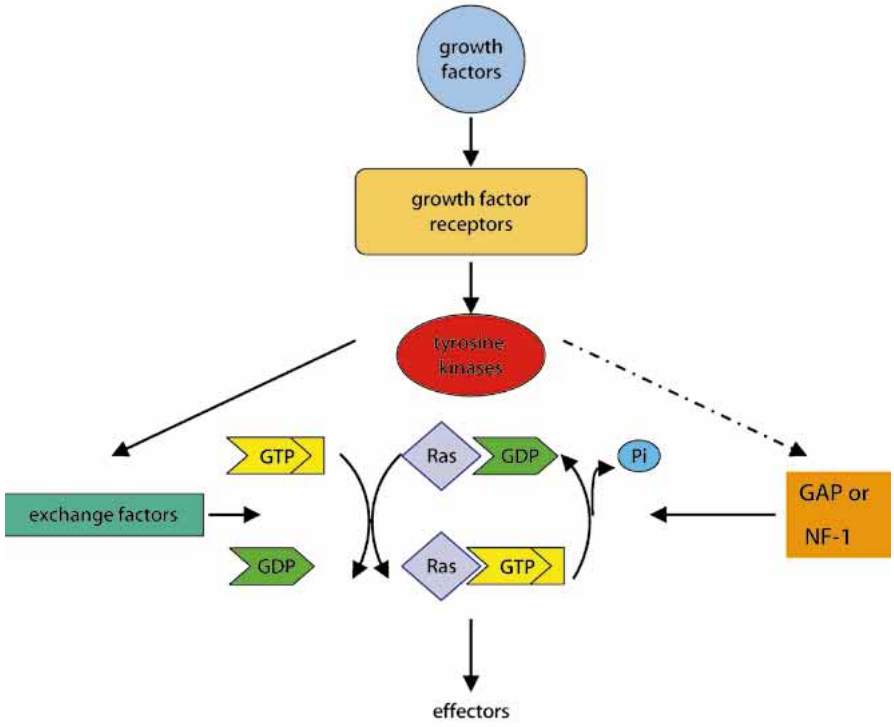
In humans, *H-RAS*, *K-RAS* and *N-RAS* are located on chromosome 11p15, 12p12 and 1p22, respectively. These three genes code for very similar proteins of 189 aa with four coding exons. Although the proteins are very similar, the genes have very different lengths due to the variable length of their introns. The expression of the three genes is essentially ubiquitous, although different tissues show differences in the levels of expression for the three genes. Analysis of the protein sequence shows that they contain domains for GTP binding, which represents a common characteristic for the whole superfamily.

The H-ras protein has been crystallized with GTP and the main regions that contact the phosphates are G1 (residues 11-17) and G3 (residues 53-62). The regions contacting the guanine ring are G4 (residues 112-119) and G5 (residues 144-146). The biochemical function of ras proteins is to hydrolyze GTP (GTPase). The other main structural feature is at the carboxyterminal end where they have the CAAX box motif (where C is a cysteine, A represents aliphatic amino acids and X is any amino acid). This box is required for the processing of the protein that is farnesylated at the cysteine, cleaved by a protease (eliminating the AAX) and carboxymethylated. This processing is essential for the protein to reach the membrane, the only place where it is functionally active. Although the first three exons of the three proteins are quite similar, there is more divergence in the fourth exon and this has resulted in different processing for K-ras versus the other two. H- and N-ras have another cysteine residue that is palmitoylated and upstream from the one that is farnesylated. K-ras lacks this cysteine, which is substituted by a run of positively charge amino acids. This feature appears to modify the way in which K-ras is brought to the membrane, and it appears to make it more resistant to inhibition by farnesyltransferase inhibitors (FTIs), a potential treatment for tumors. K-ras also has two alternative exons 4 (4A and 4B). The specificity of the function for each isoform has not been conclusively proven, but it appears to be strongly supported by gene inactivation studies. Although *N-* and *H-ras* knockouts are apparently normal, *K-ras* is embryonic lethal. However, the double knockout between *K-ras* and *N-ras* is lethal even earlier than the *K-ras* knockout, indicating that *N-ras* is providing a function that cannot be substituted by *K-ras*.

Cellular and molecular regulation

The function of the Ras proteins is to act as signal transducers between the membrane and other cellular structures, most often the nucleus. Ras molecules are on the inner face of the plasma membrane in an inactive state, which corresponds to their binding to GDP. When a stimulus has to be transduced, a Ras guanine dissociation stimulator (RasGDS or Ras GEF) interacts with Ras resulting in its dissociation from GDP. Given the much higher concentration of GTP in the cellular environment, the next nucleotide to bind to Ras is usually GTP. This results in a conformational change that activates Ras, which is now poised to transmit a signal downstream. Ras now binds to its effectors and facilitates their activation. To prevent excessive signaling, Ras has a weak intrinsic GTPase activity. To increase this GTPase activity there are molecules that enhance this GTP hydrolysis and are called GAPs (GTPase activating proteins). For Ras, two such proteins have been identified, GAP and NF1 (Fig. 1). Some mutations can abolish the GTPase activity of Ras and block its response to GAPs. This results in a constitutive activation of Ras with uninterrupted signaling, as it has been found in tumors (Fig. 2).

The previous description corresponds to the so-called Ras cycle that involves the steps from a Ras-GDP molecule to Ras-GTP and back to Ras-GDP. The linear Ras pathway describes the route followed by an outside signal through Ras and its final destination in the nucleus (Fig. 3). As an example, the signal triggered by epidermal growth factor (EGF) has been chosen. EGF is a growth factor that interacts with its specific receptor in the cell surface. This interaction triggers a dimerization and autophosphorylation of the cytoplasmic domains of the receptor. These phosphorylated residues recruit SH2-containing molecules like the adaptor Grb2. This molecule also contains an

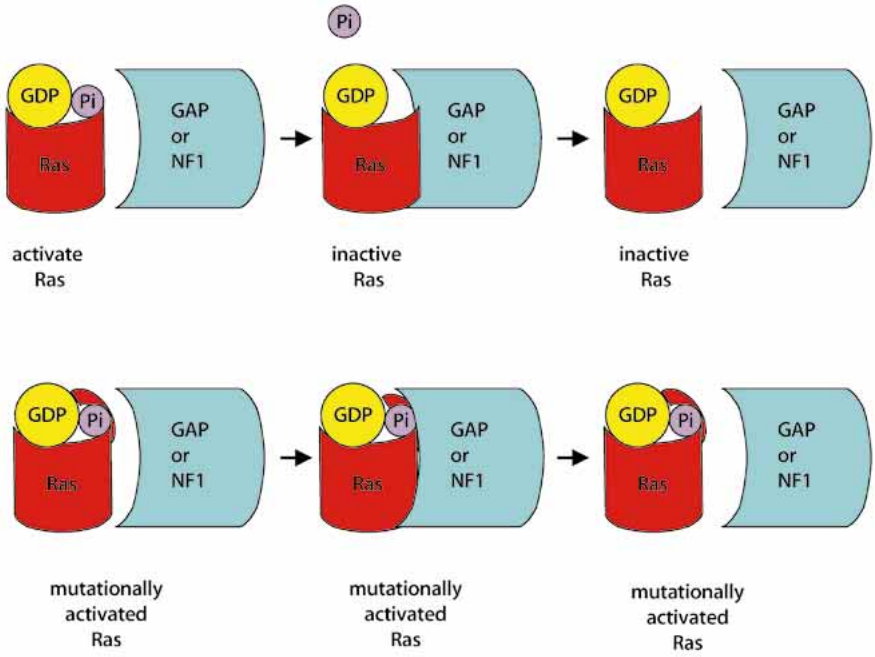


RAS. Fig. 1 – Biochemical Ras cycle. The Ras protein is a molecular switch that cycles between activated and inactive states. External signals facilitate the interaction between the exchange factor and Ras-GDP (inactive) which brings about the dissociation of the nucleotide from Ras. Given the much higher concentration of GTP in the cytoplasm, Ras binds then to GTP and becomes activated, interacting with its effectors and activating them. To prevent a constitutive activation, there are molecules that stimulate the intrinsic GTPase activity of Ras producing GTP hydrolisis andresulting on Ras bound to GDP ready to start again the cycle when another stimulus arrives.

SH3 domain that recruits Ras GEF, through its polyproline domain, to the membrane. Ras GEF activates Ras which then interacts in its GTP bound conformation with Raf1 (other important effectors are PI3 kinase and RalGDS). Raf1 is a kinase that now activates another downstream kinase called MEK, which in turn activates another downstream kinase called ERK. This kinase now translocates to the nucleus and activates a number of transcription factors like Elk, which are responsible for executing the action triggered by the original signal (EGF) through the induction of appropriate genes. It is important to mention that although the best known function of Ras is to stimulate cell proliferation, signals transduced through Ras can also induce differentiation, growth arrest and senescence, depending on the cellular context where its action is studied.

Clinical Relevance

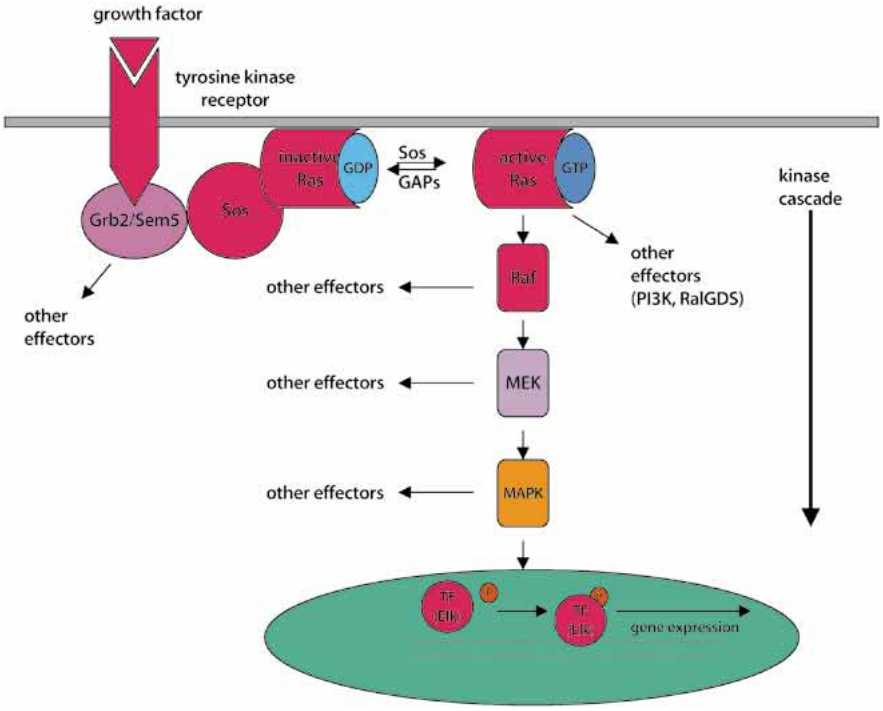
The great effort that has been devoted to study the *Ras* genes and their regulation stems from their importance in cancer. As mentioned above, two of the genes (*H-* and *K-ras*) were identified as oncogenes in an acute rodent retrovirus and the other (*N-ras*) as a cellular oncogene in a human neuroblastoma. Using DNA-mediated gene transfer and rodent cells as recipients (usually NIH3T3), a large variety of human and animal tumors were tested for the presence of oncogenes using the focus formation assay. When these assays were positive, the majority of oncogenes identified were shown to be one of the Ras isoforms. The ability of activated *Ras* genes to induce malignant transformation has its molecular basis on mutations acquired in tumor development that render the molecule constitutively active. In



RAS. Fig. 2 – Mutational activation of Ras makes it resistant to the inactivating effects of GAP proteins. A crucial biochemical difference between the normal Ras protein and the mutationally activated version found in many tumors is the fact that the abnormal version is unable to respond to the effects of GAP or NF1 by hydrolyzing GTP into GDP. Therefore this abnormality leaves the pathway constitutively activated and contributes to uncontrolled proliferation in cancer.

animal tumors induced by chemical carcinogens, it has been shown that the Ras mutations correlate with the chemical reactivity of the carcinogen, suggesting that in some cases Ras mutations could initiate tumor development. Similarly in transgenic mice, *Ras* oncogenes, under the control of tissue specific promoters, have induced tumor development in those tissues.

The most clear indication of clinical relevance for the *Ras* genes is its significant frequency of mutation in human tumors. Mutations were originally detected by cloning and sequencing the transforming genes. Following the advent of polymerase chain reaction (PCR), most detection strategies now use this technique as part of the protocol. The most popular include the use of Single Strand Conformation Polymorphism (SSCP) and the use of amplification with oligonucleotides that would produce a new restriction site if a mutation is present, followed by the digestion and resolution of the fragments in agarose gel elctrophoresis. The overall frequency of a mutationally activated *Ras* gene in human cancer has been calculated to be 20 – 30%. There is nevertheless, a large variation in the frequencies depending on the tumors to be considered. The highest frequency is in pancreatic cancer (80 – 90%). In colon carcinoma is around 40%, thyroid tumors 60%, non small cell lung cancer 50% and in some acute leukemias also around 25%. In bladder tumors there has been some controversy about the frequency of Ras activation ranging from 10 – 60%, depending on the techniques utilized for detection. The frequency of Ras mutations in many other types of human tumors is lower, and in particular in breast and prostate cancer is around 5%. Based on the ability of FTIs (farnesyltransferase inhibitors) to inhibit Ras membrane attachment, a number of clinical trials have been initiated in cancer patients using FTIs to inhibit tumor growth.



RAS. Fig. 3 – A representative signal transduction pathway where Ras is a crucial step. The signal starts from a growth factor and through its receptor eventually activates Ras. Activated Ras is able to activate a kinase cascade which last member translocates into the nucleus activating transcription factors that will be the executors of the functions that a particular growth factor induces in a particular cellular context. It is relevant to mention that the elements of the pathway in red have been shown to be oncogenic in different systems and this includes some of the transcription factors that are stimulated by the Ras pathway.

RAS. Table – Incidence of ras mutations in human tumors.

tumor type	activated *ras* gene	incidence [%]
pancreatic adenocarcinoma	*K-ras*	90
cholangiocarcinoma	*K-ras*	90
thyroid carcinoma	*H-*, *K-*, *N-ras*	60
lung adenocarcinoma	*K-ras*	50
colorectal carcinoma	*K-ras*	40
seminoma	*K-*, *N-ras*	40
endometrial carcinoma	*K-ras*	30
skin keratoacantoma	*H-ras*	30
myelodysplastic syndromes	*N-ras*	25
acute myelogenous leukemia	*N-ras*	25
acute lymphocytic leukemia	*N-ras*	20
melanoma	*N-ras*	20
bladder carcinoma	*H-ras*	15

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RAS Activation

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Definition

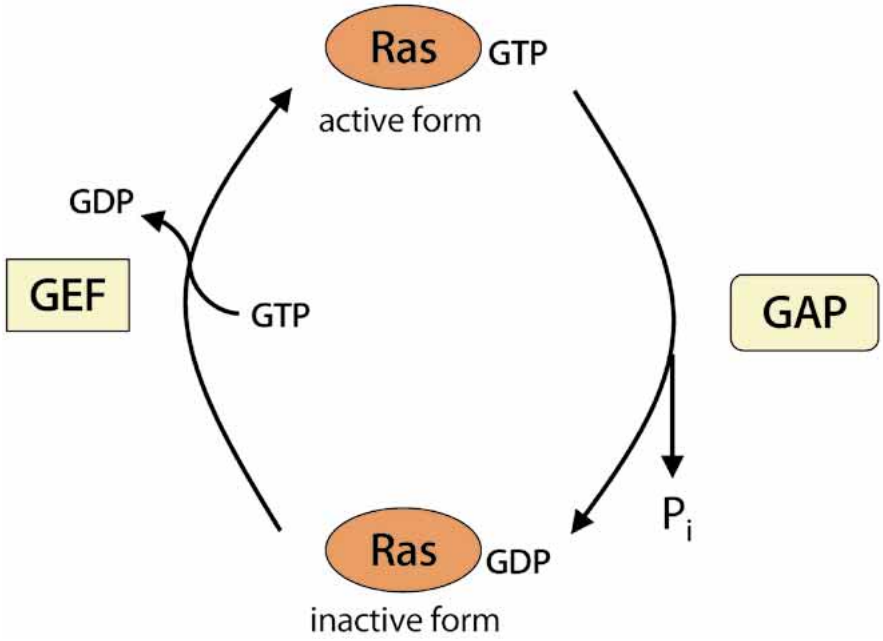
Increase of the active GTP-bound form of Ras proteins promoted by ligand-bound receptors or other mechanisms.

Characteristics

The family of → Ras proteins (Ha-Ras, N-Ras, Ki-RasA and Ki-RasB) are low molecular weight guanine nucleotide-binding proteins that play essential roles in the control of cellular growth and differentiation. Ras alternates between an active GTP-bound state and an inactive GDP-bound state. The slow intrinsic rate of GTP hydrolysis on Ras is stimulated by GTPase-activating proteins (GAPs), such as p120GAP and neurofibromin, while Ras activation requires guanine nucleotide exchange factors (GEFs) that stimulate the dissociation and exchange of bound GDP for GTP on Ras in response to upstream signals.

Cellular & Molecular Regulation

Ras proteins are localised at the inner surface of the plasma membrane where they participate in transmitting signals from → tyrosine kinase receptors (RTK) and some receptors coupled to heterotrimeric → G-proteins. It is well estab-

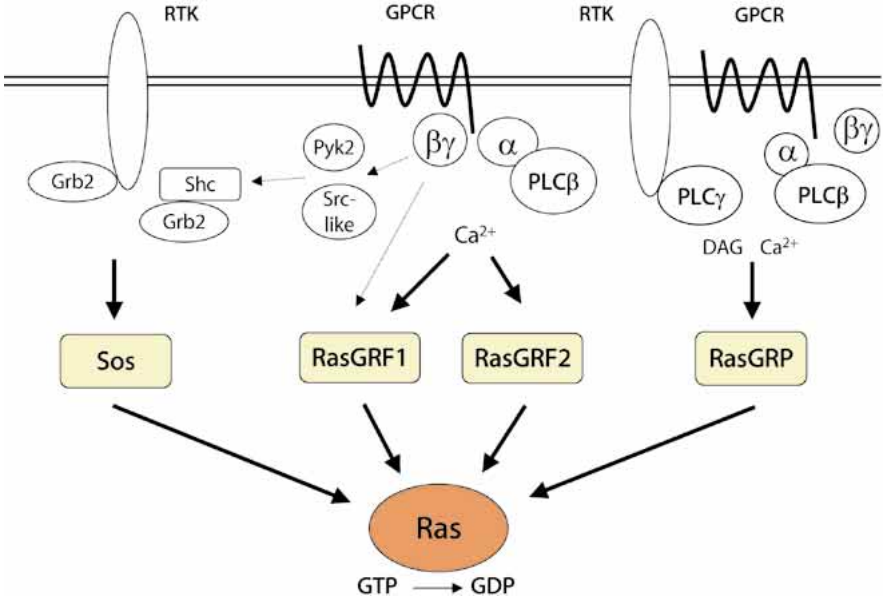


RAS Activation. Fig. 1 – Cycling of the Ras protein between the inactive GDP-bound form and the active GTP-bound form. Binding of growth factors to their receptors promote formation of active, GTP-bound Ras. This is achieved through the action of guanine nucleotide exchange factor (GEF), which stimulates the dissociation of the tightly bound GDP from Ras. Hydrolysis of bound GTP is accelerated by GTPase activating protein (GAP).

lished that ligand-bound receptor tyrosine kinases initiate Ras activation through formation of heterotrimeric complexes consisting of autophosphorylated receptors, the SH2/SH3 adaptor protein Grb2 and the guanine nucleotide exchange factor Sos. The SH3 domains of Grb2 bind to the carboxy-terminal proline-rich domain of Sos, whereas the SH2 domain binds to specific tyrosine phosphorylated sequences. Complex formation of Sos with autophosphorylated receptor tyrosine kinases via Grb2 results in the translocation of Sos from the cytosol to the plasma membrane, where its target, Ras, is localised. Interestingly, complex formation does not measurably alter the exchange activity of Sos towards Ras.

The mechanism(s) whereby G protein-coupled receptors (GPCRs) regulate Ras activation and cell proliferation is not completely understood. However, it is clear that ligand binding to GPCRs in a variety of cellular systems leads to rapid tyrosine phosphorylation of the Shc adaptor protein, followed by formation of Shc-Grb2 complexes. The candidate kinases responsible for Shc phosphorylation are Pyk2 and Src-like kinases, which link Gβγ subunits to MAP kinase activation through phosphorylation of Shc and subsequent complex formation of Shc with Grb2-Sos. Two other Ras exchange factors, the brain-specific RasGRF1 and the more ubiquitously expressed RasGRF2, also participate in the signalling events from GPCRs to Ras activation. In addition to their catalytic domain, both RasGRFs contain calcium and calmodulin-binding motifs. The third member of the Ras exchange factor family, RasGRP, is a widely expressed, calcium- and diacylglycerol-activated regulator of Ras.

Two distinct GAPs for Ras proteins have been identified; p120GAP is widely expressed, while neurofibromin is present predominantly in cells of the nervous system. Both GAPs sti-



RAS Activation. Fig. 2 – Activation of Ras following ligand-binding to receptor tyrosine kinases (RTKs) or G protein-coupled receptors (GPCRs). Three distinct GEFs specific for Ras have been identified: Sos1/2, RasGRF1/2, and RasGRP. Binding of growth factor (e.g. epidermal growth factor) to its RTK initiates dimerization and autophosphorylation of the receptor and subsequent recruitment of the Grb2/Sos complex to the plasma membrane, where Ras activation occurs. GPCRs that signal through heterotrimeric G proteins can activate all three GEFs. The Gβγ subunit may activate tyrosine kinases that through Shc phosphorylation recruit Grb2/Sos complex to Ras at the plasma membrane. In addition, the Gβγ subunit can also stimulate the activity of RasGRF1. Calcium signals triggered by GPCRs seem to be required for the activation of both Ras GRFs. Finally, RasGRP, that contains calcium- and diacylglycerol-binding domains, may also contribute to Ras activation downstream of either RTKs or GPCRs.

mulate the weak endogenous rate of Ras GTP hydrolysis, thereby negatively regulating signalling via Ras.

Clinical Relevance

Mammalian Ras proteins have been studied in great detail because mutant Ras proteins are associated with many types of human cancer. These mutant proteins are permanently in the GTP-bound, active state and can cause neoplastic transformation. The point mutations found in Ras genes fall into two functional groups; those effecting codons 12, 13, 59, 61 and 63 reduce the rate of hydrolysis of GTP on Ras protein and critically blocking its stimulation by GAPs, while those at codons 116, 117, 119 and 146 increase the rate of nucleotide exchange.

One of the Ras GAPs, neurofibromin, is the product of the NF1 gene. Damage to this gene has been implicated in the hereditary disease Von Recklinghausen neurofibromatosis, which is characterised by a number of developmental defects including benign and malignant tumours of neural crest origin, such as neurofibromas and neurofibrosarcomas.

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RAS Transformation Targets

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Synonyms

- RAS-regulated genes

Definition

The term '→ RAS transformation targets' denotes a set of genes deregulated by the expression of oncogenic RAS proteins. The target genes mediate the initiation of malignant transformation in normal cells and tissues and/or maintain the biological properties of cancer cells.

In a general way, the term 'target' defines a gene whose transcription is turned on or off by a positive or negative controller present in the nucleus (a transcriptional activator or repressor). In eukaryotic genes, the regulation is goverened by binding of transcription factors to defined transcriptional control regions. Since RAS proteins are cytoplasmic signalling molecules attached to the inner face of the plasma membrane, direct transcriptional targets do not exist. Rather, RAS proteins mediate transcriptional alterations indirectly via a cascade of cytoplasmic effector molecules.

Characteristics

Background

Since 1982, cellular oncogenes of the *RAS* gene family have been discovered in an increasing number of human and experimental tumors. Gene transfer studies (transfection experiments) have shown that mutationally activated RAS proteins (→ RAS activation) induced characteristic morphological alterations in phenotypically normal cells grown as established lines in culture. Moreover, the RAS transfected cells showed features of cancer cells, including an-

chorage-independent proliferation and rapid tumor formation following injection into mice. *RAS* activation became the paradigm for a cancer initiating process at the cellular and molecular level. Following the initial discovery of cellular *RAS* oncogenes, new experimental approaches suggested that cellular transformation mediated by *RAS* oncogenes requires more than a single genetic alteration. Firstly, more sophisticated gene transfection experiments in normal diploid cells showed that the transformation process requires cooperating oncogenes and inactivation of tumor suppressor genes. Secondly, molecular genetic analysis of tumors indicated that *RAS* activation occurs in conjunction with tumor suppressor gene loss and other changes (1). Detailled biochemical and structural analysis of RAS proteins identified a specific region or effector domain responsible for interacting with other cellular proteins (effectors). Furthermore, cellular transformation by RAS is invariably coupled with profound alterations of gene expression. For this reason, identification of the genes (targets) that respond to expression of activated *RAS* genes is of paramount importance for understanding the biological properties of cancer cells and the transformation process itself.

How do activated RAS proteins affect gene transcription?

RAS genes encode small GTP-binding proteins located at the inner face of the plasma membrane. The RAS proteins affect gene transcription in a global way by acting as major switches in signal transduction processes. Signaling pathways couple extracellular stimuli (e.g. a growth-stimulating polypeptide binding to a specific receptor on the cell surface) with transcription factors. Briefly, the signalling pathway is made of a chain (cascade) of interacting proteins located on the cell surface, in the cytoplasm and in the nucleus. In normal cells, the RAS signal transduction mediates proliferation, developmental processes and differentiation. RAS proteins are switch molecules exhibiting OFF and ON states. In resting cells, RAS proteins are inactive. RAS proteins are switched on (activated) by a complex set of protein interactions initiated by the binding of a ligand (e.g. a growth stimulating polypeptide or hormone outside the cell) to a receptor at the cell surface and terminating with the loading of GTP, catalyzed by nucleotide exchanger proteins (Fig.). RAS proteins are switched off (inactivated) by hydrolysis of GTP to GDP.

Oncogenic forms of RAS are locked in their active, GTP-bound state and transduce signals essential for cellular transformation, cell survival, angiogenesis, invasion and metastasis. There are several branches of the signaling pathway downstream of RAS. These involve the RAF/MEK/ERK cascade of cytoplasmic kinases, the small GTP-binding proteins RAC and RHO, phosphatidylinositol 3-kinase (PI3K) and others (Fig. 1). Several nuclear proteins (transcription factors) are stimulated by signaling pathways downstream of RAS. These comprise ETS-domain transcription factor ELK1, serum-responsive factor SRF, the leucine zipper protein JUN, activation transcription factor 2 (ATF2) and nuclear factor κB (NFκB). Thus, RAS expression affects a complex set of transcriptional targets (2,3).

Since many receptor tyrosine kinases that affect transcription signal through RAS, the number of transcriptional targets of the RAS pathway or RAS-induced genes may be huge. Consequently, there exists a significant overlap between RAS targets and genes regulated by individual transcription factors whose activity is modulated by RAS signalling cascades. The complexity of transcriptional activity mediated by RAS is even further complicated by the cross-talk of individual pathways downstream of RAS. Furthermore, cellular signals triggered by RAS activation can be transient (e.g. following growth factor stimulation of quiescent cells) or sustained (e.g. due to mutations that impair efficient GTP hydrolysis).

How can RAS target genes be identified?

Cells express between 5,000 and 15,000 different genes at a time depending on the state of growth, development and differentiation. How can the subset of genes regulated by RAS signalling be identified? A convenient strategy to isolate RAS targets is based on repro-

ducible expression differences between normal and RAS-transformed cells at the level of messenger RNA. Various methods of genetic manipulation and differential cloning techniques have been used to recover differentially expressed genes. In the first step, the RAS oncogene is cloned under the control of an artificial conditional or constitutive promoter and is introduced into cultured normal cells by DNA transfection. In a typical example, the inducible promoter can be activated by adding certain hormones or antibiotics to the culture medium. In the absence of inducer, the cells maintain normal properties. Following induction for defined time periods, the expression of the oncogene results in the tumorigenic conversion of the transfected cells. In the second step, RNA is prepared from normal and transformed cells and converted into double-stranded complementary DNA (cDNA). The cDNA sequences derived from both types of cells are denatured to produce single-stranded molecules, subsequently mixed and incubated at elevated temperatures. The cDNA molecules common to both cell types will anneal and form hybrids, while the cDNA molecules specific for either the normal or transformed cells remain in the single-stranded conformation. This method is called cDNA subtraction. The specific cDNA molecules are separated from the common sequences by various methods, among others by chromatography, magnetic sorting or selective enrichment using polymerase chain reaction, and cloned in bacteria. Afterwards, the cloned specific cDNAs are sequenced and their differential expression is verified in normal and RAS-transformed cells by hybridization methods. Alternatively, the impact of RAS on transcription can be monitored in cells that stably express the oncogene under a constitutive promoter and exhibit the fully transformed phenotype (4,5).

Recently, the cDNA microarray technology has been developed permitting parallel mRNA expression analysis of thousands of genes. In view of the rapid progress in sequencing entire genomes of humans and rodents, it will be possible to monitor gene expression differences at the level of the transcriptome in the near future. In addition, recent progress in protein analysis at high resolution and sensitivity will allow the extension of studies from the level of transcription to the level of translation products. Post-translational modifications of proteins are also subject to alterations during the transformation process.

RAS transformation targets

The following list of RAS-induced genes is selective rather than comprehensive (Table 1). The targets included have been chosen as examples of functional classes of genes that are likely, or already known, to contribute to the process of tumorigenesis. The number of potential targets described in the literature is much higher. An important cautionary note is that some of the target genes identified in model systems have not been thoroughly analysed in human cancers, hence their general importance is not clear. Furthermore, differential expression in transformed cells as compared to normal cells does not prove a causal role in the transformation process and verification experiments are required. For example, the functional role of matrix metalloprotease genes was verified by determing their effects on the invasive properties of cancer cells using cell biological assays. To establish a decisive role of a growth-stimulating target gene in RAS transformation, it is essential to measure its effect on growth of transfected cells. Alternatively, gene ablation experiments are needed to establish a functional role. RAS transformation is closely linked to gene expression (Table 2). Little is known about the mechanisms of down-regulation, transcriptional repressors involved and global mechanisms of gene repression by RAS signal transduction pathways.

How many RAS targets are there?

Different cell types may express different RAS targets. In addition, the transcriptional pattern induced by RAS may depend on growth conditions and other environmental factors. Therefore, the number of RAS targets directly involved in the control of transformed properties of cells is not known. A recent paper lists 393 genes diffentially expressed in normal rat fibroblasts versus a RAS-transformed derivative.

RAS Transformation Targets. Table 1 – Genes up-regulated by RAS.

gene product	functional role of gene product
ornithine decarboxylase (ODC)	polyamine biosynthesis, essential for proliferation
glucose transporter	metabolism
JUN	transcription factor, component of AP-1 transcriptional activator complex
Cyclin D1	stimulator of cell cycle
transforming growth factor β	autocrine growth stimulator
stromelysin 1 (MMP3)	metalloprotease, stimulates invasive properties
collagenase (MMP1)	metalloprotease, stimulates invasive properties
urokinase-type plasminogen activator (uPA)	controls synthesis of the serine protease plasmin, degradation of extracellular matrix
cyclooxygenase-2 (COX2)	prostaglandin synthesis, necessary for cell survival
vascular endothelial growth factor (VEGF)	stimulates neovascularization
glycoprotein CD44	induces metastasis

RAS Transformation Targets. Table 2 – Genes down-regulated by RAS.

gene product	properties
myoblast differentiation factor 1 (MYOD1)	stimulates myoblast diffentiation, commitment to form myotubes
fibronectin	structural component of extracellular matrix
smooth muscle α actin	structural component of cytoskeleton
alpha type I collagen	structural component of cytoskeleton
lysyl oxidase	extracellular enzyme, stabilizes extracellular matrix
thrombospondin	inhibits neovascularization
growth-arrest specific protein 1 (GAS1)	growth inhibitor
tissue inhibitor of metalloproteases-1 (TIMP2)	inhibits metalloproteases and invasive properties
Fas (CD95)	inducer of apoptosis

These targets include 168 up-regulated and 225 down-regulated genes (5).

Clinical relevance

RAS mutations are correlated with bad prognosis of certain tumors. However, RAS signalling can be activated in the absence of genetic damage to RAS genes. Therefore, activation of the RAS signalling cascade is a very general feature of tumorigenic cells. The malignant properties are controlled by an unknown number of RAS-induced genes that may differ among different types of cancers. Thus, genome wide identification of target genes executing the diverse repertoire of biological activities of RAS-expressing tumor cells increases our knowledge about the multi-step process of tumorigenesis and helps to define novel tumor markers. The products of the genes causally involved

in the control of aggressive tumor properties represent drug targets that may form the basis for novel therapeutic interventions.

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RAS-binding Domain

Definition

Ras-binding domain (RBD) is a protein domain in → Raf-1 and in other members of the Raf-1 family that interacts with the → Ras protein. In Raf-1 the RBD spans residues 51-131.

RB1

Definition

RB1 is a → tumor suppressor gene. Homozygous functional inactivation results in childhood → retinoblastoma.

RB2/p130

Definition

RB2/p130 is a member of the → retinoblastoma family of → tumor suppressor genes. To date, three members of the retinoblastoma family (RB/p105, p107 and RB2/p130) have been identified. One of its functions is to protect cells from uncontrolled growth.

RBD

Definition

→ Ras-binding domain.

Rcc1

Definition

Rcc1, also known as CHC1 (chromosome condensation 1), guanine nucleotide exchange factor is a protein of 421 amino acids and 44 kD. The human CHC1 or RCC1 gene locus maps at 1p36.1. Rcc1 promotes the exchange of Ran-bound GDP by GTP. It is involved in the regulation of onset of chromosome condensation in the S-phase and binds to the chromatin. The Rcc1/Ran complex (together with other proteins) acts as a component of a signal transmission pathway that detects unreplicated DNA.

RDS

Definition

→ Radioresistant DNA synthesis.

Reactive Oxygen Species

Definition

The reactive oxygen species are superoxide anion (O_2), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH), and are produced continuously in the body by the incomplete reduction of oxygen to water. Their deleterious effects are countered by the antioxidant systems of the

body (vitamin E, glutathione, superoxide dismutase, catalase etc.). The oxygen radicals such as hydrogen peroxide, hydroxyl anion radicals, superoxide anion, or singlet oxygen are produced by normal oxidative metabolism and removed by antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase. Superoxide, singlet oxygen, hydroxyl radical and hydrogen peroxide are all capable of oxidising other molecules by abstracting hydrogen or an electron from them. The hydroxyl radical, *OH, is sufficiently reactive to damage DNA; → detoxification.

REAL

Definition

The REAL (Revised European-American Classification of Lymphoid) classification, proposed in 1994, integrates information that is currently available for the grouping of lymphoid neoplasms. It takes into account morphological characteristics, immunophenotype, genetic analyses and clinical parameters; → B-cell tumours.

RecA

Definition

RecA protein is central to homologous recombination in bacteria. It forms a DNA-protein filament utilizing the energy of ATP to catalyse homologous pairing of DNA molecules and strand exchange. It is the product of the *recA* locus of *E.coli*; a protein with dual activities, activating proteases and also able to exchange single strands of DNA molecules. The protease-activating activity controls the SOS response, and the nucleic acid handling activity is involved in recombination-repair pathways.

RecA/Rad51-like Proteins

Definition

RecA/Rad51-like proteins share homology to the RecA (bacteria) and Rad51 (eukaryotes) proteins. They are involved in the repair of DNA damage by homologous recombination. In humans they include the Xrcc2, Xrcc3, Rad51L1, Rad51L2 and Rad51L3 proteins.

Receptor Tyrosine Kinases

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Synonyms

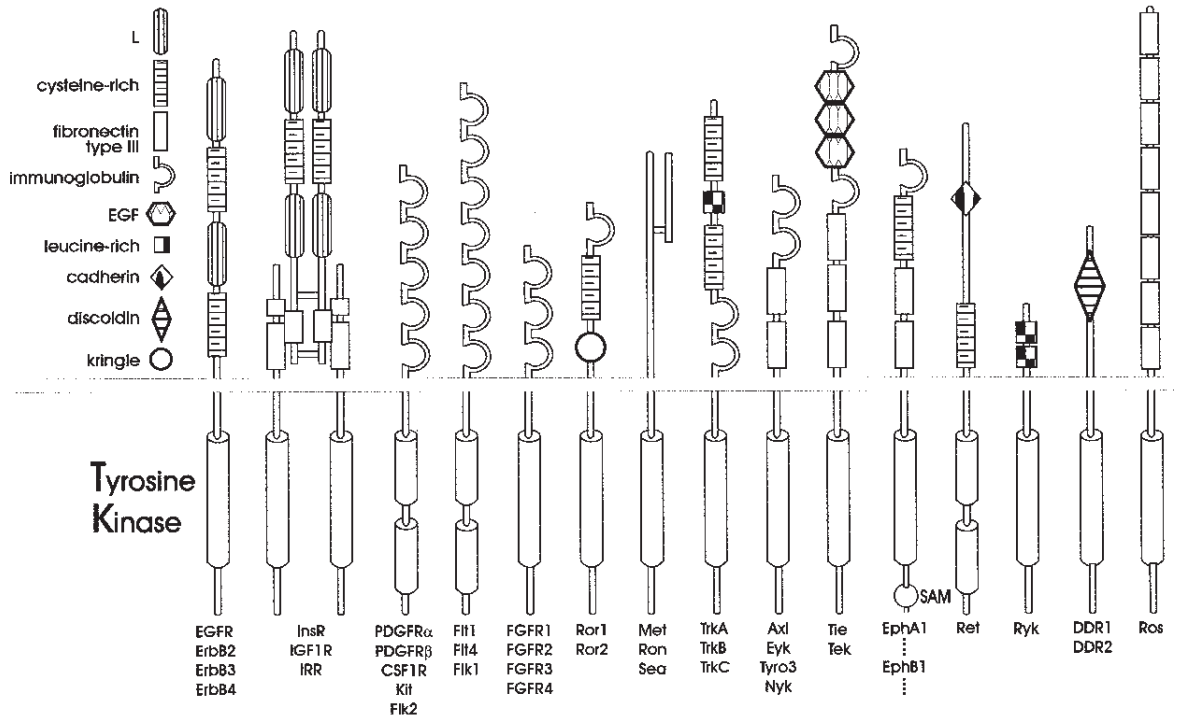
Receptor tyrosine kinases are growth factor receptors.

Definition

Receptor tyrosine kinases (RTKS) are transmembrane glycoproteins that represent key components of signaling pathways which control cell proliferation, differentiation and metabolism. Dysregulation of RTKs often results in neoplastic growth or developmental abnormalities.

Characteristics

Receptor tyrosine kinases (RTKs) show a common architecture comprising an extracellular portion that binds polypeptides ligands, a transmembrane helix and a cytoplasmic portion that displays catalytic activity (Fig. 1). Docking sites for protein-protein interactions with cytoplasmic signaling molecules are also present. The majority of RTKs are formed by a single polypeptide chain in a monomeric conformation in the absence of ligand. Met and its related receptors Ron and Sea, after proteolytic processing of a single chain precursor, consist



Receptor Tyrosine Kinases. Fig. 1 – Structure and domain organization of representative RTKs. Legend for the extracellular (top) domains is on the left side. The tk containing cytoplasmic portion of the receptor is on the bottom.

of a short extracellular α-chain that is disulfide-bonded to a membrane-spanning β-chain. Members of the insulin receptor subfamily are disulfide-linked dimers of two polypeptide chains, forming an α2β2 heterotetramer.

The extracytoplasmic portion shows considerably diversity among members of the family, usually containing a linear array of discrete folding modules such as immunoglobuline-like domains, cysteine-rich domains, fibronectin tipe III-like domains and EGF-like domains.

The cytoplasmic portion of RTKs is more conservative and uniform. Next to the transmembrane helix is located the so-called juxtamembrane region, followed by the tyrosine kinase (TK) catalytic domain and finally by the C-terminal region. Some receptors, for example the → PDGF receptor and Ret, contain a relatively large insert in the TK domain. Upon ligand binding to the receptor, autophosphorylated tyrosine residues (docking sites) can be found along the juxtamembrane and TK domains. A sterile α motif (→ SAM) is found at the C-terminus of Eph receptor subfamily members.

Mechanisms of RTKs activation

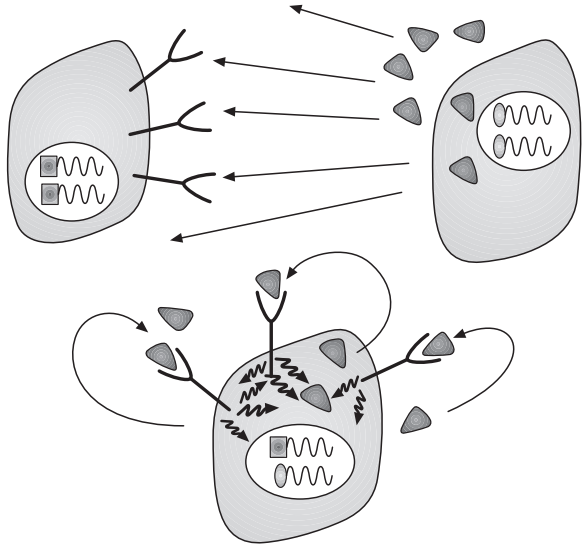
Binding of growth factors to RTKs results in receptor dimerization and activation of intrinsic tyrosine kinase activity, leading to intermolecular phosphorylation of each receptor on specific tyrosine residues. This results in the recruitment of cytoplasmic signaling molecules containing SRC homology 2 (→ SH2) domains or phosphotyrosine-binding (PTB) domains, which recognize short peptide segments containing phosphotyrosine residues on the activated receptor in a sequence-specific manner. These cytoplasmic signaling molecules include proteins with enzymatic activity, such as phospholipase Cγ (PLCγ), phosphatidylinositol 3-kinase (PI3K), the GTP-ase activating protein for Ras product (p120 GAP) and non-enzymatic proteins functioning as adaptor proteins, such as Grb2. The latter contains SH2 an SH3 do-

mains, which also function as protein-protein interaction motifs and recognize proline-rich domains in other proteins. Grb2 acts as an adaptor that binds to phosphotyrosines in activated receptors via its SH2 domain and recruits the Ras nucleotide exchange factor Son of Sevenless (SOS) to the receptor via its SH3 domain.

These proteins, through a series of protein-protein interactions in part mediated through SH2 and SH3 domains, form signaling complexes downstream of RTKs. Many of these signaling complexes regulate the activity of serine/threonine kinases, which in turn regulate, through phosphorylation, the activity of nuclear transcription factors thus completing the signal transduction pathways following ligand interaction with RTKs.

Paracrine and autocrine RTK activation

The ligand can be provided by a cell different from those expressing the related RTK or by the same cell. The latter is defined as autocrine activation whereas the former as paracrine activation (Fig. 2).



Receptor Tyrosine Kinases. Fig. 2 – Paracrine (top) and autocrine (bottom) RTK activation. The latter occurs following an inappropriate expression of a ligand for a constitutively expressed RTK, as it happens for PDGFβ and PDGF-R in dermatofibrosarcoma protuberans (see text).

Bioactivity

A number of oncogenes derive from mutant forms of RTKs. These include receptors for known growth factors, such as epidermal growth factor receptor (v-erb B), colony stimulating factor-1 receptor (v-fms), hepatocyte/scatter factor receptor (→ met), nerve growth factor receptor (→ trk), stem cell factor receptor (→ kit), heregulin co-receptor (→ HER-2/Neu) and GDNF/NTN/PSP/ART receptor (Ret).

In these oncogenes the common mechanism of activation from the physiological status to the neoplastic one implies structural changes that deregulate the receptor kinase activity, with the delivery of a continuos ligand-independent signal (Table).

Mutations that promote ligand-independent dimerization thus represent the general mechanism for oncogenic activation of RTKs. HER2/Neu can be activated by a single point mutation in the transmembrane domain. This mutation promotes receptor dimerization and kinase activation in the absence of ligand. The loss of a single cysteine residue in the extracellular domain of → Ret in MEN2A [→ multiple endocrine neoplasia type 2] syndrome, which includes medullary thyroid carcinomas and pheochromocytomas, results in receptor constitutive activation. This mutation most likely destroys an intramolecular disulfide bond that frees a cysteine residue that, through the formation of intermolecular bond, enhances receptor dimerization and results in constitutive activation of the receptor catalytic activity.

On the contrary, activation of Ret in MEN2B syndrome, which develops the same tumors as MEN2A and displays a more complex phenotype, occurs through a single and specific point mutation in the receptor kinase domain (M918T) that changes the methionine residue, specific for RTK, to threonine (often found in that position in cytoplasmic TKs). This mutation increases the basal kinase activity and alters the substrate specificity of the receptor.

Another activation mechanism of RTKs consists of gene amplification and/or overexpression of the receptor protein. This is the case

Receptor Tyrosine Kinases. Table – Receptor tyrosine kinases and cancer.
[a] belonging to Multiple Endocrine Neoplasia type 2 Syndrome and Familiar Medullary Thyroid Carcinomas. *MTC*: Medullary Thyroid Carcinoma; *PTC*: Papillary Thyroid Carcinomas; *HPRC*: Hereditary Papillary Renal Cell Carcinomas; *CMML*: Chronic Myelomonocytic Leukemia.

RTK	sporadic cancer	mechanism of activation	hereditary cancer	mechanism of activation
Ret	MTC	point mutations	MTC/pheochromocytomas[a]	point mutations
	PTC	fusion genes		
Trk	PTC	fusion genes		
Met	thyroid/gastric/colorectal	gene amplification/ overexpression	HPRC	point mutations
Kit	gastric cancers/hematopoietic	point mutations		
HER2/Neu	mammary and ovary carcinomas	gene amplification/ overexpression		
EGF-R	brain tumors	overexpression		
PDGF-R	dermatofibrosarcoma protuberans/CMML	fusion genes		

for HER2/Neu and EGFR in mammary/ovary carcinomas and brain tumors, respectively. An important class of RTKs oncogenes in human tumors, including ret, trk, met and PDGF-R, are constitutively activated following chromosomal rearrangements that juxtapose novel sequences derived from unrelated genes (activating genes) with the TK domain of the receptor. The majority of these oncogenes are fused with proteins containing a coiled-coil domain capable of promoting protein/protein interaction and thus mediating dimerization and constitutive activation of the kinase activity. This is particularly evident for the RTKs Ret and Trk involved in the pathogenesis of papillary thyroid carcinomas (PTCs). The former, in particular, has been found associated with thyroid tumors developed in children from the areas contaminated by the Chernobyl nuclear disaster. A similar activation mechanism has been described for → PDGF receptors in chronic myelomonocytic leukemia (CMML) through the production of the fused oncogene TEL/PDGFR. However, the same receptor has been found constitutively activated in dermatofibrosarcoma protuberans by the activation of an autocrine loop due to the formation of a fusion gene COLA1α/PDGFβ that results in the constitutive production of a stimulating PDGFβ product.

Clinical Relevance

- HER2/Neu gene → amplification represents an already validated negative prognostic marker for both mammary and ovary carcinomas. Moreover, an anti $p185^{erb\text{-}2}$ monoclonal antibody that has antiproliferative effects enhances the inhibition of human breast cancer cells following treatment with different compounds.
- The detection of Ret germ-line mutations offers an invaluable diagnostic tool for the identification of asymtomatic individuals at high risk to develop a MEN2-associated neoplasia.

- Moreover, fusion oncogenic proteins derived from Ret and Trk rearrangements are a distinctive diagnostic features of the papillary type of thyroid cancer. In general, this class of oncogenic products (fusion proteins) are viewed as potential tumor specific target for therapeutic interventions.
- A variety of tyrosine kinase inhibitors have been now developed and much progress has been made toward obtaining specific and potent compounds. They include, in particular, EGFR and PDGFR inhibitors that have entered clinical trials.

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Receptor-mediated Endocytosis

Definition

→ Endocytosis.

Recessive

Definition

A recessive allele is obscured in the phenotype of a heterozygote by a dominant allele, often due to inactivity or absence of the product of the recessive allele.

Recurrent Viral Infection

Definition

Recurrent viral infection is a clinical disease resulting from reactivation of latent virus.

Recycling

Definition

Recycling is the process that allows receptors to be diverted from the degradation fate and to be re-routed to the plasma membrane after → endocytosis. Recycling constitutes the default pathway for the majority of receptors undergoing constitutive internalization and involved in the uptake of nutrients. Kinetic measurements revealed that each receptor can recycle about every 20 minutes.

Regulators of G-protein Signalling

Definition

Regulators of G-protein signalling (RGS) proteins inhibit → G-protein-mediated signalling by activating the GTPase activity of defined G-protein α-subunits. The identified RGS proteins appear to predominantly interact with members of the G_i and G_q families of heterotrimeric G-proteins. The exact cellular function of RGS proteins are currently not clear, but they may play an important role in the dynamic regulation of the sensitivity of the G-protein-mediated signal transduction system.

REL

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Synonyms

- NFκB

Definition

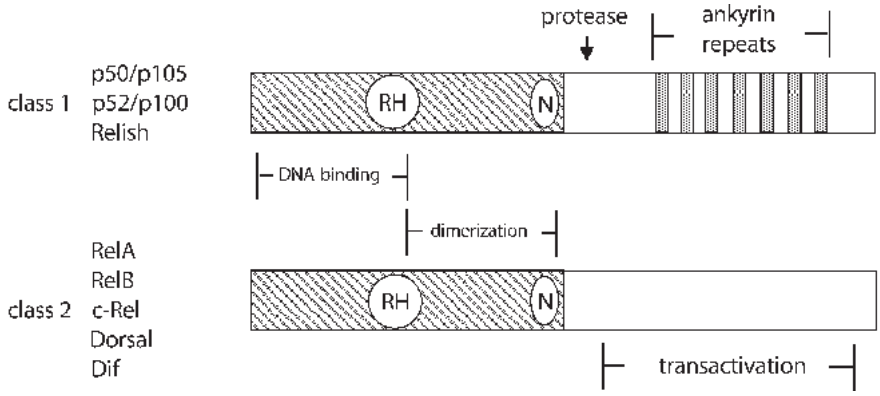
Rel can refer generically to a family of structurally related eukaryotic transcription factors. The Rel family includes the vertebrate proteins RelA, RelB, c-Rel, p50/p105 and p52/p100, a viral protein (v-Rel) and the Drosophila proteins Dorsal, Dif, and Relish (Table). Alternatively, Rel can specifically refer to v-Rel or c-Rel. The term NFκB is also often used in a general fashion interchangeably with Rel, as in Rel/NFκB transcription factors.

Characteristics

Rel family proteins are related through an N-terminal domain of approximately 300 amino acids, called the Rel homology domain, which contains sequences important for the formation of dimers, DNA binding and nuclear localization. Rel proteins must form dimers to bind to DNA and these dimers bind to a related set of DNA target sites called κB sites. The consensus sequence for the κB site is GGGRNWTTCC (where R is any purine, N is any nucleotide, and W is A or T).

Vertebrate Rel proteins can be divided into two classes based on the sequences C-terminal to the Rel homology domain (Fig. 1). The C-terminal half of one class of Rel protein (including p105 and p100) contains multiple copies of a 33 amino acid repeat, called ankyrin repeats, which causes these proteins to be inactive. However, these C-terminal inhibitory sequences can be removed to yield shorter, active DNA-binding forms (p50 and p52) that consist primarily of the Rel homology domain. A second class of Rel family proteins (RelA, RelB, c-Rel) contains C-terminal sequences that function as transcription activation domains.

Almost all Rel family proteins can form homodimers and heterodimers. The most common Rel dimer in many vertebrate cells is a heterodimer consisting of p50-RelA, which is often specifically called NFκB. The binding of Rel dimers to DNA target sites generally activates the transcription of a large variety of cellular genes, including many whose protein products are involved in immune responses (immunoglobulins), inflammation (cytokines and cytokine re-



REL. Fig. 1 – Generalized structures of Rel proteins. The Rel homology domain (RH) has sequences important for the formation of dimers, DNA binding, inhibitor (IκB) binding and nuclear localization (N). Class 1 Rel proteins have multiple copies of inhibitory ankyrin repeats, which can be removed by proteolysis to release the mature N-terminal DNA-binding proteins (p50 or p52). Class 2 Rel proteins do not undergo proteolysis and have C-terminal sequences that are required for transcription activation. Dimers can be formed within or between Rel classes (e.g., p50-p50 or p50-RelA, etc.).

REL. Table – Rel/NFκB transcription factors.

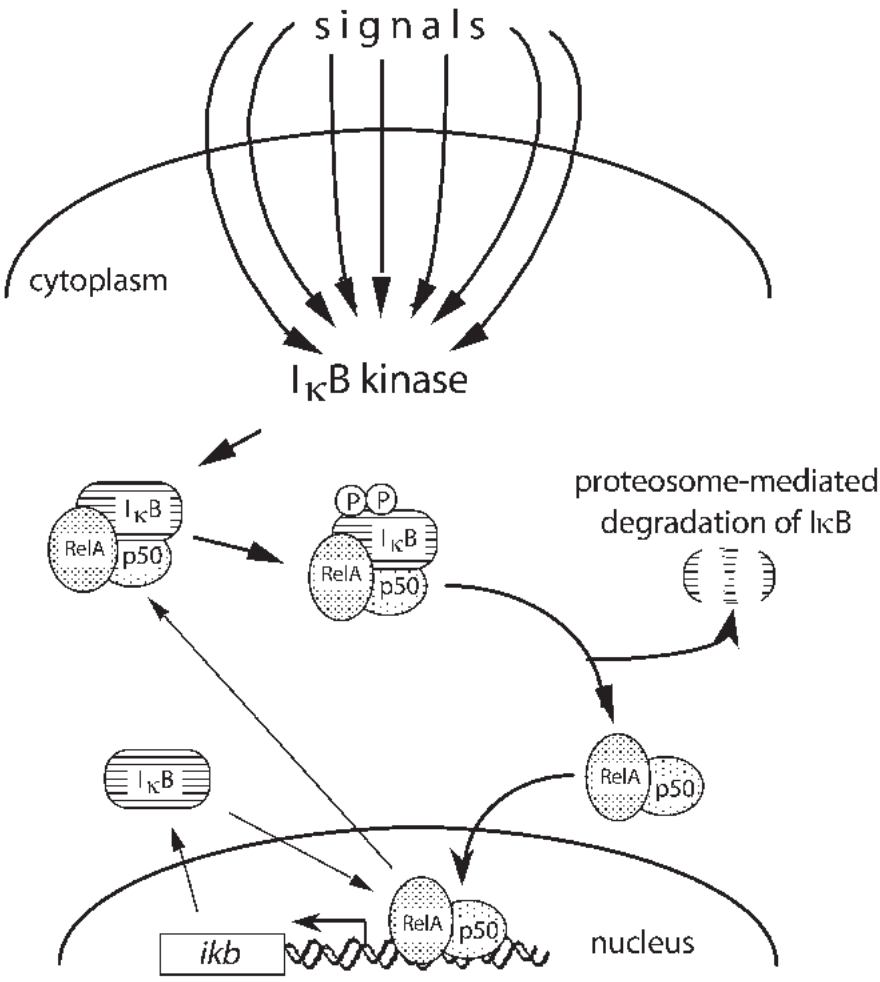
gene	protein	
Drosophila melanogaster		
dorsal	Dorsal	
dif	Dif	
relish	Relish	
Avian Rev-T retrovirus		
v-rel	v-Rel	
vertebrates		**human gene (chromosome)**
rela	RelA	*RELA* (11q12-q13)
relb	RelB	*RELB* (19q13)
c-rel	c-Rel	*REL* (2p13-12)
nfkb1	p50, p105, or p50/p105	*NFKB1* (4q23-q24)
nfkb2	p52, p100, or p52/p100	*NFKB2* (10q24)

ceptors), adhesion (cell surface attachment proteins) and programmed cell death. In addition, several viral genes (including those of human oncogenic viruses such as Human immunodeficiency virus [HIV-1], Epstein-Barr virus [EBV] and Cytomegalovirus [CMV]) are activated by Rel/NFκB dimers.

Cellular and Molecular Regulation

Rel transcription factors can be regulated at several levels:

- First, because there are five interacting members of the Rel transcription factor family, they can combine to form a diverse set of dimers with distinct DNA target site-binding specificity. The composition of these dimers is determined by the affinity of specific Rel subunits for one another, the concentration of specific subunits in a given cell, and possibly the post-translational modification of specific subunits. The composition of the dimers is important since it influences which genes they can control and which inhibitors (see below) can bind to the complexes.
- Second, in most cell types, Rel complexes are located in the cytoplasm due to interaction with an inhibitor protein called IκB. IκB proteins are members of a structurally related family of proteins that inhibit Rel complexes by masking sequences involved in nuclear localization and DNA binding. A variety of extracellular signals, including cytokines, phorbol esters, virus infection, interferon and lipopolysaccharide, can activate Rel transcription complexes by inducing their dissociation from IκB. In the best characterized example, the p50-RelA NFκB complex is held in the cytoplasm by interaction with IκBα (Fig. 2). Stimulation of the cell by an appropriate signal leads to activation of an IκBα kinase (IKK), which then phosphorylates IκBα at two N-terminal serine residues. Phosphorylation of IκBα signals it for → ubiquitination at a nearby lysine residue. Ubiquitinated IκBα is then degraded by the proteasome and the NFκB complex is free to enter the nucleus to activate transcription of specific genes. One of these genes encodes IκBα. Newly synthesized IκBα can then pull NFκB off DNA and export and resequester it in the cytoplasm. Thus, activa-



REL. Fig. 2 – Activation of the Rel/NFκB pathway. Many extracellular signals (see text) lead to the activation of the NFκB signal transduction pathway. In most cases, these signals lead to the activation of an IκB kinase, which phosphorylates (P) IkB at two nearby residues. Phosphorylation of IκB targets it for degradation by the cellular proteasome. Free NFκB (p50-RelA) then enters the nucleus, binds to specific DNA sites and turns on the expression of many cellular genes. One of these genes encodes IκB. Newly-synthesized IκB can remove NFκB from DNA and resequester it in the cytoplasm. Thus, activation of NFκB (thick arrows) is usually a transient (30 – 60 min) process, which is subject to negative auto-regulation (thin arrows).

tion of NFκB is a transient (approximately 30-60 minute) event in most cell types.

However, in some cell types NFκB is constitutively located in the nucleus. Cells with constitutively nuclear and active NFκB include some normal cells (B cells, Sertoli cells, some neurons), many cancer cell types (including breast, lymphoid cell, colon, prostate and pancreatic cancers) and cells infected with certain oncogenic viruses (HTLV-1, → EBV, Hepatitis B [→ hepatitis viruses]). In cancer cells, the constitutive nuclear NFκB activity can either be due to chronic stimulation of the cell (for example, by an autocrine signaling mechanism) or due to mutations in genes controlling NFκB signaling. For example, mutations that inactivate the gene encoding IκBα have been found in several types of Hodgkins lymphoma cells. In the case of viral infection, specific viral gene products (HTLV-1 Tax, EBV LMP-1 and HBV X proteins) can constitutively induce the degradation of IκBα, allowing NFκB to continuously enter the nucleus of infected cells.

Clinical Relevance

Rel transcription factors have been associated with oncogenesis in several settings.

First, the avian Rev-T retrovirus has a single gene, *v-rel*, that encodes the v-Rel transcription factor. Young chickens infected with high titer stocks of Rev-T succumb to leukemia/lymphoma quite rapidly, often within 7-10 days, and have multiple lymphoid cell tumors primarily in their spleens and livers. The *v-rel* oncogene arose by a recombination event between an avian → retrovirus and the turkey *c-rel* gene (→ transduction of oncogenes), such that *v-rel* encodes a chimeric protein that has internal c-Rel amino acids and retroviral (Env) amino acids at its N and C termini. v-Rel is mutated in several ways compared to the c-Rel proto-oncoprotein:

- v-Rel has the Env amino acids at both termini
- it has a deletion of two N-terminal and 118 C-terminal amino acids
- it has 18 internal mutations. These mutations affect a variety of activities of v-Rel:
- v-Rel has a reduced ability to associate with IκBα and therefore is constitutively located in the nucleus of cells;
- v-Rel has an altered DNA-binding site specificity;
- v-Rel has an altered ability to form dimers. These acquired activities contribute to the oncogenicity of v-Rel, and it is likely that v-Rel malignantly transforms cells by forming homodimers that freely enter the nucleus, bind DNA and turn on the transcription of genes that enhance cell growth and block apoptosis.

Second, alterations in Rel transcription factor structure or regulation are seen in several human cancers. As mentioned above, several cancer cell types have constitutive nuclear NFκB activity. It is thought that this chronic NFκB activity endows the cancer cells with a proliferate advantage and resistance to apopotsis. In addition, genetic alterations in *rel* family genes, including amplifications, point mutations and chromosomal translocations, have been identified in several human cancers, especially hematopoietic cell cancers. One of the most commonly described alterations of this type occurs in many lymphoid cell cancers and results in deletions of the 3′ region of the human *NFKB2* gene, leading to the expression of a C-terminally truncated p100 protein with altered activity.

Pharmacologic or molecular agents that target the Rel signal transduction pathway have been much sought after as anti-inflammatory and anti-cancer therapeutics. Many common anti-inflammatory agents, including aspirin, glucocorticoids and green tea, act, at least in part, by inhibiting NFκB. For example, aspirin appears to act by binding directly to IκB kinase, thus blocking activation of NFκB. Similarly, many recent anti-cancer therapies seek to use molecular or pharmacological agents to sensitize tumor cells to chemotherapeutic agents by inhibiting the anti-apoptotic activity of NFκB. For example, expression of a super-repressor form of IκBα, which cannot be released from NFκB, sensitizes several tumor cell types to anti-tumor agents.

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Relapse

Definition

Relapse defines the re-emergence of disease symptoms after a complete or partial remission. Relapse can be only biological (re-appearence or increase of biological disease markers) or clinical (re-appearence of clinical symptoms of the disease). Whatever the malignant condition, relapse is usually associated with a poorer prognosis.

Remission

Definition

Remission is the reduced severity of disease symptoms. Complete remission, e.g. in leukemia, refers to a hematologic state that is defined by normal bone marrow, peripheral blood characteristics and a normal performence status, with the consequence that the patient feels perfectly normal. Partial remission refers to a lessening of severity of disease symptoms without reaching a normal state.

Renal Carcinoma

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Definition

Renal carcinoma is a cancer, arising from the epithelial cells of the kidney and must be distinguished from tumors of the renal pelvis.

Characteristics

World wide about 150 000 people develop renal carcinoma each year, and approximately 78 000 individuals die from the disease. Most cases of renal carcinoma are sporadic, that is most renal carcinomas occur without an inherited predisposition to develop renal cancer. About 1-5% of renal carcinomas are the consequence of an inherited tendency to develop renal cancer.

Cigarette smoke (→ tobacco carcinogenesis) is a recognized environmental factor that can lead to renal cancer. Exposure to high doses of trichloroethylene, an industrial solvent, may also lead to renal cancer. Renal tumors occur more commonly in men than woman. The male/female ratio for renal cancer is about 2/1.

Early diagnosis

Currently, there are no accepted methods for the early diagnosis of renal cancer. Although population-based screening by renal ultrasound would undoubtedly detect renal tumors in asymptomatic individuals, this procedure is not considered cost effective. No serological tests are currently available for the early detection of renal cancer.

Treatment

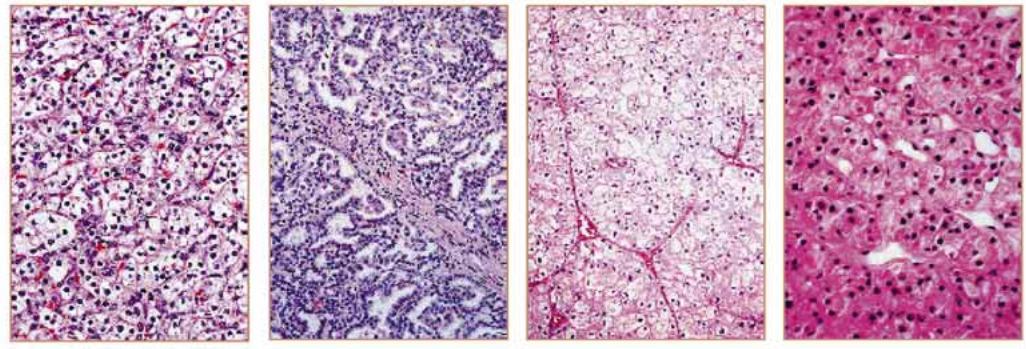
Surgical removal of the renal cancer, usually by nephrectomy, is so far the best treatment of renal cancer. Treatment of patients with metastatic disease is difficult; there are no treatments available that consistently eradicate metastatic renal carcinoma.

Types of renal cancer

Renal cancer is not one disease as such. There are at least four types of epithelial renal cancer, which can be distinguished by their histological appearance (Fig.):

- clear cell renal carcinoma (75% of sporadic renal carcinomas),
- papillary renal carcinoma (15%),
- chromophobe renal carcinoma (5%),
- renal oncocytoma (5%).

There is evidence, suggesting a correlation between the histological type of renal cancer and prognosis.



	clear cell	papillary	chromophobe	oncocytoma
type:				
genes:	VHL	MET	?	?
mutation freq (%):	50	13	?	?

Renal Carcinoma. Fig. – Frequency of mutations in sporadic human renal epithelial neoplasms.

Renal cancer genes

Studies of families with inherited forms of renal cancer have led to the identification of two genes that, in their mutant form, predispose individuals to the development of renal neoplasia, the von Hippel-Lindau (*VHL*) tumor suppressor gene and the *MET* proto-oncogene. Current studies are aimed at identifying a third renal cancer gene - the *BHD* gene, responsible for the Birt Hogg Dube syndrome.

The *VHL* and the *MET* gene are mutated in sporadic renal carcinomas. The *VHL* gene is mutated in about 50% of sporadic clear cell renal carcinomas. It is inactivated by hypermethylation in about 15% of sporadic clear cell renal carcinomas. The *MET* proto-oncogene is mutated in about 15% of sporadic papillary renal carcinoma.

Types of inherited renal cancer

The currently recognized forms of inherited renal carcinoma are: von Hippel-Lindau disease (VHL), inherited balanced translocations involving human chromosome 3, hereditary papillary renal carcinoma (HPRC) type 1, the Birt Hogg Dube (BHD) syndrome and familial clear cell renal carcinoma.

Clinical features of renal cancer suggesting hereditary renal cancer

Several characteristics of inherited renal cancers make them distinct from sporadic renal cancers. Frequently, other family members are affected with renal tumors. In general, the renal tumors are multiple and often involve both kidneys. Individuals with renal tumors need to determine whether their tumors are single or multiple and whether other family members have had renal cancer. In cases of hereditary renal cancer, it is essential to test other, apparently healthy, family members for occult renal tumors.

Mechanisms of development of inherited renal cancers

The *VHL* gene is a classic tumor suppressor gene. In the von Hippel-Lindau disease and in sporadic clear cell renal carcinoma, renal tumors develop as a consequence of the loss or inactivation of both copies of the VHL gene. The result of an inactivated *VHL* gene is the lack of the VHL protein in proximal renal tubular epithelial cells.

The von Hippel-Lindau disease is a rare inherited disorder characterized by a predisposition to develop tumors in multiple organs:

brain, spinal cord, eye, adrenal gland, kidney, pancreas, and epididymis. There are 3 distinct clinical types of VHL:

- type 1 is characterized by renal carcinoma, brain, spinal and eye tumors without tumors of the adrenal glands (pheochromocytoma);
- type 2A is characterized by pheochromocytoma, eye and brain tumors without renal cell carcinoma;
- type 2B is characterized by pheochromocytoma, renal cell carcinoma, eye and brain tumor.

VHL types 2A and 2B are produced by germline missense mutations in the VHL gene; VHL type 1 is produced germline mutations that produce truncated VHL proteins.

The proto-oncogene *MET* encodes the receptor for hepatocyte growth factor/scatter factor. Renal tumors in patients with hereditary papillary renal carcinoma type 1 as well as a subset of sporadic papillary renal carcinomas, develop as a consequence of the constitutive activation of the MET cell surface receptor. Hereditary papillary renal carcinoma type 1 is characterized by an inherited predisposition to develop multiple, bilateral papillary renal carcinomas. Founder effects have been observed in several families with HPRC. All germline and somatic mutations, identified to date in the *MET* gene, were missense mutations and were located in the tyrosine kinase domain of the gene.

The Birt Hogg Dube syndrome is an inherited disorder, characterized by a predisposition to develop multiple tumors of the hair follicle (fibrofolliculomas). Usually, the fibrofolliculomas occur on the face and neck. Furthermore, a predisposition a predisposition to develop spontaneous pneumothorax and renal cancer has been described. Pathologists have had difficulty classifying the renal tumors associated with the Birt Hogg Dube syndrome, they appear to be either chromophobe renal carcinomas or renal oncocytomas.

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Renaturation

Definition

Renaturation is the association of denatured complementary single strands of a DNA double helix.

REP

Definition

The rep gene of → AAV encodes Rep (replication) proteins.

Repair of DNA

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Characteristics

The repair of DNA comprises a group of distinct biochemical pathways by which various types of damage to DNA, especially damage to the nitrogenous bases A, T, C and G, is repaired. Such damage can occur spontaneously in living cells as the result of the generation of products of oxidative metabolism that interact with the bases of DNA. Base damage in DNA also transpires as the result of interactions between environmental agents, especially cancer-

causing chemicals, with DNA. DNA repair of base damage can be conveniently classified as follows:

1. *Reversal of Base Damage.* This mode of DNA repair involves the direct reversal of selected types of base damage. A good example is the restoration of thymine dimers in DNA to their native monomeric state. Thymine dimers (T↔T) are formed by the abnormal chemical joining of adjacent thymine bases (T, T) in DNA and frequently occurs in cells that are exposed to ultraviolet (UV) radiation, such as sunlight. These lesions in DNA can interfere with normal DNA functions such as DNA replication and/or transcription. One way that thymine dimers are repaired in cells is by the action of an enzyme called DNA photolyase, which directly reverses the chemistry of the thymine dimers so that they are once again in their monomeric native state (T↔T→ T, T).
2. *Removal of Damage.* This mode of DNA repair involves the enzymatic excision of damaged bases.
   - One biochemical pathway of excision repair involves the excision of damaged bases as the free base and is therefore called base excision repair. Base excision repair is employed by cells to remove simple chemical modifications such as those produced by → oxidative damage to DNA or by simple chemicals, including alkylating agents that are used to treat various cancers.
   - A second biochemical pathway for the removal of damage involves the excision of a relatively large piece of one strand of the DNA duplex that includes the damaged base. This is called nucleotide excision repair. Nucleotide excision repair operates against thymine dimers in DNA (in addition to DNA photolyase) as well as in the removal of many other types of chemical altered bases.
   - A third mode of excision repair involves the excision of bases in DNA that are incorrectly paired (mispaired bases). This type of excision repair is called → mismatch repair.

Repair of DNA and Cancer

Defective DNA repair allows the persistence of damaged bases in the genome. When such DNA is copied during DNA replication mistakes are made leading to permanent mutations. Mutations that affect oncogenes or tumor suppressor genes can lead to cancer. Hence, defective DNA repair is an important cancer predisposing factor. Patients who suffer from the hereditary disease xeroderma pigmentosum (XP) are unable to carry out nucleotide excision repair of DNA. Hence, when exposed to sunlight, the thymine dimers generated in the DNA of their skin cells accumulate and result in a very high incidence of skin cancer. Patients who are genetically prone to colon cancer suffer from a disease called hereditary nonpolyposis colon cancer (→ HNPCC) and are often defective in mismatch repair. Thus, they accumulate mutations that lead to cancer of the colon.

Base excision repair often removes bases that are altered by alkylating agents used for cancer treatment. In principle therefore, inhibiting base excision repair in cancer (but not in other normal) cells would be expected to improve the therapeutic efficacy of alkylating agents. For this reason DNA repair enzymes are strategic targets for rational design of anticancer agents.

Repair of DNA Strand Breaks

Strand breaks can be produced in DNA after exposure to ionizing radiation (X-rays) and can also occur spontaneously in cells as a result of oxidative damage. Specialized repair pathways operate to repair both single- and double-strand breaks in DNA. The proteins encoded by the breast cancer genes → BRCA1 and → BRCA2 are believed to be involved in the repair of strand breaks in DNA.

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Repair Endonucleases

Definition

Repair endonucleases, such as formamidopyrimidine glycosylase (FPG) or endonuclease III, often combine two enzymatic activities; a lesion-specific glycosylase that removes the damaged base and an AP-endonuclease that recognises the resulting baseless sugar and cuts the DNA.

Repetitive DNA

Definition

Repetitive DNA in a reassociation reaction behaves as though many (related or identical) sequences are present in a component, allowing any pair of complementary sequences to reassociate.

Replication Error Positive

Definition

Replication error positive (RER^+) is defined as alterations (deletions or expansions) in the length of short tandem repeats in tumor DNA compared to constitutive DNA from the same patient. The analysis is performed by comparing → microsatellite sequences, in DNA from the tumor and from normal tissue of the same individual, amplified by PCR using specific primers that flank the microsatellite. The detection of microsatellite changes can be visualized by silver- and ethidium bromide-staining of polyacrylamide gels, radioactive labeling of PCR products or automated fluorescence detection. A multicenter study proposed that at least five microsatellite loci should be analyzed (including mono- and dinucleotide repeats) and → microsatellite instability should only be diagnosed if >20% of loci studied display alterations of the band pattern.

Replication Forks

Definition

Replication forks are the protein machine that translocates along double-stranded DNA, replicating both strands as it moves (→ replication licensing system).

Replication Licensing System

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Definition

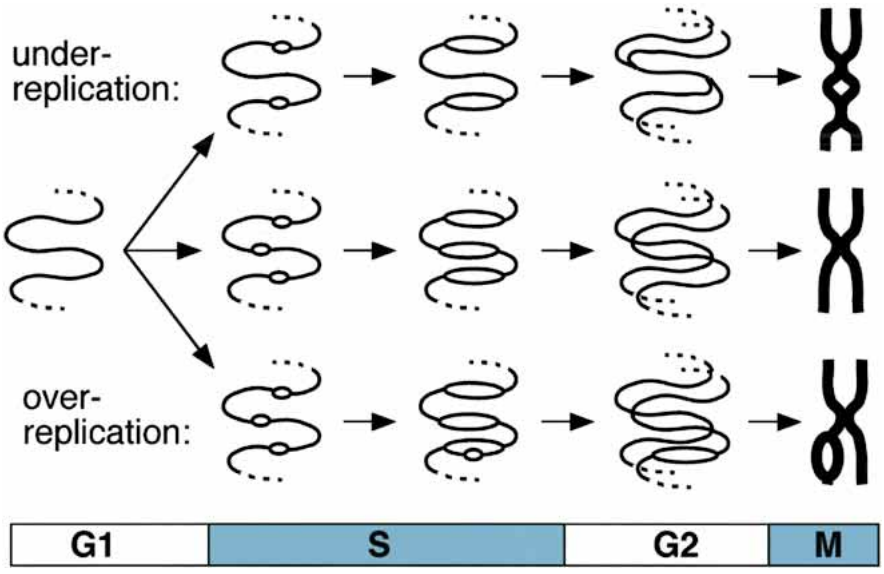
The replication licensing system ensures that no sections of chromosomal DNA are replicated more than once in a single cell cycle. It loads complexes of → MCM/P1 proteins onto → replication origins early in the cell cycle, which 'license' the origin for a single initiation event in the subsequent S phase. As a consequence of replication, the MCM/P1 proteins are removed from the DNA, thus ensuring that re-replication of DNA does not occur in a single cell cycle.

Characteristics

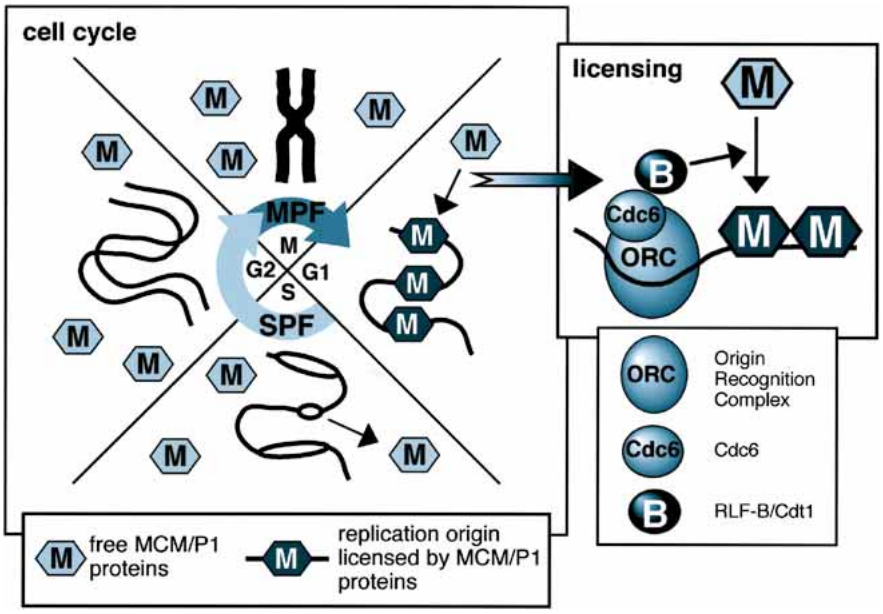
In order to minimise the risk of potentially cancer-causing mutations occurring whenever a cell divides, the duplication of chromosomal DNA must be as accurate as possible. All the genome must be replicated, with nothing left unreplicated and nothing replicated more than once. This problem is made complicated

by the way that eukaryotes undertake genome duplication. → Replication forks progress along double-stranded DNA and copy both strands at a rate of 10-100 base pairs per second. However, a single replication fork would take years to duplicate a typical genome and so thousands of replication forks are used, initiated from replication origins scattered throughout the genome (Fig. 1). To ensure the complete replication of eukaryotic chromosomes, these origins must be sufficiently closely spaced so that all the intervening DNA can be replicated before entry into mitosis occurs. Failure to replicate even small sections of DNA could lead to disastrous consequences as the sister chromatids are pulled apart during mitosis (Fig. 1, 'under-replication'). On the other hand, to prevent any section of DNA being replicated more than once in a single S phase, each origin must fire no more than once in each cell cycle. Over-replication of DNA is likely to be very harmful as it would represent an irreversible genetic change, potentially leading to the risk of recombination and amplification occurring in the duplicated region (Fig. 1, 'over-replication'). The replication licensing system permits replication origins to be used efficiently, whilst preventing them from firing more than once in single cell cycle.

Precise chromosome duplication is achieved by separating the initiation of DNA replication into two phases (Fig. 2, left-hand panel). In the first phase, which occurs early in the cell cycle, replication origins are 'licensed' by loading the RLF-M complex of MCM/P1 proteins (shown as 'M' in Fig. 2). In the second phase, → S-phase promoting factor (SPF) becomes active and promotes the initiation of a single pair of replication forks at each licensed origin. S-phase promoting factors probably consist of various → cyclin-dependent kinases (CDKs) as well as the → Cdc7/Dbf4 protein kinase. As each origin initiates its MCM/P1 proteins are removed, thereby resetting the origin to the unlicensed state. To ensure that no re-initiation occurs, the ability to license origins must completely cease before any replication forks are initiated; even a small amount of licensing activity present during S phase could lead to the re-replication of part of the genome. The way this regulation is achieved is currently poorly understood.



Replication Licensing System. Fig. 1 – Ensuring precise chromosome replication. A small segment of chromosomal DNA, replicated from 3 origins is shown during the cell cycle. Middle panel: successful duplication. Top panel: under-replication due to the failure of one of the origins to fire. Bottom panel: over-replication, due to one of the origins firing a second time in S phase. The four stages of the cell cycle – G1, S (DNA synthesis), G2 and M (mitosis) – are shown below.



Replication Licensing System. Fig. 2 – Left-hand panel: a small segment of genomic DNA containing three replication origins is shown during the cell cycle. The factors that drive cell cycle progress (SPF and MPF) are shown by arrows. In late mitosis and early G1, replication origins are 'licensed' by binding the MCM/P1 complexes (hexagons). During S phase, S-phase-promoting factor (SPF) induces replication forks to initiate at licensed origins, and the MCM/P1 proteins are displaced. In G2 phase, all replication forks have terminated, all origins are unlicensed, and all DNA is replicated. Mitosis-promoting factor (MPF) then triggers the condensation of DNA into chromosomes and its segregation to the two daughter cells. Right-hand panel: the licensing reaction requires ORC, Cdc6 and RLF-B/Cdt1, and results in multiple copies of the MCM/P1 complex being assembled onto each replication origin.

Molecular composition

The replication licensing system consists of two components: The RLF-M complex of MCM/P1 proteins, which constitutes the licence, and RLF-B/Cdt1, which is required to load RLF-M onto chromatin early in the cell cycle. Other proteins are also required on the DNA that allow it to serve as a substrate for the replication licensing system. These include the Origin Recognition Complex (ORC) and the Cdc6 protein (Fig. 2, right-hand panel). This group of proteins form the '→ pre-replicative complex' present at replication origins during late mitosis and G1. All of these are potentially under cell-cycle regulation to prevent the re-licensing of replicated DNA.

There are 6 MCM/P1 proteins, termed Mcm2, 3, 4, 5, 6 and 7, which are in the form of a hetero-hexamer in the active RLF-M complex. However, a number of other MCM/P1 protein complexes also exist, which are probably intermediates in an assembly/disassembly pathway. In vertebrates they are abundant nuclear proteins, and 10 – 20 copies of the RLF-M hetero-hexamer are loaded onto each replication origin. The precise role of the MCM/P1 proteins in DNA replication is currently unclear, though they have been shown to have helicase (DNA unwinding) activity. This helicase activity may be involved in allowing the replication fork proteins to load onto or move along the DNA. Various lines of evidence suggest that the MCM/P1 proteins are substrates of the Cdc7/Dbf4 kinase that is involved in activating replication origins during the cell cycle. As a consequence of MCM/P1 protein phosphorylation by Cdc7/Dbf4, other replication factors such as DNA polymerase α are then recruited to the replication origin.

RLF-B/Cdt1 acts to promote the loading of the RLF-M complex onto DNA, but thereafter does not appear to be required for the maintenance of the licensed state. Its precise molecular activity is currently unknown. RLF-B/Cdt1 is under tight cell cycle control and this regulation is likely to be important in preventing re-replication of DNA in a single cell cycle.

The Origin Recognition Complex (ORC) consists of 6 polypeptides termed Orc1, 2, 3, 4, 5 and 6. Binding of ORC to DNA appears necessary for that place to be defined as a replication origin. In addition to its role in establishing replication origins, ORC also appears to be involved in establishing repressive chromatin states at specific sites on DNA.

The binding of Cdc6 to chromatin is dependent on the presence of ORC. It is required for the origin to become licensed but is not for the maintenance of the licensed state once licensing has occurred. In mammalian cells, Cdc6 is exported from the nucleus at the end of G1 and does not regain access to the chromatin until the end of the subsequent mitosis. In yeast, Cdc6 is degraded at the end of G1. These features are likely to contribute to mechanisms that prevent re-licensing of DNA once S phase has started.

Regulation

Since re-replication of segments of chromosomal DNA is likely to cause very significant genetic changes to the cell, it is obviously crucial for eukaryotic cells to prevent this from happening. In order for the licensing system to operate properly and ensure that no DNA re-replicates, its activity must completely cease before S phase starts (Fig. 2). Recent work suggests that cells may possess multiple redundant mechanisms for ensuring this. One important consideration is that the components required to load the MCM/P1 proteins onto DNA (ORC, Cdc6, RLF-B/Cdt1) are not required for the continued binding of the MCM/P1 proteins. Therefore in principle, licensing can be prevented by inhibiting the activity of either ORC, Cdc6 or RLF-B/Cdt1/Cdt1, whilst the licensed state is maintained. Although it is currently not possible to describe in molecular detail how licensing is prevented late in the cell cycle, three important themes have emerged: regulation by changes in subcellular localisation, regulation by cyclin-dependent kinases and regulation by geminin.

Early experiments that characterised the replication licensing system showed that in order for replicated nuclei from the G2 phase of the cell cycle to undergo a further round of DNA replication, they had to undergo a transient permeabilization of their nuclear envelope. This suggested that re-replication of chromosomal DNA was regulated at least in part by compartmentalization of regulatory components between the nucleus and cytoplasm. Recent work has confirmed this idea, but has shown that different organisms actually regulate different components of the licensing system by this mechanism. In mammalian cells, the Cdc6 protein, which is required on the DNA for licensing to occur, is nuclear in late mitosis and G1 and is exported from the nucleus during S phase and G2. The lack of Cdc6 in the nucleus late in the cell cycle is likely to play an important role in preventing the re-licensing (and hence re-replication) of the DNA. A different version of this story is seen in the yeast *Saccharomyces cerevisiae*, where the MCM/P1 proteins, rather than Cdc6, are present in the nucleus only in late mitosis and early G1 and are exported from the nucleus at later stages of the cell cycle. Physically separating essential licensing components from their DNA substrate by nuclear export would seem to be a powerful way of preventing the inappropriate licensing of replicated DNA.

Another important aspect of the regulation of the replication licensing system involves cyclin-dependent kinases (CDK). These kinases promote the major transitions of the eukaryotic cell division cycle, including entry into S phase (SPF) and entry into mitosis (MPF) (Fig. 2). During late mitosis and early G1, however, CDK activity is low and this is the period when replication licensing can occur. Research in a number of different experimental systems have shown that CDKs, either directly or indirectly, can inhibit origin licensing. The nuclear exclusion of yeast MCM/P1 proteins and mammalian Cdc6 appears to be promoted by high

CDK activity. CDKs may also have other inhibitory effects on the activity of the licensing system. For example, in *S. cerevisiae*, the CDK-dependent degradation of the Cdc6 protein occurs during later stages of the cell division cycle. This idea provides an elegant explanation for cell cycle regulation of chromosome replication, with an early pre-replicative phase where CDK activity is low and licensing occurs, followed by progression into S phase where CDK activity is high and licensing is inhibited. Another key licensing regulator that has recently been identified is geminin, a small protein whose abundance changes dramatically during the course of the cell cycle: it builds up in S phase and G2, but is degraded on exit from metaphase. Geminin tightly binds and inhibits the RLF-B/Cdt1 component of the licensing system, and may represent the major activity preventing re-licensing of replicated DNA late in the cell cycle of higher eukaryotes.

Clinical relevance

When cells terminally differentiate or enter quiescence, their replication origins do not remain licensed and the MCM/P1 proteins are lost from the chromatin. The MCM/P1 proteins therefore can be used as unique markers for proliferative cells. Antibodies to the MCM/P1 proteins been shown to be useful in analyses of cervical smears and urine samples to detect abnormal proliferative cells. More speculatively, failures of the replication licensing system may underlie the chromosome rearrangement and gene amplification observed in many types of cancer. The replication licensing system as a whole also represent a potentially powerful target for anti-proliferative drugs, though this potential has yet to be explored.

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Replication Origin

Definition

The replication origin is the site on DNA where → replication forks are initiated.

Reporter Gene

Definition

A reporter gene is a coding unit whose product is easily assayed (such as chloramphenicol transactylase or green fluorescence protein). It may be connected to any promoter of interest so that expression of the gene can be used to assay promoter function.

Residual Disease

Definition

→ Minimal residual disease.

Respiration Rate

Definition

Respiration rate, also known as O_2 consumption rate or O_2 uptake rate, is the amount of oxygen consumed by a tissue or organ per unit time.

Restriction Enzymes

Definition

Restriction enzymes recognize specific short sequences of (usually) unmethylated DNA and cleave the duplex (sometimes at the target site, sometimes elsewhere, depending on restriction enzyme type).

Restriction Fragment Length Polymorphism

Definition

Restriction fragment length polymorphism (RFLP) refers to inherited differences in sites for restriction enzymes in DNA, for example caused by base changes in the enzyme target site. These base changes result in differences in the length of fragments generated by cleavage with appropriate restriction enzymes. In case two alleles differ in a target site for an enzyme, the two homologous chromosomes can be individually identified at this allelic site by the different DNA fragment length in an agarose gel. RFLPs have been widely used for genetic mapping to link the genome directly to a conventional genetic marker. Today, mapping is done more efficiently by → microsatellite analysis.

Restriction Landmark Genomic Scanning

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Definition

Restriction Landmark Genomic Scanning (RLGS) is a two-dimensional gel electrophoresis method that is used as a genome scanning technique to survey genomes for copy number changes as well as changes in DNA → methylation status of promoter sequences.

Description

RLGS was developed as a high speed genome scanning technique for the identification of DNA amplification as well as DNA methylation changes (Fig.) (3). For RLGS, genomic DNA is restriction digested with a landmark enzyme, usually a rare cutting enzyme (e.g. *Not*I). The restriction ends are endlabeled in a fill-in reaction using [α-^{32}P] labeled nucleotides. In the following second restriction digest (e.g. *Eco*RV), the DNA fragments are cut into smaller fragments suitable for separation in an agarose disc gel electrophoresis (1st dimension). The next step is an in-gel restriction digestion with a third enzyme (e.g. *Hinf*I) that generates fragments with a size range between 50 to 1000 basepairs. DNA fragments are separated in a polyacrylamide gel (2nd dimension). The gel is dried and exposed to an X-ray film. The resulting RLGS profile display highly reproducible patterns of RLGS fragments in which each fragment represents an end-labeled *Not*I site. Up to 2,000 end-labeled rare cutting restriction sites are displayed in a single RLGS profile. Most of the rare cutting restriction enzyme sites with GC-rich recognition sequences are located in the promoter region of genes, resulting in a selective display of gene sequences rather than random genomic sequences. The sequence of RLGS fragments can be determined through standard PCR-based or direct cloning procedures. If methylation sensitive enzymes are used as restriction landmarks, the method is able to provide a search for altered DNA methylation. Methylation in a *Not*I site would inhibit the restriction digest resulting in lack of endlabeling at this particular site. As a result this fragment would be missing from the RLGS profile. Since RLGS profiles are highly reproducible, this system allows the comparison of profiles derived from different tissues (e.g. normal versus diseased tissue) or between different individuals.

Identification of low level DNA amplification in human cancers

The endlabeling step in the RLGS procedure guarantees a correlation between RLGS fragment intensity and copy number of the particular sequence in the genome. The technique is sensitive enough to distinguish diploid (full intensity fragments) and haploid (half intensity fragments) copies. The majority of fragments in a human RLGS profile (about 90%) show diploid intensity, while a much smaller fraction of fragments (about 10%) have haploid intensity. In addition, 10-15 fragments show markedly enhanced intensities (approximately 50 to 200 times the intensity of a diploid fragment). It was shown that these fragments are derived from rDNA sequences, present at higher copy numbers in the genome. Such quantitative capacity allows the use of RLGS to scan tumor genomes for regions of DNA amplification (1). RLGS profiles established from tumor DNA are compared to profiles of matching normal tissue or blood of the patient. The tumor profile is analyzed for fragments that show an enhancement relative to the normal profile. Using this strategy *CDK6* amplification in gliomas (1) and low level amplification of *MYCN* and *NAG* was identified in medulloblastomas for the first time.

Global scanning for DNA methylation changes in human cancers

If methylation sensitive restriction enzymes are used for the RLGS analysis, it is possible to scan genomes for differences in methylation patterns. The restriction landmark enzyme *NotI* is methylation sensitive. If a genomic *NotI* site is methylated, the enzyme does not cut and the site will not be endlabeled, resulting in a loss of this fragment in the RLGS profile. On the other hand, if the *Not*I site is unmethylated, the site is cut, the restriction ends are end-labeled and the fragment is present in the profile. → CpG islands frequently contain gene promoters and/or exons and are usually unmethylated in normal cells. Since *Not*I sites are mainly located in CpG islands, RLGS is a method that allows the determination of the methylation status in thousands of CpG islands at one time. It was shown that methylation of CpG islands is associated with delayed replication, condensed chromatin and inhibition of transcription initiation in cancer. In a recent study, aberrant CpG island methylation in multiple human cancers was studied using RLGS (2). In this global analysis, the methylation status of 1184 unselected CpG islands in 98 primary human tumors was tested. An estimated average of about 600 CpG islands (range of 0 to 4,500) of the 45,000 in the genome were aberrantly methylated in the tumors. This study also showed patterns of CpG island methylation that were common to several types of tumors together with patterns and targets that displayed distinct tumor type specificity.

Detection of imprinted genes

RLGS was used in two studies to identify allele specific methylation in the mouse genome. Allele specific methylation is associated with genes that show imprinted expression (→ imprinting). Imprinted genes are either expressed exclusively from either the paternal or maternal allele and possess important functions during development. The screen is based on the identification of polymorphic RLGS fragments between two mouse inbred strains. Allele-specific methylation in a polymorphic fragment results in the presence of this fragment in one cross, but its absence in the reciprocal cross. RLGS was the first method that allowed a systematic screen for loci that showed allele specific methylation. The cloning of those sequences that were methylated gave access to novel imprinted loci. So far two novel imprinted genes (*U2afbprs* and *Grf1*) were identified in an RLGS scan (4, 5).

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Restriction Map

Definition

A restriction map is a linear array of sites on DNA cleaved with various restriction enzymes.

Restriction Point

Definition

The restriction point (R-point) represents the most important regulatory mechanism controlling cell cycle progression of mammalian cells in response to external mitogenic or growth inhibitory signals. The R-point is located in late G_1 and is governed by the → retinoblastoma protein pathway (INK4 - → cyclin D - CDK4/6 - Rb - → E2F). Beyond the R-point, cell-cycle progression is largely independent of extracellular signals.

RET

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Definition

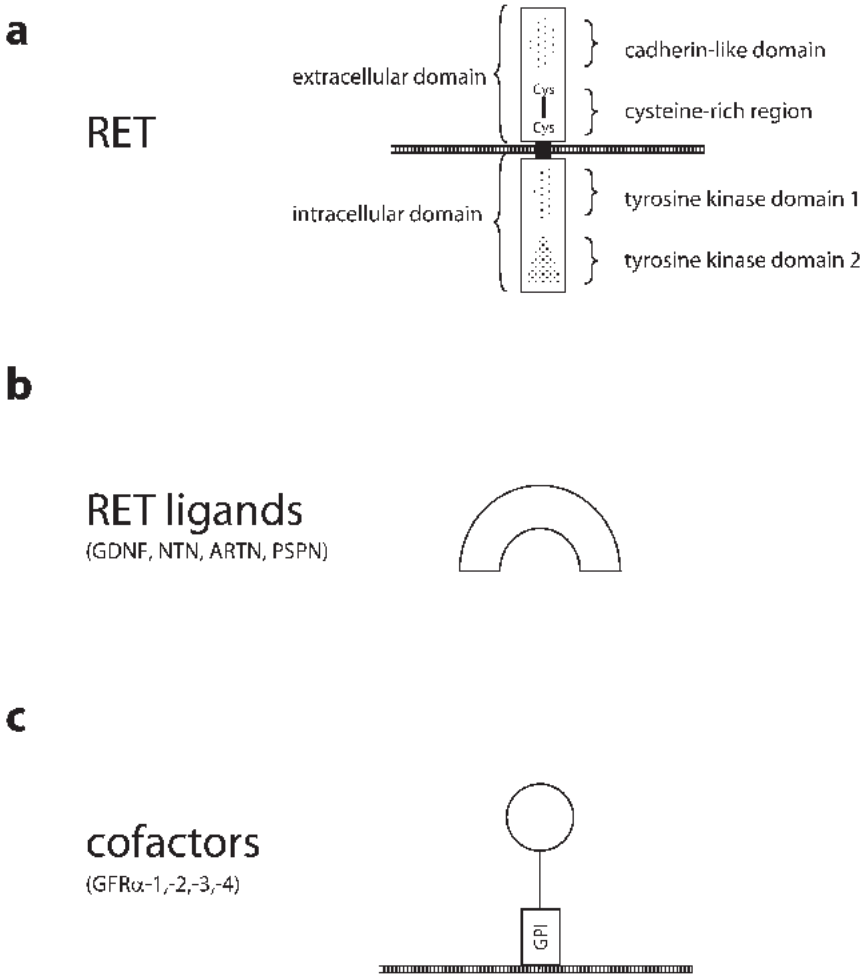
RET is the abbreviation for a gene that was found to be rearranged during transfection studies of NIH 3T3 cells with human lymphoma DNA. It is a receptor tyrosine kinase, for which the ligand is glial cell line derived neurotrophic factors (GDNF). It was originally detected as an oncogenic version from a T-cell lymphoma.

Characteristics

RET is located on chromosome sub-band 10q11.2 and consists of at least 21 exons. Alternative polyadenylation sites and alternative splicing results in different transcript sizes. It is not known how many protein isoforms exist, however, two major isoforms differ in their number of C-terminal amino acids (1072 and 1114 amino acids). The gene encodes for a transmembrane tyrosine kinase receptor. The first 10 exons and maybe part of exon 11 encode the extracellular domain, exon 11 encodes the transmembrane domain while the remaining exons encode the intracellular or cytoplasmic domain (Fig. 1a). The extracellular domain contains a cadherin-like domain and a cysteine-rich region. Whether the cadherin-like domain is of functional importance is unknown. The cysteine residues are bound to each other via intramolecular disulfide bonds. Two tyrosine kinase domains have been identified in the intracellular domain.

Cellular and molecular regulation

RET expression is largely restricted to neural, neuroendocrine and nephrogenic tissues. During embryogenesis, RET is expressed in developing kidneys, the presumptive enteric neuroblasts of the developing enteric nervous system,



RET. Fig. 1 – Putative structure of RET, its ligands and their cofactors.

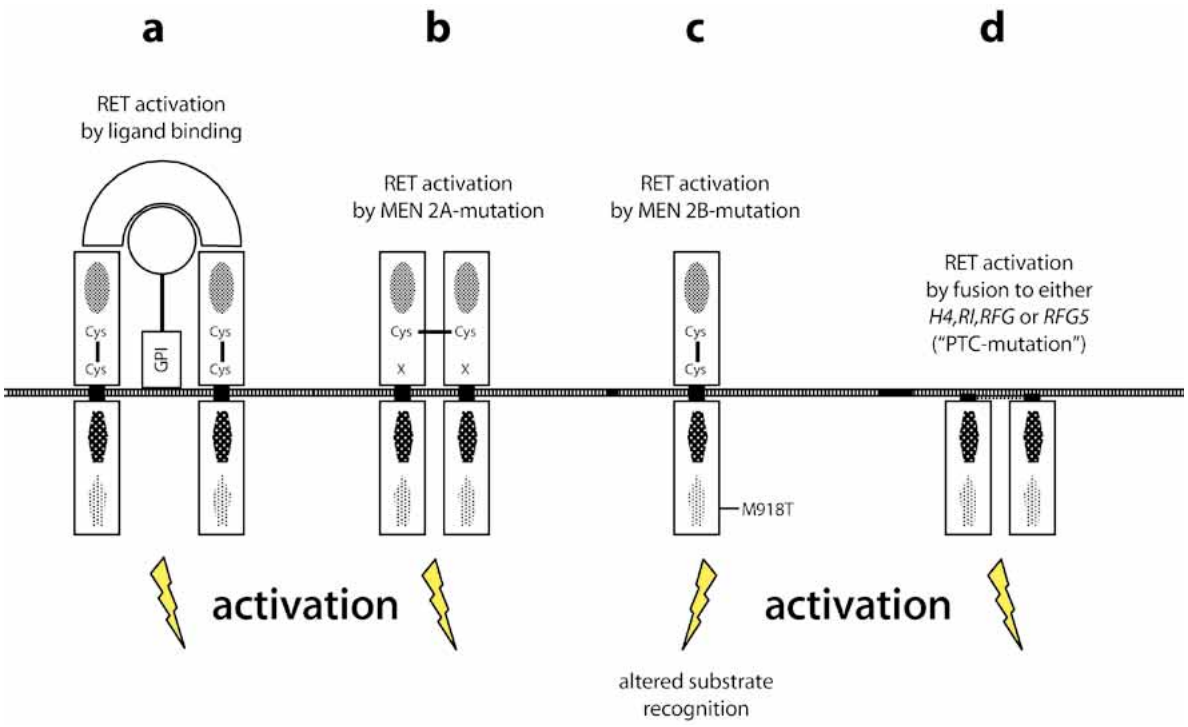
cranial ganglia and in the presumptive motoric neurons of the spinal cord.

RET can be activated by binding to one of its ligands. Ligand binding leads to dimerization of the RET-receptor and subsequent phosphorylation of tyrosine residues (Fig. 2a). At least four ligands

1. glial cell line derived neurotrophic factor (GDNF)
2. neurturin (NRTN or NTN)
3. persephin (PSPN or PSP)
4. artemin (ARTN or ART)

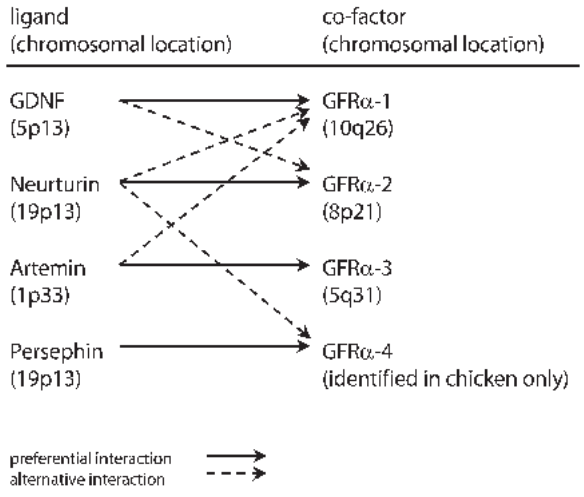
have been identified (Fig. 3, Fig. 1b). Recently, a potentially fifth ligand, enovin (EVN), has been described which, however, turned out to be identical to artemin. All ligands belong to the GDNF family. The identity and similarity between the various members is between 40 – 60%. The ligands can not bind directly to RET but rather require the presence of a membrane bound glycosyl-phosphatidylinositol (GPI)-linked cofactor. Four cofactors, named GFRα-1, -2, -3 and -4, have been identified (Fig. 1c, Fig. 3). GFRα-4 has only been identified in the chicken. GFRα-1 is also known as GDNFR-α, RETL1 and TrnR1; GFRα-2 is also known as GDNFR-β, NTNR-α, RETL2 and TrnR2. Each ligand preferentially binds to one cofactor, however, alternative binding has been observed *in vitro* (Fig. 3). The prevalence of these alternative interactions *in vivo* is unknown.

RET -/- knockout mice die early after birth due to agenesis of kidneys and lack of enteric neu-



RET. Fig. 2 – Mechanisms of RET activation.

rons distal to the stomach. In addition, *RET* knockout mice show depressed ventilatory response to inhaled CO_2. *GDNF* -/- and *GFRα-1* -/- mice also die perinatally due to failure to develop enteric neurons and kidneys, similar to mice lacking *RET*. The GDNF-GFRα-1 system has also been shown to be critical for survival of subpopulations of sensory neurons in the dorsal root ganglion and in the nodose ganglion. Apparently the survival of neurons in the central nervous system does not solely depend on the GDNF-GFRα-1 system since only a few or no deficits have been found. The critical role in kidney development emphasizes that classifying these molecules as being strictly neurotrophic is difficult. Of interest, approximately 30% of *GDNF* +/- mice lack one kidney, while *RET* +/- and *GFRα-1* +/- mice have two normal kidneys. In contrast, *NTN* -/-, *GFRα-2* -/- and *GFRα-3* -/- mice have a relatively mild phenotype. They present with ptosis (*NTN* -/-, *GFRα-2* -/- and *GFRα-3* -/-), i.e. drooping of the eyelids, and deficits in the enteric nervous system, i.e. reduced density of acetylcholine esterase (AChE) fibers (*NTN* -/- and *GFRα-2* -/-), parasympathetic nervous system (*NTN* -/-, *GFRα-2* -/-), e.g. absence of parasympathetic innervation to the lacrimal gland, and sympathetic nervous system (*GFRα-3* -/-), e.g. severe defect in superior cervical ganglion (SCG) de-



RET. Fig. 3 – Interaction between various RET ligands and cofactors.

velopment. Knockout mice of *ART*, *PSP*, and *GFRα-4* have not been described.

To date, the downstream signaling pathways of RET have not been well defined, but there is evidence that RET signals are, at least in part, transduced by intracellular serine/threonine kinases designated MAPK (mitogen activated protein kinases). Other potential pathways involve phosphatidylinositol-3-kinase (PI3K), Jun N-terminal kinase (JNK) and phospholipase-C (PLC)γ.

Two types of mutations have been identified in RET: mutations associated with loss-of-function and mutations associated with gain-of-function. Mutations causing loss-of-function of RET are associated with Hirschsprung disease (HSCR). All kinds of mutations have been found; missense mutations, non-sense mutations, frame-shift mutations, splice-site mutations as well as small and large insertions and deletions. Depending on the mutations, RET function may only be slightly impaired or completely lost. Mutations causing gain-of-function of RET have been shown to be associated with a hereditary syndrome named multiple endocrine neoplasia type 2 (MEN 2) as well as with papillary thyroid carcinoma (PTC).

Most mutations found in MEN 2 are missense mutations. Germline RET mutations affecting one of six cysteines (Cys609, 611, 618, 620, 630, 634) within the cysteine-rich region (Fig. 1a) are responsible for the vast majority of two hereditary syndromes, multiple endocrine neoplasia type 2A (MEN 2A) and familial medullary thyroid carcinoma (familial MTC or FMTC), both of which are part of MEN 2. It has been shown that replacement of these cysteines by an alternate amino acid abrogates the intramolecular disulfide bonds and leads to aberrant intermolecular disulfide bonds with subsequent homodimerization and autophosphorylation (Fig. 2b).

A variety of other mutations affecting the intracellular domain have been found (e.g. E768D, L790F, Y791F, V804M, V804L, A891S). Most of them have been found in patients with FMTC but some of them have also been found patients with MEN 2A (e.g. V804L, L790F). The precise mechanism of RET activation caused by these mutations is unknown.

Missense mutations in two other codons (M918T, A883F) of RET affecting the tyrosine kinase domain have been found in patients with multiple endocrine neoplasia type 2B (MEN 2B), which is also part of the MEN 2 syndromes. By far, M918T is found in most patients with MEN 2B. It has been shown that the point mutation M918T causes RET activation (Fig. 2c) but also changes the substrate specificity of the tyrosine kinase. Dimerization does not occur.

Of interest, Rb/p53 knockout mice show a high incidence (about 40%) of MTC. Some of the mice acquire a somatic RET mutation, analogous to activating mutations seen in humans with MEN 2A and FMTC. However, Rb mutations have not yet been described in human MTC, and p53 mutations are only found in a minority of cases of this tumor.

In contrast to mutations found in MTC, specific somatic rearrangements (translocations and inversions) of RET have been found in PTC. The tyrosine kinase domain of RET is fused to the 5′-terminal region of heterologous genes. The resulting chimeric molecules are named RET/PTC. Currently, at least five types of RET rearrangements have been found in PTC (RET/PTC1-5). The fusion partners of RET are the genes H4, RI, RFG (ELE1), and RFG5 in the case of RET/PTC1, 2, 3 and 5, respectively. In the case of RET/PTC4, the fusion of RET and RFG includes the region encoding the transmembrane region of RET. The promoter of these genes, which substitutes the RET promoter, is able to drive expression of RET in the thyroid gland. Even though the extracellular domain of RET is missing, coiled-coil domains in the fusion partners cause a constitutive dimerization (Fig. 2d). The subsequent activation of RET in this manner seems to be restricted to PTC; only one study reports RET rearrangement in one thyroid adenoma. The frequency of RET rearrangements in PTC varies between about 10 – 40% with regional differences. Irradiation seems to be able to cause RET rearrangements. Mainly RET/PTC3 has been found in PTCs from patients following the Chernobyl nuclear accident in 1986, when a dramatic increase of PTCs was observed. Irradiation is able to cause RET/PTC1 rearrangements in cul-

tured cells (thyroid carcinoma cells and fibrosarcoma cells) *in vitro*. Transgenic mice with RET/PTC1 develop PTC if driven by a thyroid-specific promoter such as the thyroglobulin promoter.

The strict differentiation between mutations associated with loss-of-function and mutations associated with gain-of-function is not correct, since some mutations can cause both loss and gain-of-function depending on the time of expression, i.e. embryonic versus adult, and tissue of expression. This seemingly contradictory observation has been explained as follows. For example, the mutation C609W leads to a drastic reduction of mature RET product and subsequently reduces the level of functional receptors at the cell surface. Consequently, during formation of the enteric nervous system, the overall signal transmitted by the RET/GDNF and the RET covalent dimer complex is below the threshold required for the survival of enteric neurons. On the other hand, the ligand-independent constitutive signaling activity of the RET covalent dimer is high enough to lead to proliferation of thyroid C-cells with subsequent development of MTC. Biochemical analysis revealed that mutations affecting codon 618 and 620, and to a lesser extent codon 609, result in marked reduction of the level of RET on the surface.

Bioactivity

RET has become the paradigm for molecular medicine in the management of familial cancer syndromes. Germline RET mutations have been found in more than 95% of all patients with hereditary MTC as part of → MEN 2. The knowledge of RET as the disease-causing gene helps identifying individuals at risk of developing MTC.

Somatic RET mutations have been found in sporadic MTC. Depending on the mutation analysis technique, mutations have been found in 30 – 70%, most often the MEN 2B-specific mutation M918T in exon 16. The identification of this mutation in DNA extracted from cells obtained performing a fine needle aspiration cytology can help in making the diagnosis of MTC. However, the absence of this mutation in the cytologic specimen does not exclude the presence of MTC, either hereditary nor sporadic. Also, the presence of the M918T mutation does not allow determining whether the MTC is sporadic or hereditary. Additional germline mutation analysis of RET is required.

Similar to MTC, somatic RET rearrangements can be diagnosed in cells obtained performing a fine needle aspiration cytology in patients with PTC. However, since the frequency of RET rearrangements in PTC (10 – 40%) is even lower than the frequency of RET mutations in sporadic MTC (30 – 70%), no conclusion can be drawn in the absence of RET rearrangements. The identification of RET/PTC, however, justifies the diagnosis PTC.

RET is highly expressed in the presumptive enteric neuroblasts of the developing enteric nervous system. Humans harboring inactivating germline RET mutations may lack enteric neurons and have HSCR. Interestingly, despite high levels of RET expression in presumptive motoric neurons of the spinal cord and developing kindeys, no clinical evidence of spinal cord involvement has been shown in patients with inactivating RET mutations, and renal agenesis has been reported in only a few patients with HSCR. HSCR has been reported to be associated with congenital central hypoventilation syndrome (CCHS or Ondine'e curse). Despite the fact that RET -/- mice show depressed ventilatory response to inhaled CO_2, no RET mutations have been reported in patients with CCHS.

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Retinoblastoma, cancer genetics

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Synonyms

- glioma retinae

Definition

Retinoblastoma (Rb) is a malignant tumor that originates from developing retina. Diagnosis is based on clinical signs and symptoms and is usually made in children under the age of five. Mutational inactivation of both alleles of the *RB1* gene is a prerequisite for tumor formation. In most patients with sporadic unilateral Rb, both mutations have occurred in somatic cells and are not passed to offspring (non-hereditary Rb). Almost all patients with sporadic bilateral and virtually all patients with familial Rb are heterozygous for an oncogenic *RB1* gene mutation and transmit Rb predisposition autosomal dominantly (hereditary Rb).

Characteristics

Diagnosis of Rb

Mostly, the first presenting sign of the disease is a white pupillary reflex (leukocoria). Strabismus is the second most common sign and may accompany or precede leukocoria. Diagnosis of Rb is usually made during early childhood. In children with bilateral Rb diagnosis is made earlier that in children that develop Rb in one eye only. Usually, Rb diagnosis is established by examining the fundus of the eye using indirect ophthalmoscopy. Additional diagnostic tools such as computer tomography (CT), magnetic resonance imaging (MRI) and ultrasonography may be required for differential diagnosis and staging. If tumor material has been obtained, histopathology can confirm diagnosis of Rb.

Presentation and family history

Most patients (approximately 60%) have Rb in one eye only (unilateral Rb). Occasionally, multiple tumor foci can be found (unilateral multifocal Rb). In the remaining 40% of patients, both eyes are affected (bilateral Rb), that is, usually with more than one focus per eye (bilateral multifocal Rb).

Unilateral Rb is mostly spradic, i.e. no other case of retinoblastoma has been noted in their family. About 75% of Rb patients with bilateral Rb are also sporadic, the remaining 25% have a positive family history (familial Rb). Examination of the fundus of the eye in all first degree relatives of children with Rb is required to identify retinal scars or quiescent tumors (retinomas). The presence of such lesions in a relative indicates familial disease.

Therapy and prognosis

Treatment of Rb depends on the tumor stage, the number of tumor foci (unifocal, unilateral multifocal or bilateral disease), localization and size of the tumor(s) within the eye, presence of vitreous seeding and the age of the child. Treatment options include enucleation, external-beam radiation, cryotherapy, photocoagulation and brachytherapy with episcleral plaques. Novel treatment options include systemic chemotherapy combined with local therapy. Following successful treatment, children require frequent follow-up examination in order to detect new intraocular tumors early. If the tumor(s) have not invaded extraocular tissues, treatment is usually successful but metastasizing Rb is letal in most patients.

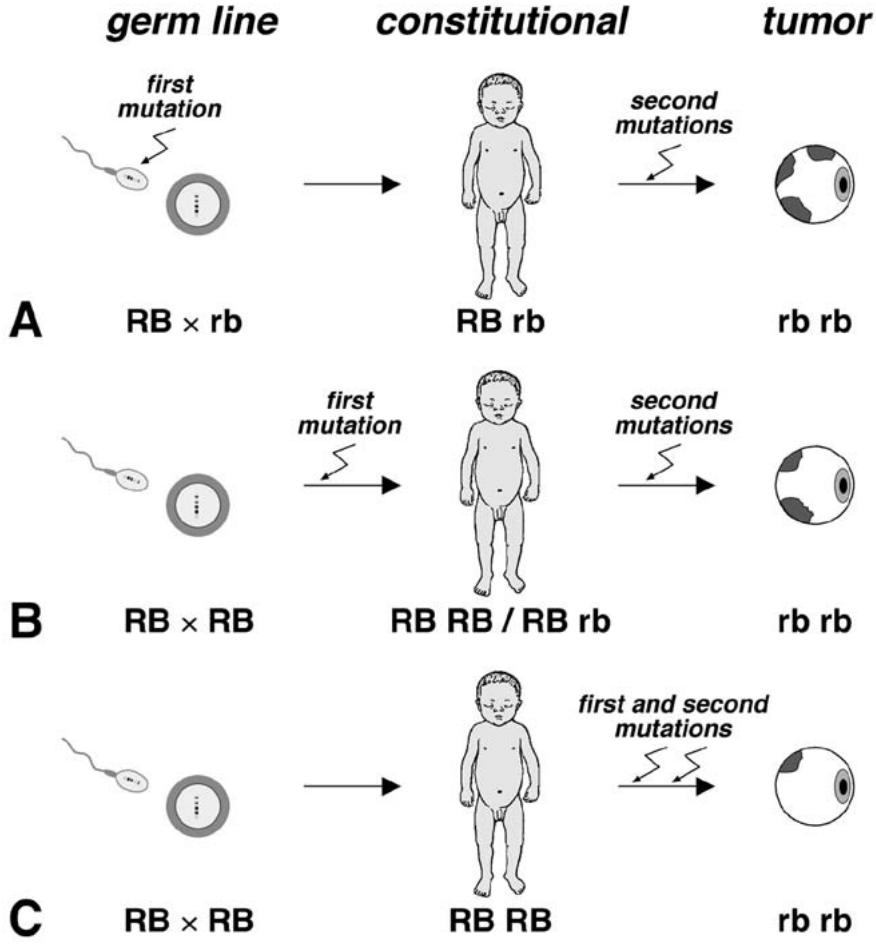
Secondary tumors.

Patients that are predisposed to Rb have an increased risk of specific neoplasms outside of the eye (secondary tumors). The spectrum of those includes osteogenic sarcoma, soft tissue sarcoma and malignant melanoma. Secondary tumors are preferentially observed in patients that have received external beam radiation as a treatment of bilateral Rb.

Molecular Genetics

Rb is caused by two mutations. As hypothesized by Knudson's → two-hit model, two mutational events are a prerequisite for Rb development (Fig.). These two mutations affect both copies of the retinoblastoma → tumor suppressor gene (*RB1*) and result in loss of function.

- In patients with familial Rb, one *RB1* gene mutation is inherited via the germ line. All non-tumorous cells are heterozygous for this mutation. The second mutation occurs in a somatic cell and results in the inactivation of the normal allele (Fig., A).
- In most patients with sporadic bilateral Rb, the first mutation has occurred de-novo in one of the parents and has been transmitted via the germ line. As in familial Rb, all non-tumorous cells are heterozygous and the second mutation is of somatic origin (Fig., A).
- In few patients with sporadic bilateral or unilateral Rb, the first mutation has occurred de-novo during embryonal development. Tumor development is initiated if a second mutation inactivates the normal al-



Retinoblastoma, cancer genetics. Fig. – Genetics of retinoblastoma.

lele in a cell that is part of the mutant sector (Fig., B).
- In most patients with sporadic unilateral Rb, the two mutations that are necessary to alter both copies of the *RB1* gene have occured in somatic cells (Fig., C).

Spectrum of RB1 gene mutations

- Large deletions: conventional cytogenetic analysis of peripheral blood lymphocytes shows deletions involving 13q14 in < 10% of patients with bilateral and in < 5% of patients with sporadic unilateral retinoblastoma. Using Southern blot hybridiziation, smaller deletions are identified in about 10% of patients with bilateral or familial retinoblastoma.
- Point mutations: more than 70% of the mutations detected in peripheral blood DNA from patients with hereditary retinoblastoma are single base substitutions and small length mutations (database of *RB1* gene mutations: http://www.d-lohmann.de/Rb/mutations.html). Most of these mutations result in premature termination codonsa in consequence of nonsense or frameshift alterations.
- In most tumors, the inactivation of one of the RB1 alleles is accompanied by the loss of constitutional heterozygosity (LOH) at polymorphic loci on chromosome 13. LOH results from deletions or one of several chromosomal mechanisms, such as mitotic recombination and non-disjunction.
- Another type of mutation that is particular to tumors, is hypermethylation of the, usually unmethylated, → CpG-island at the 5′-end of the RB1 gene. → Hypermethylation is observed in about 10% of retinoblastomas.

Genotype-phenotype associations

In heterozygous carriers, *RB1* gene mutations that cause premature termination codons, almost invariably result in bilateral tumors (complete → penetrance). Distinct missense changes, promoter mutations and in-frame deletions are asscociated with incomplete penetrance and reduced → expressivity (preponderance of unilateral retinoblastoma).

Risk Prediction

Inheritance: predisposition to Rb is transmitted autosomal dominantly. Most mutant alleles show near complete penetrance (> 99%) and result in bilateral Rb. Some mutant alleles are associated with incomplete penetrance (typically 50 – 75%) and reduced expressivity (preponderance of unilateral Rb).

Familiar risk: relatives of all patients with RB are more likely to carry a predisposing RB1-gene mutation and, consequently, are at higher risk of developing a tumor (Table 1).

Predictive testing: in many cases, accurate risk prediction in relatives depends on the identification of the Rb causing mutation in the index patient. In familial cases, segregation analysis of linked DNA markers can be used for risk prediction (Table 2).

Retinoblastoma, cancer genetics. Table 1 – Risk for Rb in family members.

clinical presentation of index case	risk to siblings	risk to offspring
sporadic unilateral Rb	=1%	2-6%
sporadic bilateral Rb	=2%	close to 50%
familial bilateral Rb (one parent affected)	close to 50%	50%
familial Rb, incomplete penetrance type	< 40%	< 40%

Retinoblastoma, cancer genetics. Table 2 – Laboratory testing used for predictive testing in relatives of patients with Rb.

clinical presentation	genetic mechanism	genetic analyses
sporadic unilateral RB	in >90% of patients, tumor development results from somatic mutations that are not detected in peripheral blood; <10% of patients have predisposing mutation that can be detected in peripheral blood DNA	mutation identification in DNA from tumor; two mutations have to be identified
sporadic bilateral RB	>90% of patients are heterzygous for an oncogenic *RB1* allele that originated from a new mutation in the parental germ line; <10% of patients are mutational mosaics because the predisposing mutation has occurred during embryonal development	identification of the predisposing mutation in DNA from peripheral blood or from tumor; because of mutational mosaicism in some patients, analysis of tumor DNA is preferred
familial RB	patients have inherited an oncogenic *RB1* gene mutation	genotyping of linked polymorphic loci to identify cosegregating marker alleles mutation identification in DNA from peripheral blood of patients that have inherited a mutant allele

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Retinoblastoma Protein, biological and clinical functions

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Definition

The retinoblastoma protein, pRB, was discovered because it suppresses the formation of the rare tumor of the retina, retinoblastoma. However, pRB is also an important transcription factor that controls both cell cycle progression and differentiation. Cell-cycle control is mainly accomplished by repression of other transcription factors that promote expression of genes required for cell-cycle progression. Effects of pRB on differentiation are accom-

plished by activation of transcription in a cell type specific manner. pRB is widely expressed in adult tissues and is expressed specifically when differentiation events occur in certain developing tissues, including the brain, muscles, lens, retina and hematopoietic cells. Absence of pRB at critical times of differentiation results in apoptosis. However, in human developing retina, loss of pRB initiates the tumor retinoblastoma.

Characteristics

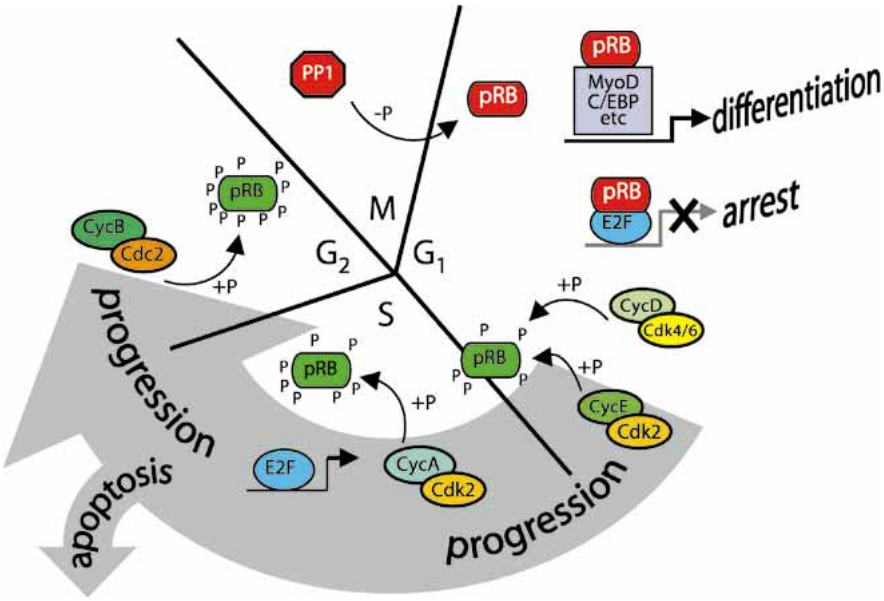
pRB is a nuclear phosphoprotein encoded by the RB1 gene. The activity of pRB is regulated by phosphorylation (Fig.). In the G_0/G_1 phase of the cell cycle, hypophosphorylated pRB actively represses transcription of genes required for S phase. At the G_1/S transition, pRB is inactivated by phosphorylation on serine and threonine residues through the action of cyclin-dependent kinases (cdks). Cyclins D and E form complexes with cdk4/6 and cdk2, respectively, at the G1/S transition. Progressive hyperphosphorylation of pRB continues through S phase by cyclin A/cdk2 and in mitosis by cyclin B/cdc2. Dephosphorylation and activation of pRB occurs after the beginning of mitosis due to the activity of phosphoprotein phosphatase type 1 (PP1).

Cellular and molecular regulation

The diversity of action of pRB is evident from the vast number of proteins to which it binds, facilitating a number of different functions (Table). Re-introduction of pRB into some cell lines that lack functional pRB suppresses growth, confirming the clinical observation that pRB suppresses specific tumors. At the cellular level, pRB is central to regulation of the cell cycle, differentiation and apoptosis.

pRB and the cell cycle

The importance of pRB in cell-cycle control was first evident from the binding of DNA tumor virus oncoproteins to pRB. pRB binds the regions of simian virus 40 (→ SV40) large T anti-



Retinoblastoma Protein, biological and clinical functions. Fig. – pRB regulates cell cycle progression, differentiation and apoptosis. Hypophosphorylated pRB represses genes necessary for S phase entrance by repressing transcriptional activation by E2F, causing cell cycle arrest, and activates genes necessary for differentiation by binding cell type specific transcription factors. Phosphorylation of pRB by cyclin D-cdk4/6 and cyclin E-cdk2 kinases in late G_1, releases E2F allowing cell cycle progression. Continual phosphorylation of pRB occurs by cyclin A-cdk2 and cyclin B-cdc2 complexes in S and G_2 phases. pRB is activated in late mitosis by de-phosphorylation. Inappropriate entry into S phase in the absense of pRB induces apoptosis.

Retinoblastoma Protein, biological and clinical functions. Table – Proteins that bind pRB.

function	pRB-binding proteins
Cdk inhibitor	p21
chromosome scaffold	H-Nuc
co-repressor	CtIP, RbAp48, RBP1
cyclins	cyclins D1,2,3
histone deacetylase	HDAC1
molecular chaperone	hsp75
nuclear matrix	lamin A, p84
oncoprotein	E1A, E7, large T Ag
phosphatase	PP-1α2
Pol I transcription factor	UBF
Pol II transcription factor	TAFII250
Pol III transcription factor	TFIIIB
Potential tumor suppressor	prohibitin
protein kinase	RbK
regulator of p53 stability	MDM2
replication licensing factor	MCM7
repressor	HBP1
Ser/Thr kinase	cdc2, cdk2
signaling molecule	Raf-1
transcription factor	ATF-2, BRG1, c-Jun, c-Myc, N-Myc, C/EBP, E2F-1, Elf-1, hBRM, Id-2, MyoD, myogenin, NF-IL6, PU.1, Sp1
transformation inducer	Bog
tyrosine kinase	c-Abl
viral transcription factor	EBNA-5, HCMV IE2
zinc finger protein	RIZ

gen, → Adenovirus E1A and → human papillomavirus 16 E7 proteins that are critical for transformation. Large T antigen specifically binds the hypophosphorylated, active form of pRB and may inactivate pRB by displacing cellular pRB binding proteins.

→ E2F is the best characterized cellular pRB binding protein that is released when viral oncoproteins bind to pRB. When not binding pRB, E2F activates transcription of genes necessary for S phase entrance, such as DNA polymerase α, thymidylate synthase, proliferating cell nuclear antigen and ribonucleotide reductase. pRB does not directly bind to DNA, but is brought to promoters through its interaction with E2F (see Fig.).

A number of other transcription factors bind to pRB, including ATF-2 and SP1, and cell type specific transcription factors such as Elf-1, PU-1, MyoD and NF-IL6. Generally, the binding of pRB results in repression of transcriptional activity, however, positive regulation of factors such as c-Jun, MyoD, C/EBP, and NF-IL6 correlates with differentiation of specific cell types,

although the precise mechanism of activation is unknown.

One mechanism by which pRB represses the activity of factors such as E2F is to bind and recruit histone deacetylase to the promoter. This activity is thought to encourage nucleosome structures, thereby blocking access of the transcription factor to the promoter. In addition, pRB binds a number of co-repressors including RbAp48, RBP1 and C-terminal interacting protein (CtIP), which function by directly repressing transcription or by recruiting HDAC1 to the promoter. However, not all promoters are regulated by histone deacetylase activity, therefore there must be other mechanisms by which pRB represses transcription. pRB binding transcription factors can block access of the transcription factor to the basal transcriptional machinery, or pRB itself may directly interact with members of the basal transcriptional machinery to inhibit transcription. Recently, pRB was shown to prevent recruitment of the preinitiation complex to the promoter by E2F. Furthermore, the binding of pRB blocked the ability of the TATA-binding protein-associated factor TAFII250 to phosphorylate itself, the RAP74 subunit of TFIIF and possibly other targets.

Transcriptional regulation by pRB is not limited to regulation of polymerase II genes. pRB regulates polymerase I genes by binding to and inhibiting the ability of an upstream binding factor to bind the ribosomal DNA promoter. Regulation of polymerase III genes such as tRNA, 5s RNA and TATA-box containing genes such as U6 occurs by binding of pRB to the general polymerase III transcription factor TFIIIB.

The physical association of the signaling molecule Raf-1 with pRB provides a direct link between mitogenic signaling and cell cycle regulation. Inactivation of Ras in the presence of pRB efficiently inhibits DNA synthesis, while if pRB is disrupted, inhibition of DNA synthesis is defective, suggesting that the status of pRB dictates whether cells stop cell cycle progression in response to inactivation of Ras. Raf-1 binding to pRB can be detected within 30 minutes of serum stimulation, and this binding may be one mechanism of transducing mitogenic signals from the cell surface receptors to the cell cycle machinery. Activated Ras may function through pRB to allow cell-cycle progression in cancer.

pRB, differentiation and development

A more detailed picture of the function of pRB was obtained from experiments that knocked out pRB in mice. The *RB1*$^{-/-}$ mice die early in gestation with abnormalities in hematopoiesis and neurogenesis. There is significant increase in the number of immature nucleated erythrocytes and massive cell death and ectopic mitoses in the central nervous system (CNS) and peripheral nervous system (PNS). Partial rescue of *RB1*$^{-/-}$ mice uncovers defects in lens development and myogenesis that suggest a role of pRB in differentiation. These defects are consistent with the high level of expression of the *RB1* gene in these tissues.

The neural retina is normal in the absence of pRB in early gestation, but inactivation of pRB in later retinal development results in ectopic mitoses and cellular degeneration. However, no retinoblastomas were observed, suggesting that loss of pRB during the late development of the retina results in cell death rather than increased proliferation. Mice carrying one normal *RB1* allele developed pituitary tumors in which a second mutation had occurred in the remaining *RB1* allele, similar to the loss of both alleles of *RB1* that occurs in human retinoblastoma. However, *RB1*$^{+/-}$ mice do not develop tumors of the retina. In the human retina pRB may be specifically required, while in the mouse the other members of the pRB family may play a significant role. Loss of both p107 and pRB in the retinoblasts of chimeric mice resulted in retinoblastoma which showed features of amacrine cells.

The importance of pRB in differentiation can be demonstrated using cell culture systems. pRB positively regulates terminal adipocyte differentiation of murine embryonic fibroblasts by binding to and activating the CCAAT/enhancer-binding proteins (C/EBPs). pRB also binds NF-IL6, another member of the C/EBP family, during differentiation of U937 cells into the monocyte/macrophage lineage following treat-

ment with phorbol 12-myristate 13-acetate. Production and maintenance of the terminally differentiated phenotype of muscle cells in the presence of MyoD requires pRB, which binds to MyoD both *in vitro* and *in vivo*. Also, pRB/c-Jun complexes are observed in human keratinocytes during differentiation of the cells but not in non-synchronized cycling cells. These culture experiments are consistent with the idea that the role of pRB in differentiation involves activation of genes that are needed for cell type specific differentiation.

Although pRB binds a number of proteins, the importance of its interaction with E2F is clear in development in mice lacking both proteins. The loss of E2F-1 in the *RB1*$^{+/-}$ mouse resulted in reduced frequency of pituitary and thyroid tumors and increased lifespan. Loss of both alleles of *RB1* and *E2F-1* resulted in later embryonic lethality than loss of *RB1* alone.

pRB and apoptosis

Among the diverse functions of pRB is its ability to protect cells from → apoptosis. This role in apoptosis was first evident by the excessive cell death in certain tissues in the absence of pRB. The apoptosis resulting from the lack of pRB in the lens was shown to be p53-dependent; there was a 94% reduction in the amount of → TUNEL-stained cells in the lens of a mouse with no pRB or p53 compared to a mouse with no pRB alone. Central nervous system apoptosis was also p53-dependent, but apoptosis in the sensory ganglia of the peripheral nervous system was independent of p53, suggesting that at least two pathways of induction of apoptosis are inhibited by pRB. Experiments in fibroblast cells showed that deregulated expression of E2F-1 resulted in S phase entry followed by apoptosis, and addition of pRB prevented induction of apoptosis. Excess pRB also overcame p53-mediated apoptosis in Hela cells. Apoptosis in the absense of pRB is dependent on the activity of E2F-1, evident by the absence of apoptosis in the lens of mice which have no pRB or E2F-1, suggesting that E2F-1 is a crucial target in the ability of pRB to protect cells from apoptosis.

An abundance of data connects p53 and E2F-1, two proteins generally thought to be in different pathways. Coexpression of E2F-1 with wildtype p53 enhanced the ability of E2F-1 to induce apoptosis, whereas a transdominant negative mutant p53 inhibited the ability of E2F-1 to induce apoptosis. E2F-1 has also been shown to induce cell death in a p53-dependent manner by increasing the stability of the p53 protein. This may be achieved by transcriptional activation of $p14^{ARF}$ ($p19^{ARK}$ in mouse), which binds to the MDM2-p53 complex and promotes the rapid degradation of MDM2, thereby preventing the degradation of p53 by MDM2. MDM2 itself also stimulates the transcriptional activity of E2F-1/DP1 which could potentiate apoptosis through → $p14^{ARF}$ regulation of MDM2 in an autoregulatory loop. Thus transactivation by E2F-1 is important in induction of p53-dependent apoptosis. Consistent with this interpretation, E2F proteins containing deletions in either the DNA binding or the transactivation domains were unable to induce apoptosis in Rat-1a cells that contain wildtype p53.

However, transactivation does not appear to be important in p53-independent induction of apoptosis by E2F-1. E2F-1 has been shown to induce apoptosis in a p53-independent fashion in Saos-2 cells that lack p53 and pRB. Transfection of E2F-1 proteins that are mutant in transactivation into Saos-2 cells induced apoptosis, whereas the DNA binding defective E2F-1 mutant was incapable of inducing apoptosis. The pRB inhibition of p53-independent induction of apoptosis by E2F-1 requires direct binding of pRB to E2F-1, and is not affected by MDM2. This suggests that induction of p53-independent apoptosis by E2F-1 occurs through a different mechanism than induction of p53-dependent apoptosis, and may rely on relief from repression of promoters rather than on transcriptional activation by E2F-1.

The p53-independent apoptosis in Saos-2 cells was inhibited by viral inhibitors of apoptosis including crmA, a pox virus inhibitor of initiator caspases, and E8-FLIP, a herpes virus apoptotic inhibitor that binds the death receptor-associated adapter protein FADD, suggesting that the apoptosis was occurring through death receptor-dependent mechanisms. Death receptors are cell surface receptors which,

when stimulated by a 'death ligand', can transmit apoptotic signals and activate the caspases which lead to apoptosis. E2F-1 was also shown to potentiate cell death induced by a death receptor, TNFα, and does so by inhibition of NFκB activity. This inhibition most likely occurs by preventing activation of the I-κB kinases α and β (IKK). IKK phosphorylates I-κB, thereby releasing its interaction with NFκB in the cytoplasm and allowing NFκB to traverse to the nucleus and activate transcription of anti-apoptotic genes.

Clinical Relevance

Retinoblastoma arises as a result of the loss of both copies of the *RB1* gene and can be either heritable or non-heritable. Children who inherit a mutant *RB1* allele usually have multiple tumors present in both eyes following loss of heterozygosity or mutation of the second allele in individual retinoblasts. However, unilateral retinoblastoma occurs in children with normal *RB1* alleles who have rare somatic mutations in both copies of the *RB1* gene in one retinoblast. Most mutations inducing retinoblastoma lead to loss of functional RB protein. Inheriting one mutant copy of *RB1* also predisposes an individual to secondary tumors, most common being osteosarcoma.

However, mutations in *RB1* have also been associated with malignant progression of a number of tumors including small cell lung carcinomas, prostate carcinomas, breast carcinomas, some types of leukemias and cervical carcinomas. Some tumors may have normal pRB but mutations in other proteins involved in the pRB pathway such as cyclin D1 and p16. Alternatively, other tumors affect pRB function by overexpressing regulators of pRB, eg. cyclin E, effectively neutralizing pRB.

The molecular characterization of the *RB1* gene has helped families with retinoblastoma by providing a means of detecting the mutation. Genetic tests can determine if a new child will develop retinoblastoma. Such testing is beneficial as it avoids the repeated conventional examination of young children, often under general anesthetic, to identify and treat small tumors. Once the *RB1* mutation is found in a family, individuals can be screened and only those with the mutation need continue surveillance.

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Retinoblastoma Protein, cellular biochemistry

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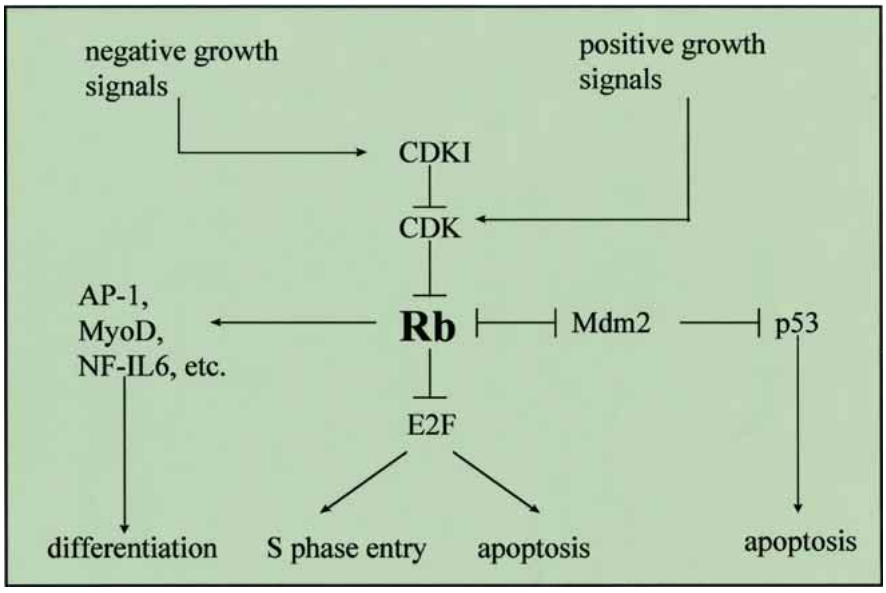
Definition

Retinoblastoma protein is a 110KD nuclear phosphoprotein (→ tumour suppression). The gene locus maps to 13q14.1-q14.2. Retinoblastoma is an embryonic neoplasm of retinal origin. Young children with a germline mutation in one Rb1 allele have a 95% chance of developing a retinoblastoma tumour in their eyes. Mutation in the *Rb1* allele also predisposes patients to develop other tumours, such as osteosarcomas and fibrosarcomas. Almost two-thirds of the secondary tumours arising in patients with retinoblastoma are mesenchymal in origin. Most mutations in *Rb1* lead to premature termination of translation.

Characteristics

pRb and its family members

The pRb, p107 and p130 proteins form the 'pocket protein' family that is crucial in cell-cy-



Retinoblastoma Protein, cellular biochemistry. Fig. – pRb integrates negative and positive signals in regulating the cell-cycle clock. Mitogenic signals activate cyclin-dependent kinase activity and inactivate pRb function by phosphorylation. In reverse, inhibitory signals, such as TGFβ and contact inhibition mobilise CDK inhibitors (CDKI) to maintain active pRb in its hypophosphorylated form. pRb has at least two distinct activities in tumour suppression: by inactivating downstream E2F activity, and by augmenting transcription activities of various transcription factors for cellular differentiation. Mdm2 acts as a mediator bridging together both the p53 and pRb pathways.

cle regulation. pRb and p107/p130 have five domains of high conservation. pRb shares between 30 – 35% sequence identity with p107/p130. Most of the conserved sequences lie within the so-called 'pocket region' (amino acid 379-792), which is composed of two subdomains, known as the 'A' and 'B' box. Viral oncoproteins, such as → SV40 large T antigen, → adenovirus E1A and 'high risk' → human papillomavirus early protein E7, all target the pocket domain, displacing cellular proteins that interact with the pocket, thereby leading to loss of pocket protein function.

At least three distinct protein binding activities of pRb have been described to be important for its function: the large pocket (amino acid 395-876) binds to → E2F, the small pocket (amino acid 379-792) binds to the LXCXE (L = leucine, C = cysteine, E = glutamic acid, X = any amino acid) peptide and the C pocket binds to tyrosine kinase c-Abl and oncoprotein Mdm2. Viral oncoproteins (e.g. E1A, E7, SV40 LT) and some cellular proteins (e.g. histone deacetylase (HDACs), cyclin D) possess an LXCXE motif in their protein sequence that enables them to bind to the pocket. The three-dimensional structure of the pRb A/B pocket bound to a LXCXE-containing peptide from E7 has been solved. The peptide binds a highly conserved groove on the B box portion of the pocket, and the A box is required for the stable folding of the B box. The A/B box interface is highly conserved, suggesting an additional protein binding region. Furthermore, both E2F and E7 peptides occupy distinct sites in the pocket. Indeed, HDACs that contain an LXCXE-like motif may form a trimeric complex with pRb and E2F. c-Abl was also reported to bind to pRB when the pocket region is occupied by E2F. Taken together, the evidence suggests a model for pRb as a 'molecular matchmaker' in mediating protein complex formation.

Despite the close similarities among the family members, only *Rb* has been shown to be mutated in tumour cells. Neither *p107* nor *p130* have been found to be mutated in naturally occurring tumours. In order to study the specific cellular function of pRb, p107

and p130, knockout mice in *Rb*, *p107* and *p130* have been developed. The phenotypes indicated both distinct and overlapping functions among the family members. The absence of pRb in mice causes embryonic lethality, although $p107^{-/-}$ and $p130^{-/-}$ mice survive to term, possibly as a result of functional redundancy between p107 and p130. This is consistent with the phenotype of the $p107^{-/-}$: $p130^{-/-}$ mice which exhibit embryonic lethality. Interestingly, $Rb^{+/-}$ mice do not suffer from retinoblastoma, but develop tumours of the pituitary and thyroid origin. This difference may be attributed to the physiology of human and mice. Bilateral, multifocal retinal dysplasia is observed in $Rb^{+/-}$: $p107^{-/-}$ mice. This suggests that pRb and p107 may share overlapping functions in controlling cellular homeostasis in the murine retina, and loss of both is required for tumour formation. $p107^{-/-}$: $p130^{+/-}$ and $p107^{+/-}$: $p130^{-/-}$ mice were developed to investigate if p107 and p130 possess tumour suppression function. However, analysis of such animals did not reveal any obvious tumour phenotype. An alternative explanation is that, unlike the mutations in pRb, mutations in p107 and/or p130 may not be advantageous for tumourgenesis. Therefore mutations in other components of the pathway such as p16, which is frequently found to be mutated in human tumour(s), contribute to the inactivation of p107/p130 tumour suppression function, without abrogating other necessary functions of p107/p130.

Cell-cycle regulation of pRb

The pRb protein is ubiquitously expressed in most cycling and resting cells. pRb acts as a negative regulator for cellular proliferation, sequestering a variety of nuclear proteins involved in cellular growth. The most studied pRb target is the family of transcription factors known as E2F. E2F consists of a heterodimer of an E2F protein bound to a DP partner. Together, they regulate the timing and levels of expression of many genes involved in cell-cycle progression. E2F target genes encode proteins involved in DNA replication (for example, DNA polymerase a, thymidine kinase, dihydrofolate reductase and cdc6), chromosomal replication (for example, replication origin-binding protein HsOrc1, MCM proteins) and cell-cycle regulation (for example, cyclin A, E and D1, p107, cdc2, E2F1, 4 and 5 and $p19^{ARF}$). During G0/G1, hypophosphorylated pRb binds to E2F, inactivating and thereby preventing cell-cycle progression. Cyclin D/cdk4/6 and cyclin E/cdk2 progressively phosphorylate pRb at late G1 to early S phase. In S phase, pRb phosphorylation is maintained by cyclin A/cdk2. Progressive phosphorylation of pRb results in reduced affinity for E2Fs, the release of free E2F and thereafter induction of E2F transcription.

Different pocket proteins have a preference for binding to different E2F family members. The E2F family now consists of six members (E2F-1 to -6). E2F-1, -2 and -3 exhibit high affinity binding towards pRb, but bind weakly to p107/p130. In contrast, E2F-4 and -5 show greater specificity for p107/p130, but also bind pRb. Complexes between the pRb family members and E2F form at different phases of the cell cycle. In general, p130/E2F complexes are mainly found in quiescent or differentiated cells and p107/E2F complexes predominate in S phase cells. pRb/E2F complexes are most evident during G1/S phase transition, but also exist in quiescent or differentiated cells.

pRb can modulate E2F transcription activity in at least two distinct ways: First, pRb binds to the transcription activation domain in E2F and directly inhibits E2F transcription activity. Using *in vitro* transcription and footprinting assays, it has been demonstrated that pRb blocks the recruitment of the transcription initiation complex by E2F. Second, pRb was shown to bind to HDACs, which is believed to deacetylate histones on the promoter, and actively repress transcription via chromatin remodelling. It has been shown that active repression of pRb/E2F complex is important in mediating G1 arrest triggered by transforming growth factor-β (TGFβ), p16 and contact inhibition.

There is evidence that phosphorylation of the pRb C -terminus by CDK4/6 causes successive intramolecular interactions between the C-terminus and the central pocket region. The initial interaction is thought to disrupt HDAC

binding and therefore relieve active repression by pRb. This event then facilitates the phosphorylation of the pocket region by cyclin E/CDK2, thereby disrupting the pRb/E2F interaction. These intramolecular interactions provide a molecular basis of how phoshporylation of pRb progressively inactivates its growth suppression function.

Apart from regulating E2F transcription activity, pRb proteins also regulate E2F protein stability and apoptotic function. Overexpression of pRb family members can stabilise E2F-1 and E2F-4. E2F-1 was shown to be degraded by a ubiquitin-dependent proteasome pathway through an SCF-like complex. E2F-1 mutants which cannot be degraded by the SCF pathway drive cell-cycle progression into S phase followed by apoptosis. E2F-1-mediated apoptosis occurs independently of the transcription activation domain, but requires DNA binding. Furthermore, E2F-1-mediated apoptosis is both p53-dependent and p53-independent. Overexpression of pRb in tissue culture cells and analysis of the $Rb^{-/-}$ mouse embryos versus the $E2F\text{-}1^{-/-}$:$Rb^{-/-}$ embryos indicated pRb can suppress E2F-1-mediated apoptosis. E2F-1-mediated apoptosis can, at least in part, occur through a death receptor-dependent mechanism by inhibiting activation of anti-apoptotic signals including NFκB.

Role in terminal differentiation

The role of pRb in differentiation was suggested from the phenotype of $Rb^{-/-}$ mice, which show a pronounced defect in erythroid, neuronal and lens development. Although loss of pRb function allows the initiation of differentiation, the embryos fail to achieve a fully differentiated state, indicating that pRb is likely to play an important role in achieving and maintaining the post-mitotic state. Furthermore, the aberrant cell-cycle entry observed in the CNS and the PNS of the $Rb^{-/-}$ embryo causes elevated levels of → apoptosis. Again this implies that pRb may function in protecting cells from apoptosis. Indeed, $Rb^{-/-}$: $E2F\text{-}1^{-/-}$ mice show reduced levels of ectopic cell-cycle entry and apoptosis in both the CNS and the lens at 13.5 d.p.c. as compared to $Rb^{-/-}$ embryos, suggesting that these defects are in part due to de-regulated E2F-1 activity. However, this mechanism is tissue type specific, as loss of E2F-1 has less of an effect on cell-cycle entry and apoptosis in the PNS.

It is clear that pRb interacts with other non-E2F targets. This is best demonstrated in the analysis of pRb mutants that show reduced binding to E2Fs, but are still capable of augmenting MyoD transcriptional activity and inducing tissue specific gene transcription. Furthermore, these mutants still retain certain tumour suppressor functions. Therefore, at least part of the tumour suppression function of pRb correlates with its ability to promote tissue differentiation.

Although the pRb protein level does not change dramatically upon differentiation, pRb is found to be hypophosphorylated during cell-cycle exit. Hypophosphorylated pRb augments the transcriptional activity of various transcription factors important for tissue differentiation, including MyoD for myogenesis and C/EBPα for adipogenesis. Also, pRb was shown to directly bind to C/EBPα, NF-IL6 and → AP-1 transcription factors and enhance their DNA binding activity.

Other targets of pRb

Low penetrance pRb mutants, such as substitution of Trp for Arg 661 in the B pocket of pRb (661W) which are inactive in both E2F and LXCXE binding, still retain tumour suppressor activity. In cell based assays, the 661W mutant was shown to inhibit G1/S progression. Furthermore, C pocket mutations in full length pRb also reduce pRb function. Taken together, these suggest non-E2F targets of pRb contribute to pRb tumour suppression function.

It was shown that pRb can bind to the oncoprotein → Mdm2 through a C-terminal region in pRb, and Mdm2 overcomes pRb-mediated growth suppression. Direct interaction of pRb and Mdm2 can overcome both the anti-apoptotic function of Mdm2 and the Mdm2-dependent degradation of p53.

The C-terminus of pRb also interacts with the c-Abl tyrosine kinase. The c-Abl kinase is ubiquitously expressed in both the cytoplasm and nucleus. The nuclear kinase activity of c-

Abl is under cell-cycle control, being activated during cell-cycle progression. Interestingly, c-Abl can simultaneously interact with pRb when the pocket region is occupied by E2F.

The human homologue of yeast SNF/SWI2 proteins, hBrm/hBrg, can interact with pRb in a pocket-dependent manner. hBrm/hBrg has chromatin remodelling activity and co-operates with pRb in the transcriptional activation of the glucocorticoid receptor. Together with the observation that pRb also recruits HDACs, it is likely that pRb regulates transcription by altering chromatin structure.

pRb also interacts directly with the largest TATA-binding protein associated factor, $TAF_{II}250$, through multiple regions in each protein. Apart from being part of the basal transcriptional machinery, $TAF_{II}250$ possesses intrinsic histone acetyltransferase activity and kinase activity. Mutagenesis studies suggested $TAF_{II}250$ is a cell-cycle regulated protein and its acetyltransferase activity is required for cell-cycle progression. pRb was reported to inhibit the kinase activity of $TAF_{II}250$. These findings point to an additional mechanism of pRb regulating transcription by modulating the activity of the basal transcription apparatus.

Evidence exists that pRb can modulate transcription that is mediated by RNA polymerase I and III. During differentiation of U937 myeloid porgenitor cells, pRb becomes localised to nucleoli, which are the major sites of ribosomal gene transcription by RNA polymerase I. Immunoprecipitation experiments demonstrated that pRb can associate with transcription factor UBF1 and this interaction compromised the transcriptional activity of UBF1, thus leading to the down-regulation of Pol I transcription activity.

Loss of pRb function also correlated with up-regulation of Pol III transcription in tumour cells. $Rb^{-/-}$ fibroblasts have elevated Pol III activity compared to $Rb^{+/+}$ fibroblasts. Immunoprecipitation and co-fractionation experiments demonstrated pRb interacts with TFIIIB, and TFIIIB was identified as a target of repression by pRb.

Animal models

$Rb^{-/-}$ mice fail to develop to full term, dying in utero at about 12-13 days post-coitum. The embryonic lethality is due to the failure of certain cell lineages to undergo terminal differentiation, particularly in the haemopoietic and central nervous systems (CNS). These observations suggest that pRb plays a crucial role in controlling cellular differentiation during development. Interestingly, $Rb^{+/-}$ mice do not develop retinoblastoma. Instead, 95% of the Rb heterozygotes die between day 300 to 400 after birth with tumours of the intermediate lobe of the pituitary.

Summarising remarks

The RB tumour suppressor protein is an essential component of the cell-cycle clock, integrating both positive and negative signals for cellular growth and proliferation with the transcription machinery. The pRb protein exerts its function in → tumor suppression by both antagonising and synergising with downstream effectors such as E2F. pRb has two modes of action: it can inactivate E2F transcription activity, and assemble an active repression complex with E2F. Apart from E2F, pRb synergies with various factors to promote cellular differentiation. The differentiation property of pRb at least contributes partly to its tumour suppressor function. The pRb-c-Abl and pRb-Mdm2 interactions also adds to its growth suppression function, though the mechanisms remain to be elucidated.

It is also clear that pRb is a master regulator for transcription. It can both activate and repress transcription in a context-dependent manner. pRb interacts directly with histone acetyltransferase, deacetylase and hBrm/hBrg, all of which are classes of proteins involved in chromatin remodelling. It will be important to investigate how pRb regulates transcription in a chromatin environment. Last but not the least, pRb regulates transcription from Pol I, II and III, thereby integrating the cell-cycle clock with the biosynthetic capacity of the cell. pRb is indeed a bona fide master regulator of the cell.

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Retinoic Acid

Definition

Retinoic acid is a derivative of vitamin A that interacts with retinoid receptor proteins, a class of ligand-inducible transcription factors.

Retroperitoneum

Definition

The retroperitoneum is the area of the inside of the abdomen which is nearest to the back. This area contains the pancreas, the part of the small intestine called the duodenum, many important blood vessels, plexuses (networks) of nerves and the kidneys.

Retroposon

Definition

A retroposon is a transposon that mobilizes via an RNA form. The DNA element is transcribed into RNA and then reverse-transcribed into DNA, which is inserted at a new site in the genome.

Retrovirus

Definition

A retrovirus, formerly referred to as RNA tumor virus, belongs to the group of RNA viruses. RNA viruses have an RNA, not a DNA genome. Following the virus entry into a cell, this RNA is transcribed by the enzyme reverse transcriptase into one single strand of DNA, which in turn serves as the template for the host cell DNA polymerase to synthesize the complementary DNA strand, eventually forming double stranded (ds) DNA. The ds DNA is stably inserted into the DNA of the host cell as a provirus, providing the source for an RNA template that is identical to the initial virus RNA genome. Viral proteins can now be synthesized. The provirus therfore behaves like any of the other normal genes within the host genome and, during cell division, is regularly passed on to the daughter cells. Synthesized viral proteins, together with the newly sythesized RNA, can be packaged and to yield new virus particles. Upon their release by the cell, in a process referred to as 'budding' new cells can be infected.

Usually the retrovirus infects a somatic cell and the provirus is integrated into the genome of that particular cell. If a virus infection occurs in a germ cell (a cell destined to become a sperm or egg cell) the resulting provirus can be passed on to the progeny and is inherited like a normal gene. Such genetic elements are called endogenous proviruses; they occur and are detectable in vertebrate DNA, including that of humans. Although in some species,

such as mouse, thousands of endogenous proviruses exist, they are not transcribed, a fact at least partly due to heavy → methylation. Endogenous proviruses can however, be induced artificially to produce virus particles.

Retroviruses were first isolated as agents that can cause cancer in chickens, the prototypical retrovirus being the Rous sarcoma virus (RSV). Some naturally occuring cancer diseases in humans are associated with retroviruses. The human T-cell leukemia virus I (HTLV-I) cause a relatively rare, but invariably fatal cancer of T lymphocytes. Another retrovirus, the human immunodeficiency virus, HIV (also known as HTLV-III or LAV) has been identified to cause acquired immunodeficiency syndrome (AIDS).

The study of retroviruses has laid the basis to identify → oncogenes.

The analysis of the → transduction of oncogenes has directly led to the concept of cancer as the result of a malady of genes. In pursuit of defining the role of oncogenes in cancer development, fundamental insights into the molecular basis of cancer and cellular growth control have been achieved. Retroviruses that cause, within short time after infection, cancer in animals (acutely transforming retroviruses), possess an oncogene (viral oncogene, v-onc) that is responsible for cellular transformation. Such viral oncogenes are originally not viral. Instead they but have been taken up from the host cell by the provirus, followed by their incorporation into the virus genome. Viral oncogenes therefore, have once been normal cellular genes (proto-oncogenes or cellular oncogenes). They are evolutionary conserved and are present in all mammalian cells, often even in non-mammalian cells. Alteration of these cellular oncogenes (by → mutation, → translocation or → amplification) is one of the main contributions to cancer in all mammals, including humans.

Reverse Transcriptase

Definition

Reverse transcriptase is an enzyme generating a DNA strand from an RNA template, typically in the cytoplasm of a cell infected by a retrovirus. It is carried with a retroviral particle, and originally identified in the context of a → retrovirus. → Telomerase is a cellular reverse transcriptase.

Reverse Transcription

Definition

Reverse transcription is the synthesis of DNA from a RNA template. The process is achieved by the enzyme → reverse transcriptase.

RFLP

Definition

→ Restriction fragment length polymorphism.

RGD

Definition

RGD is a tripeptide of arginine-glycine-aspartic (RGD) acid that is a common integrin-binding sequence in extracellular matrix proteins and other adhesive proteins.

RGS Proteins

Definition

→ Regulators of G-protein signalling.

Rhabdoid Tumor

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Definition

A highly malignant tumor that primarily affects infants and young children. Rhabdoid cells contain large nuclei with prominent nucleoli and abundant eosinophilic cytoplasm that often contains filamentous cytoplasmic inclusions. In tumors of the central nervous system (CNS), areas of rhabdoid cells may be seen in juxtaposition to areas of primitive neuroepithelial cells resembling primitive neuroectodermal tumor, as well as mesenchymal tissue and/or epithelial tissue. The designation atypical teratoid/rhabdoid tumor (AT/RT) is used to describe the CNS tumor.

Characteristics

The true incidence of rhabdoid tumors in the population is not known due to the fact that many of these malignancies, especially in the brain, have been misdiagnosed. The most common sites of presentation for rhabdoid tumor are the kidney and brain. Rhabdoid tumors account for approximately 1-2% of childhood renal tumors and 2% of pediatric CNS tumors. Extrarenal tumors are less common and may arise in a wide variety of sites, such as the liver, orbit, skin and other soft tissues. Most children are less than 2 years of age at diagnosis, and there is a slight male predominance (3:2). There are reports of adult patients with rhabdoid tumors, although cytogenetic or molecular genetic studies have not yet confirmed similar genetic alterations in these cases as compared to classic rhabdoid tumors of childhood. Rhabdoid tumors are highly aggressive malignancies and there have been few long term survivors.

The cell of origin for rhabdoid tumor is unknown. Variable histologic features and immunoreactivity to epithelial, neural, glial and/or myogenic markers may obscure the diagnosis of rhabdoid tumor. In the brain, the rhabdoid components of AT/RT usually exhibit positive staining for epithelial membrane antigen and vimentin, which helps distinguish it from medulloblastoma or supratentorial primitive neuroectodermal tumor. Approximately 40% of AT/RTs originate in the posterior fossa, notably in the cerebello-pontine angle. Forty percent of tumors are supratentorial, 5% are located in the pineal and the remainder are brain stem or multifocal. The tumors are highly metastatic, spreading rapidly throughout the cerebrospinal pathway.

To date, six families have been reported in which a child with a rhabdoid tumor has had a first or second degree relative with a rhabdoid or CNS tumor. The small number of families with multiple affected individuals is likely due to the fact that few survivors have reached child bearing age. A genetic predisposition to rhabdoid tumor was originally supported by the finding that approximately 10 – 15% of infants with renal rhabdoid tumors had second primary tumors of the CNS. There is also one report of an infant with a rhabdoid tumor of the liver and a CNS malignancy. In past reports, the predominant histology of the brain tumors was consistent with a medulloblastoma or other primitive neuroectodermal tumor. It is now recognized that the majority of the brain tumors in these infants are AT/RT. The occurrence of two primary rhabdoid tumors (e.g. bilateral kidney tumors or rhabdoid tumors of both the kidney and brain) predicts the worst prognosis.

Genetics

The genetic predisposition to rhabdoid tumor and AT/RT is associated with a germline mutation in the *hSNF5/INI1* gene. Cytogenetic and molecular studies of AT/RT, renal and extrarenal rhabdoid tumors demonstrated consistent deletions within chromosome band 22q11.2. Positional cloning strategies were employed to identify a candidate gene for rhabdoid tumor, which recently led to the identification of *INI1* as a rhabdoid → tumor suppressor gene. INI1 is a member of the SWI/SNF chromatin-remodeling complex and may regulate

transcription of specific target genes involved in both growth and differentiation.

Germline mutations of *INI1* have been reported for patients with renal and extrarenal rhabdoid tumors, as well as AT/RTs. Germline mutations have been identified in at least six children with primary tumors of both the brain and kidney, as well as an infant with bilateral kidney tumors. Loss of the wildtype allele or mutation in the second allele has been documented in the tumor cells of these individuals, consistent with a two-hit model for a tumor suppressor gene. In several of the affected children, the second hits arose through different mechanisms in the two tumors, providing further evidence for distinct primary malignancies and not metastases.

Bi-allelic inactivation of *INI1* is also observed in the majority of sporadic rhabdoid tumors. Homozygous deletion has been documented in rhabdoid tumors from all anatomic sites, although the highest frequency appears to be among extrarenal rhabdoid tumors. Monosomy 22 appears to be more common in AT/RT than renal rhabdoid tumors, both in infants with predisposing germline INI1 mutations and in children with sporadic disease. Two AT/RTs and one kidney tumor demonstrated compound heterozygous somatic mutations in *INI1*, also consistent with the two-hit model.

In the majority of rhabdoid tumors, loss of one allele of *INI1* is accompanied by a mutation in the remaining copy. Although some missense mutations have been reported, most of the mutations are point mutations or frameshifts that introduce a novel stop codon and thus predict premature truncation of the protein. The *INI1* gene contains nine exons. The mutations are distributed throughout the coding sequence of *INI1*, although exons 3 and 8 are clearly under-represented. Identical mutations have been observed in rhabdoid tumors of the kidney and brain, demonstrating that most mutations are unlikely to be site specific.

The total number of *INI1* mutations reported is less than 100, and early predictions regarding potential hot-spots or specificity of *INI1* mutations may change as more data is accumulated. A single base pair deletion in codon 382/383 of exon 9 has been observed in nine AT/RTs. To date, it has not been observed as a germline mutation or in a renal or extrarenal rhabdoid tumor. Two tumors with this exon 9 mutation were originally classified as medulloblastoma or primitive neuroectodermal tumor. Both tumors were subsequently re-classified as AT/RT. This clearly demonstrates the clinical utility for molecular genetic analysis in diagnosis of patients with rhabdoid tumors.

Two mutations, C472T (R→Stop) in exon 4 and C601T (R→Stop) in exon 5, also appear to be present at an increased frequency. The C472T mutation was identified in constitutional DNA from children with renal or extrarenal rhabdoid tumors, as well as a child who had a malignant rhabdoid tumor of the kidney and a sibling with a primary CNS tumor. This mutation has also been observed as a somatic mutation in sporadic renal tumors and AT/RTs.

Likewise, a germline C601T mutation was identified in a patient with a renal rhabdoid tumor and a CNS malignancy, and as a somatic change in several sporadic rhabdoid tumors of the kidney and AT/RTs. This exon 5 mutation was also found in a CNS tumor classified as choroid plexus carcinoma.

The specificity of *INI1* alterations for rhabdoid tumors is subject to debate. Mutations of *INI1* have been reported in embryonal rhabdomyosarcoma, as well as → medulloblastoma, supratentorial primitive neuroectodermal tumor and choroid plexus carcinoma. Each of these types of tumors may be included in the differential diagnosis of rhabdoid tumor or AT/RT in children with extrarenal or CNS malignancies, respectively. The question of whether identification of an *INI1* mutation is sufficient to make a clinical diagnosis of rhabdoid tumor or if there are several related tumors that may have overlapping molecular genetic alterations, will ultimately be addressed in large clinical and molecular genetic correlative studies. At present, the demonstration of an *INI1* mutation is considered a poor prognostic feature and aggressive therapy is indicated.

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Rhabdomyosarcoma

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Definition

Sarcomas are malignant tumors thought to be derived from mesenchymal cells and many contain cells that resemble those of connective tissues in the body. Rhabdomyosarcomas (RMS) are sarcomas resembling skeletal muscle and show different stages of skeletal muscle cell development (myogenesis). They are a heterogeneous group of tumors and although considered rare, they are more prevalent in children than adults. Historically they are broadly divided into two major histological subtypes, with further subdivisions and an additional minor subtype:

- Alveolar RMS (ARMS) - cellular architecture resembling the alveolar spaces of the lungs. Based on molecular evidence, a solid variant of ARMS has recently been described that does not show alveolar-like spaces. Is most frequently found in the extremities and the trunk.
- Embryonal RMS (ERMS) - more frequent group and classical ERMS are seen predominantly in young children. Most common sites are the head, neck and genitourinary area. Variants include botryoid, most frequently associated with the genitourinary tract and spindle cells.

In addition, a rarer pleomorphic subtype exists that is predominant in adults and most often found in the extremities and the trunk.

Characteristics

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood and accounts for 4-8% of all childhood cancer cases. Males are more commonly affected, with an approximate ratio 1.3:1. The embryonal subtype accounts for around 70% of RMS cases and most frequently affects young children. The alveolar subtype is more prevalent in older children. Although RMS can arise anywhere in the body, they occur most commonly in 3 regions; i. the head and neck, ii. the genitourinary tract and retroperitoneum, and iii. the upper and lower extremities. The locations are more likely to be associated with particular ages and the histologies, as indicated above.

Overall, more than 70% of RMS patients survive long term. Treatment of RMS usually involves 2 or more modalities (multi-modal therapy that can include surgery, chemotherapy or radiotherapy). The survival rate depends significantly on tumor size, location, histology and whether metastasis has occured. Accurate diagnosis of more undifferentiated RMS and tumors without distinct histological features may be difficult. ARMS are characterized by small round cells that may be difficult to distinguish from other so-called small round cell tumors, including neuroblastomas and the Ewing Family of tumors. ARMS and especially the solid variant may also be difficult to distinguish from ERMS. Development of improved disease-specific therapeutic strategies, with conco-

mitant improvements in prognosis, have rendered accurate diagnosis and classification of paramount importance. Distinctive cytogenetic and corresponding molecular changes associated with the various small round cell tumors can be useful diagnostic markers in addition to standard immunohistochemical and morphological features.

The expression of the → MRF(muscle related factor) family of transcription factors and the PAX3 and PAX7 genes in RMS is consistent with their morphological resemblance to developing skeletal muscle. These genes are considered to play a key role in myogenesis. The *PAX* genes are involved in ARMS through specific translocations; t(2;13)(q35;q14) and variant t(1;13)(p36;q14) result in *PAX3-FKHR* and *PAX7-FKHR* fusion genes, respectively. The fusion genes in RMS are thought to affect downstream targets of *PAX3* and *PAX7* or possibly novel targets. Generally, ARMS is associated with a more aggressive phenotype. In addition, data suggests that the *PAX3-FKHR* fusion is associated with a poor prognosis and the *PAX7-FKHR* is associated with better outcome. → Amplification of genes such as *MYCN*, *MDM2*, *CDK4*, and *PAX7-FKHR* are features mainly of the ARMS, whilst specific chromosomal gains including chromosomes 2, 8, 12, and 13 are associated with ERMS. Loss of alleles and imprinting at 11p15.5 and disruption of genes such as *IGF2*, *ATR*, *PTC*, *P16* and *TP53* have also been implicated in RMS development. The increased incidence of RMS associated with → Li-Fraumeni syndrome (involving germ line *TP53* mutations and increased risk to other tumors including breast cancer) and also → Beckwith-Wiedemann syndrome (associated with fetal overgrowth and other embryonal tumors) are linked to the involvement of *TP53* genes and 11p15.5, respectively.

A better understanding of RMS tumorigenesis may ultimately result in novel therapeutic strategies that increase the overall cure.

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Rho Family Proteins

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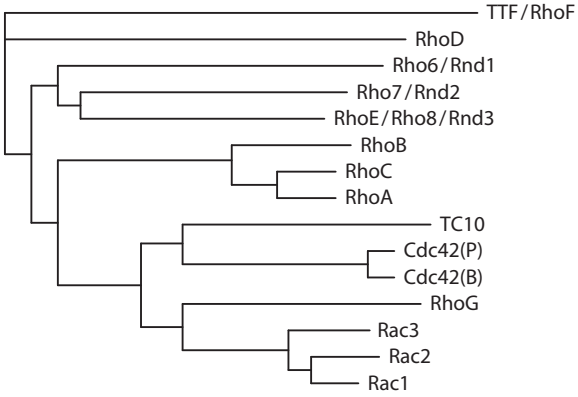
Definition

Rho family proteins are low molecular weight → G-proteins that are → Ras homologous proteins (Ras-related small GTPases).

The Rho family proteins are members of a major branch of the Ras superfamily of small GTPases. Currently, 17 mammalian members are known, with homologs present in invertebrates [*S. cerevisiae* (5), *S. pombe* (3), *C. elegans* (6), Drosophila (5), Dictyostelium (8), Aplysia (1), plants (2)] (Fig. 1). The best known and most widely characterized human Rho family proteins are Rac1, RhoA and Cdc42. These proteins function as GDP/GTP-regulated binary switches which regulate signal transduction pathways that control actin cytoskeletal organization, gene expression and cellular proliferation.

Characteristics

Rho family proteins are approximately 200 amino acids in length and a molecular weight of approximately 21 kD. They share approximately 30% amino acid identity with the Ras oncoproteins and between 50 to 90% identity within the family. All members share three distinct amino acid sequence elements: First, they possess consensus GDP/GTP binding motifs shared with other GDP/GTP-binding proteins.

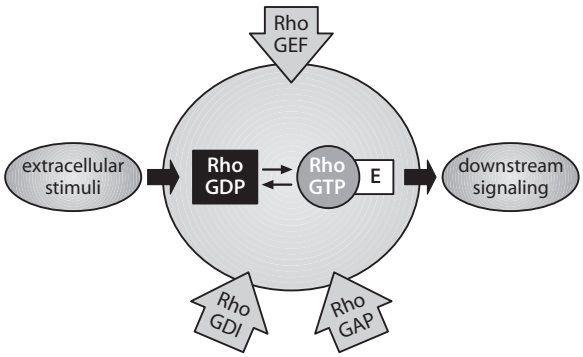


RHO Family Proteins. Fig. 1 – The Rho branch of the Ras superfamily. Sequence comparison of mammalian Rho family proteins. To date, 14 distinct mammalian Rho family proteins have been identified. A multiple sequence alignment of human Rho family proteins and H-Ras was generated, using the dynamic algorithm program ClustalW and used to construct the Rho dendrogram. The branch lengths in the dendrogram are proportional to the estimated divergence along each branch.

Like Ras proteins, Rho family proteins possess high affinity binding for guanine nucleotides (GDP and GTP). Their biological functions are controlled by cycling between active GTP-bound and inactive GDP-bound states. Second, all members terminate with a CAAX tetrapeptide sequence (C = cysteine, A = aliphatic amino acid, X = terminal amino acid). The CAAX motif signals three posttranslational modification steps; the addition of either a C15 farnesyl (when X = M) or C20 geranylgeranyl (when X = L, F) isoprenoid lipid group to the cysteine of the CAAX motif, proteolytic removal of the AAX residues and carboxylmethylation of the now terminally prenylated cysteine residue. These modifications increase the hydrophobic nature of the protein and facilitate their association with membranes. Third, sequences corresponding to Ras residues 32-40 represent the core effector domain and these sequences are involved in interaction with downstream effector targets. Sequences flanking these residues, as well as other sequences throughout Rho family proteins, are also involved in effector interactions. Finally, Rho family proteins possess a short sequence, designated the Rho insert sequence, that is not present in other Ras superfamily proteins and may also be involved in effector interaction.

Cellular and molecular regulation

The GDP/GTP cycling of Rho family proteins is controlled by three distinct functional classes of regulatory proteins (Fig. 2). Guanine nucleotide exchange factors (GEFs) stimulate the weak intrinsic exchange activity of Rho family proteins to cause an exchange of the bound GDP for GTP to promote formation of active Rho-GTP. Rho GEFs are also called → Dbl family proteins. Dbl family proteins (named after the founding member, a transforming protein identified from a human **d**iffuse **B**-cell **l**ymphoma) share a tandem Dbl homology (DH) domain and pleckstrin homology (PH) domain. The DH domain is a catalytic domain that stimulates GDP/GTP exchange. The PH domain is believed to regulate DH domain catalytic activity and can also serve to promote Dbl protein association with the plasma membrane. A number of Dbl family proteins were initially identified in gene transfer screening searches for transforming (e.g., Dbl, Vav, Ect2, Lsc) or invasion-inducing (Tiam1) genes. Others were identified as proteins with other catalytic functions, such as the breakpoint cluster region protein



RHO Family Proteins. Fig. 2 – Rho family proteins function as GDP/GTP-regulated binary switches. In response to extracellular stimuli, Rho GEFs stimulate formation of active Rho-GTP, which in turn forms complexes with various downstream effectors (designated E) to initiate downstream signaling events. Rho GAPs and GDIs serve as negative regulators of the GDP/GTP cycle.

(BCR). BCR is the translocation partner of the Abl tyrosine kinase present in Philadelphia chromosome positive human leukemias, and this genetic rearrangement causes the formation of a chimeric → BCR-Abl fusion oncoprotein. To date, at least 30 distinct human Dbl family proteins have been identified. Since they function as Rho GEFs, their transforming actions are due to chronic activation of Rho GTPases.

The second class of regulators of Rho family proteins are GTPase activating proteins (GAPs [→ GTPase-activating protein]) that stimulate the weak intrinsic GTP hydrolysis activity of Rho family proteins to promote formation of the inactive GDP-bound protein. Presently, at least 16 Rho GAPs have been identified (e.g., chimerins, BCR). The third class of regulators are the Rho guanine nucleotide dissociation inhibitory factors (GDIs). Rho GDIs can inhibit GDP dissociation as well as GAP-stimulated GTP hydrolysis. Rho GDIs also regulate the association of Rho family proteins with membranes. To date, 3 distinct Rho GDIs have been identified.

Structural analyses of Ras and Rho family proteins reveal that the GDP- and GTP-bound proteins differ in conformation in two regions, designated switch I and II. The conformation of the GTP-bound protein results in increased binding affinity for downstream effector proteins. Each Rho family protein recognizes multiple effectors and some effectors are recognized by multiple Rho family proteins. Rho family protein interaction and activation of effector function leads to the stimulation of effector-mediated signaling pathways that regulate the diverse functions of Rho family proteins. The effectors for RhoA, Rac1 and Cdc42 have been the most intensively studied and characterized. The multitude of effectors identified for each Rho family protein reflects the complex and diverse functional properties of these proteins.

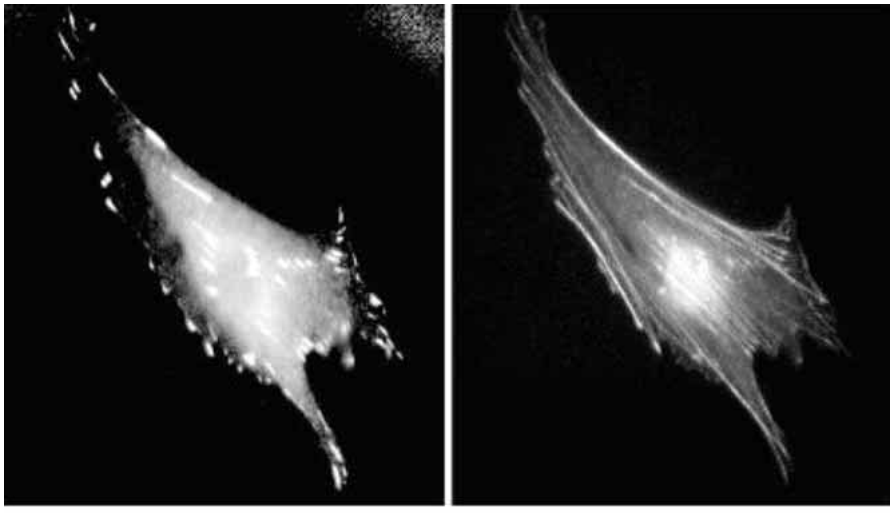
Genetically engineered structural mutants of Rho family proteins have provided very useful research reagents to evaluate the biochemical and biological functions of Rho family proteins. The first class of mutants are gain-of-function mutants. Single amino acid substitutions at residues analogous to those that convert normal Ras proteins into highly oncogenic, constitutively activated proteins (at Ras residues glycine 12 or glutamine 61) also create constitutively activated mutants of Rho family proteins. These mutations render Ras and Rho proteins insensitive to GAP stimulation and thus these proteins persist in the GTP-bound state. The second class of mutants are dominant-negative mutants that contain a serine to asparagine substitution at the residue analogous to amino acid 17 of Ras proteins. These mutants can prevent activation of specific Rho family proteins, presumably by forming inactive complexes with specific Dbl family proteins. The third class of mutants are effector domain mutants that possess single amino acid substitutions in the core effector domain. These impair interaction of Rho family proteins with downstream effectors, thus leading to impairment in biological activity. Since a particular effector domain mutation leads to differential impairment of effector interaction, such mutants have been very useful reagents in establishing the specific contribution of different effector targets to Rho family protein function.

Functions

Much of the functional information on Rho family proteins has come from studies of Rac1, RhoA and Cdc42. A diverse spectrum of extracellular stimuli via interaction with → receptor tyrosine kinases (RTKs), → G-protein-coupled receptors (GPCRs) or integrins cause activation of specific Rho family proteins, presumably via activation of specific Rho GEFs. For example, GPCRs that lead to activation of the heterotrimeric Gα13 subunit then results in direct interaction and activation of the p115 RhoGEF/Lsc Dbl family protein. This Dbl family protein is a specific GEF for RhoA. Platelet-derived growth factor (PDGF) stimulation of the PDGF RTK causes activation of Rac1 via a pathway that has not been fully elucidated. Bradykinin stimulation of a GPCR causes activation of Cdc42. These various extracellular signals cause a transient increase in the GTP-complexed protein that then rapidly cycles back to the GDP-complexed form to terminate the signal.

Rho family proteins are regulators of diverse cellular processes. Perhaps their best characterized function involves the regulation of specific filamentous F-actin organization. The actin cytoskeleton is a highly dynamic cytoplasmic structure that is reshaped and reformed in response to diverse extracellular stimuli. Specific Rho family proteins regulate distinct changes in actin cytoskeletal assembly and function. RhoA promotes the formation of stress fibers and focal adhesions, whereas RhoE/Rnd3 and Rnd1 cause the disruption of these structures. Rac1 promotes lamellipodia, curtain-like extensions that consist of thin protrusive actin sheets. Membrane ruffles represent lamellipodia that have lifted from the substratum at the leading edge of migrating cells. Cdc42 and TC10 cause formation of filopodia, which are thin, finger-like cytoplasmic extensions that contain tight actin bundles and may be involved in recognition of the extracellular environment. In some cells, these small GTPases act coordinately as part of a cascade. For example, in Swiss 3T3 and other cells, Cdc42 activation causes Rac1 activation, and Rac1 activation then causes RhoA activation. Mutated and activated Ras can also activate Rac1, and consequently, Rac1 activation of RhoA.

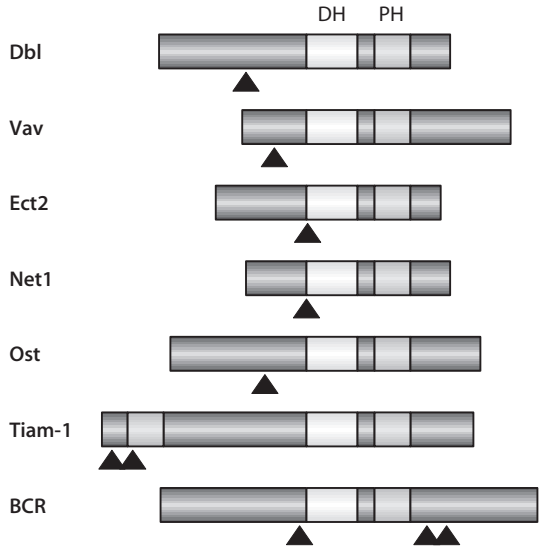
- Rho family proteins regulate actin cytoskeletal organization. For example, extracellular stimuli that activate cell surface G protein-coupled receptors (e.g., lysophosphatidic acid, thrombin) or integrins (e.g., fibronectin) cause activation of cytoplasmic signaling cascades that promote the formation of actin stress fibers and focal adhesions (Fig. 3).
- A second major function of Rho family proteins involves the stimulation of cytoplasmic signaling pathways that regulate the activity of nuclear transcription factors. These transcription factors include those that regulate genes involved in the regulation of cell growth, differentiation and apoptosis. For example, Rac1 and Cdc42 activate the Jun NH_2-terminal kinases (JNKs; also called SAPKs), and activated Jun can stimulate transcription from promoters containing AP-1 DNA binding motifs. RhoA, Rac1 and Cdc42 are activators of the NFκB tran-



RHO Family Proteins. Fig. 3 – Rho family proteins regulate actin cytoskeletal organization. Extracellular stimuli that activate cell surface G protein-coupled receptors (e.g., lysophosphatidic acid, thrombin) or integrins (e.g., fibronectin) cause activation of cytoplasmic signaling cascades that promote the formation of actin stress fibers and focal adhesions. Shown are immunofluorescence analyses of NIH 3T3 mouse fibroblasts, expressing exogenously introduced activated RhoA. Filamentous actin is shown by using FITC-phalloidin. The focal adhesion protein vinculin was stained with 7f9 anti-vinculin monoclonal antibody, followed by TRITC-conjugated goat anti-mouse IgG.

scription factor. NFκB regulates the expression of genes that serve an anti-apoptotic function. Rho family proteins activate the serum response factor (SRF), which forms a complex with ternary complex factors (e.g., Elk-1) at the serum response DNA element in promoter sequences of growth factor early response genes (e.g. *fos*).

- A third major function of Rho family proteins involves their regulation of cellular proliferation. RhoA, Rac1 and Cdc42 have been shown to be essential components required for cells to progress through the G1 phase of the cell cycle. Constitutively activated mutants of some Rho family proteins can promote G1 progress and DNA synthesis in quiescent cells, and growth transformation of rodent fibroblasts. As described above, extracellular signals that regulate cell proliferation cause transient activation of specific Rho family proteins. For example, platelet-derived growth factor is a potent growth factor for many cell types and is an activator of Rac1 function. Hence, Rho family proteins may facilitate the mitogenic actions initiated by extracellular stimuli.



RHO Family Proteins. Fig. 4 – Dbl family proteins are activators of Rho family proteins. Dbl family proteins serve as Rho GEFs and promote formation of GTP-bound Rho. All possess the tandem DH (~180 amino acid) and PH (~100 amino acid) domains. A number of Dbl family proteins have been identified as potent transforming oncoproteins, activated by truncation of NH_2-terminal residues (designated by arrows).

Clinical relevance

There is presently considerable experimental evidence linking Rho family proteins to cancer (Fig. 4). First, it was found that the Ras oncoproteins require Rho family protein function to cause growth transformation. Ras is the most frequently mutated oncoprotein in human cancers, and mutated and constitutively activated versions can be found in 30% of human cancers. Second, Rho family protein function also contributes to the transforming actions of other oncoproteins, including tyrosine kinases, Dbl family proteins, G protein-coupled receptors and heterotrimeric G protein α subunits. Third, the aberrant activation of Rho family proteins can also cause growth transformation, invasion and metastasis in experimental models of carcinogenesis. Although mutated and constitutively activated versions of Rho family proteins have not, to date, been found in human cancers, Ras and other oncoproteins may stimulate signaling pathways that lead to their aberrant up-regulation. Two Dbl family proteins have been found to be rearranged and mutated in human leukemias (BCR and LARG). Hence, their aberrant functions may contribute to the development of leukemia. Consequently, antagonists of Rho family proteins may represent novel therapeutic approaches for cancer treatment. Finally, recently developed → farnesyltransferase inhibitors (FTIs), although initially developed as anti-Ras drugs, may block tumor cell growth by blocking the function of farnesylated Rho family proteins (e.g., RhoB). FTIs have demonstrated potent anti-cancer activity in preclinical animal models and are currently being evaluated in phase II clinical trials.

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Ribavirin

Definition

Ribavirin is a synthetic 1-beta-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide used against → hepatitis C virus.

Ribozyme

Definition

Ribozyme is a RNA molecule with sequence-specific endonucleolytic acitvity; → gene therapy.

RING Finger

Definition

Really interesting new gene (RING) is a specific zinc-binding motif which is commonly found in proteins that interact with ubiquitin-conjugating enzymes, E2 [→ Ubiquitin pathway enzymes]. Many ubiquitin-protein ligases, E3's, contain RING finger motifs. It is still unclear whether all RING fingers are associated with → ubiquitination.

RLGS

Definition

→ Restriction landmark genomic scanning.

RNA Polymerase

Definition

The enzyme RNA polymerase is responsible for the synthesis of RNA from a DNA template (→ transcription). Prokaryotic gene expression involves one type of RNA polymerase. In eukaryotes, three different types of RNA polymerase exist that each transcribe specific sets of genes.

1. RNA polymerase I is located in the nucleolus and synthesizes only a single type of product, the ribosomal RNA (rRNA). RNA polymerase I accounts for the majority of RNA synthesis within the cell, transcribing all genes coding for rRNA.
2. RNA polymerase II is located in the nucleoplasm and synthesizes the messenger RNA (mRNA) precursor, heterogenous nuclear RNA (hnRNA). All protein coding genes of the cell, whether they are constitutively expressed, developmentally regulated or restricted to certain types of tissues, are transcribed by RNA polymerase II.
3. RNA polymerase III synthesizes transfer RNA (tRNA) and other small RNAs. Some viral genes, such as some adenovirus and → Epstein-Barr virus genes, are also transcribed by RNA polymerase III. RNA polymerase III transcripts are generally very short (less than 300 nucleotides), and never encode any proteins.

RNA polymerases I, II and II can be distinguished by their α-amanitin sensitivity, which is not sensitive, sensitive and species-specific, respectively.

RNA Tumor Virus

Definition

The RNA tumor virus is a member of the family of → retroviruses with the ability of inducing tumor growth. These viruses are only known

from animal systems, ranging from chicken to monkeys; they are not known in humans; → transduction of oncogenes; → oncogenes.

RNase H

Definition

RNase H ('H' for hybrid) or ribonuclease H is an endoribonuclease that specifically degrades the RNA of DNA-RNA hybrids, producing 5′ phosphate-terminated ends. RNase H does not degrade single-stranded nucleic acids, duplex DNA or double-stranded RNA.

Robertsonian Translocation

Definition

Robertsonian translocation, also known as centric fusion, is a special class of translocation in which the break occurs in the centromeric region of two different chromosomes and whole chromosome arms are exchanged between these two chromosomes.

RON

Definition

RON is the receptor for human → macrophage-stimulating protein; → macrophage-stimulating 1 receptor.

ROX

Definition

→ MNT.

R-point

Definition

→ Restriction point.

R-RAS

Definition

R-ras is a member of the → *Ras* family genes, coding for a p23 GTP-binding protein. Mutated *R-Ras* induces transformation of NIH/3T3 fibroblasts without morphological changes.

RTK

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Synonyms

- → Receptor tyrosine kinases (RTK).

RT-PCR

Definition

RT-PCR is a recombinant DNA technique that allows the rapid amplification of a complementary copy of a stretch of DNA (cDNA) starting from RNA. The first step is to reverse transcribe the RNA into a first strand cDNA copy using the enzymes reverse transcriptase. Thereafter, a standard polymerase chain reaction (PCR) is performed, using specific DNA primers and DNA polymerase, to rapidly amplify large amounts of cDNA.

S

Saccharomyces cerevisiae

Definition

Saccharomyces cerevisiae is a unicellular fungi (yeast) that is a useful tool in biotechnology (production of bread, beer and wine) and model organism in cell biology, e.g. in the study of the cell cycle or trafficking (like → endocytosis or → autophagy).

SAM

Definition

Sterile alpha motif (SAM) domain is a protein interaction domain of around 70 amino acids present in many diverse signaling proteins. SAM domains have been shown to self associate in some systems.

Sanctuary Site

Definition

A sanctuary site is any location in the body that is poorly penetrated by drugs. Presumably, the brain is protected from antileukemic drugs by the blood-brain barrier.

SAPK

Definition

Stress-activated protein kinase (SAPK), also known as JNK, is responsible for phosphorylation of c-Jun and ATF-2 proteins in response to cellular stress; → AP-1.

SARA

Definition

Smad anchor for receptor activation (SARA) is a cytosolic protein anchored in membranes close to TGFβ receptors. This protein presents unphosphorylated R-Smads to TGFβ-activated receptor complexes.

Sarcoma

Definition

Sarcoma is a malignant tumor of mesenchymal origin.

Satellite DNA

Definition

Satellite DNA consists of many tandem repeats (identical or related) of a short basic repeating unit.

Scatter Factor

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Synonyms

- hepatocyte growth factor (HGF)

Definition

Scatter factor (SF), also known as hepatocyte growth factor (HGF), is a multifunctional cytokine that participates in various biologic processes, including: embryonic development (→ morphogenesis), oncogenesis (tumor formation), → angiogenesis (new blood vessel formation), and the regulation of → apoptosis (programmed cell death). SF was originally characterized as a protein secreted by mesenchymal cells (e.g. fibroblasts) that disperses (or 'scatters') contiguous sheets of epithelium and stimulates cell motility. HGF was identified as a serum-derived protein that stimulates the proliferation of adult rat hepatocytes. Subsequent studies revealed that SF and HGF are identical. HGF is the ligand of a → tyrosine kinase receptor encoded by a → proto-oncogene (c-→ Met), HGF binding causes receptor activation.

Characteristics

SF is a heparin-binding glycoprotein composed of a 60 kD α-chain and a 30 kD β-chain. The α-chain is composed of an N-terminal hairpin loop, followed by four → kringle domains (looped structures that mediate protein interactions). The β-chain resembles protein-degrading enzymes such as trypsin, but SF lacks protein-degrading activity due to two key amino acid substitutions at the catalytic center. The binding of heparin to SF modulates its biologic activity, protects it from degradation and allows it to be stored in the extracellular matrix. Several shortened forms of SF containing only the N-terminal loop and the first kringle domain (NK1) or the first two kringle domains (NK2) are sufficient to bind with high affinity to the c-Met receptor. NK1 and NK2 are produced as a result of mRNA editing, and they can function as partial agonists or antagonists of SF. However, structure-function analyses indicate that the entire SF molecule, including the β-chain, is required to generate the full spectrum of SF's biologic activity. The human SF gene maps to the long arm of chromosome 7 (7q11.2-21).

SF is synthesized as a 728 amino acid precursor (preproSF) that is converted within the cell to its secreted form (proSF) by cleavage of a short segment (→ signal sequence). However, the secreted proSF is not biologically active, and must undergo an internal cleavage within the linker region between the α-chain and β-chain. Cleavage of proSF occurs outside of the cell and results in the production of the mature, two-chain biologically active SF. Thus, the cleavage of proSF to SF is a potential control point for the regulation of SF activity. Several enzymes capable of cleaving and activating SF have been identified. These enzymes include the plasminogen activators (urokinase and tissue plasminogen activator), proteins that convert plasminogen - an enzyme that circulates in blood in inactive form - into its active form, plasmin. Plasmin is the major enzyme responsible for dissolving blood clots. Another enzyme capable of converting proSF into active SF is called '→ HGF activator'. HGF activator is a novel protein-degrading enzyme structurally related to a blood clotting factor (factor XII, or Hageman factor). Interestingly, HGF activator is itself produced in an inactive ('pro-enzyme') form. It may be activated by blood coagulation factors, such as thrombin. The physiologic processes that regulate SF activation have not been fully elucidated, but there is evidence to suggest that an enzymatic cascade that results in activation of HGF activator and then SF is triggered by tissue injury.

The SF family

SF is not related to classic growth factors (eg., fibroblast growth factor or platelet-derived growth factor), but is a member of the kringle

domain (see above) protein family, which includes blood coagulation and fibrin-degrading enzymes (eg., plasminogen, prothrombin, factor XII, urokinase, tissue plasminogen activator) and a macrophage-stimulating protein (MSP). Within this family, SF is most closely related to the plasminogen and MSP, with which it shares a similar αβ chain structure, a similar activation mechanism (i.e. cleavage between the α and β chains), and a high degree of amino acid sequence identity (38% and 50%, respectively). MSP was formerly called HGF-like protein and is the closest relative of SF. The MSP receptor, a tyrosine kinase receptor encoded by the Ron gene, is closely related to the SF receptor,but these two proteins do not cross-activate each other's receptor. MSP circulates in the bloodstream as an inactive profactor (proMSP) which, when activated, causes macrophages to become competent and to undergo chemotaxis and phagocytosis.

SF receptor (the *c-Met* proto-oncogene product)

The *c-Met* proto-oncogene was originally discovered in rearranged form as a carcinogen-induced transforming oncogene (*Tpr-Met*) generated by a transposition between human chromosomes 1 and 7, resulting in fusion of a powerful promoter from chromosome 1 ('**T**ransposed **p**romoter **r**egion') to a portion of the *c-Met* proto-oncogene (at 7q21-31) that codes for the intracellular region of the receptor. The *Tpr-met* oncogene product is a membrane-bound tyrosine kinase that is constitutively active (ie., does not require SF for activation). The full-length *c-Met* proto-oncogene encodes a growth factor receptor-like tyrosine kinase that consists of an extracellular SF-binding domain, a transmembrane domain, and an intracellular portion containing a kinase domain and sites that associate with various cytoplasmic signaling proteins.

The binding of SF to c-Met triggers a molecular events similar to those triggered by the interactions between classic growth factors and their receptors. The receptor undergoes a change in three-dimensional conformation, resulting in:

- activation of the catalytic kinase domain
- dimerization (association of two c-Met receptors)
- cross-phosphorylation of the two receptors on multiple tyrosines
- initiation of a signal cascade ('signal transduction') causing transfer of information from the cell surface to the nucleus. Understanding signal transduction from c-Met will provide the key to understanding SF's biologic actions.

Signal initiation involves the interaction of phosphorylated tyrosines internal to the kinase domain of the activated c-Met with regions known as → SH2 domains (src-homology domain-2) of proteins that act as signaling intermediaries. Most c-Met signaling involves the interaction of these signaling intermediaries with a 'multi-functional docking site' involving two tyrosine (Y) residues located at amino acids 1349 and 1356:1349YVHVXX-X1356YVNV. This unique site associates with many signaling proteins, including phosphatidyl-inositol-3′-kinase [→ PI3K], phospholipase C-γ, pp60c-src, c-Cbl, and the Grb2/Sos complex, which binds p21Ras. Amino acid sequences similar to the multi-functional docking site of c-Met are found in the two related receptors: Ron and c-Sea (a tyrosine kinase receptor whose ligand has not been identified). Similar sequences are not found in the receptors for the epidermal growth factor, platelet-derived growth factor, or other factors.

The manner in which SF binding to c-Met can result in different physiologic consequences depending upon the cell type and context (see below) is just beginning to become unraveled. For example, it was recently found that SF-induced epithelial morphogenesis (ie., the formation of a three-dimensional network of branching tubules) specifically requires association with c-Met at cell-cell junctions and phosphorylation of a protein known as Gab1 (the Grb2-associated binder). Grb2 binds to the tyrosine-1356 site of c-Met *via* its SH2 domain, while another portion of the Grb2 protein (the SH3, or src-homology-3 domain) binds to Gab1. Gab1 is a member of the family of the 'multi-substrate docking proteins', which in-

cludes IRS-1 (insulin-responsive substrate-1), a cytoplasmic protein that is a major mediator of the biologic effects of the insulin-like growth factor → IGF-1.

Cellular and molecular regulation

SF producer and responder cell types. *In vitro* studies initially suggested that SF is produced predominantly by cells of mesenchymal (connective tissue) origin, including: fibroblasts, vascular smooth muscle, endothelial cells, glial cells, macrophages, activated lymphocytes, and other cell types. However, based on subsequent *in vivo* studies (immunohistochemistry and *in situ* hybridization), it is now apparent that a variety of epithelial cell types, including keratinocytes, mammary epithelial cells, and many carcinoma cells may also produce SF. For reasons not understood, cultured epithelial and carcinoma cells lose the ability to produce SF when placed in culture, although they often retain the c-Met receptor. A variety of cell types express the c-Met receptor and are biologically responsive to SF, including (but not limited to): keratinocytes, hepatocytes, mammary epithelium, vascular endothelial cells, melanocytes, glial cells, and the corresponding malignant cell types.

Regulation of SF production. The complexity of the regulatory mechanisms for SF production is becoming increasingly apparent as the list of known and partially characterized factors that regulate SF production continues to grow. In addition to well-known pro-inflammatory (IL-1α, IL-1β, TNF-α) or anti-inflammatory (TGF-β) cytokines that enhance or inhibit production of SF by fibroblasts, a group of partially characterized scatter factor-inducing factors distinct from IL-1 and TNF stimulate SF expression in fibroblasts and other SF-producer cell types. SF-inducing factors are secreted by various carcinoma cell lines, and they appear in the serum of rats following a subtotal hepatectomy. When co-cultured with epithelial cells, fibroblasts cease to express SF mRNA and protein, again by a regulatory mechanism that has not been elucidated. Heparin and heparan sulfate proteoglycans, which are known to bind to SF, also appear to stimulate its production. However, these molecules may simply function to stabilize the SF protein and to prevent its degradation.

Biologic responses induced by SF and their regulation

The major biologic responses induced by SF fall into four broad categories:

- motility
- proliferation
- morphogenesis
- cell survival (or more properly, protection against apoptotic cell death)

The c-Met receptor can transduce each of these biologic functions. These biologic responses may overlap (eg., morphogenesis involves a component of cell migration through extracellular matrix); and they appear to be determined by the extracellular environment and by cell-specific programs of differentiation. For example, Madin-Darby canine kidney (MDCK) epithelial cells cultured on flat surfaces are scattered, while cells cultured in collagen gels respond to SF by forming networks of branching tubules similar to those found in the kidney.

Activation of specific pathways for motility, proliferation, morphogenesis, and/or cell survival. may be determined at the receptor level or more distally. The specific pathways that activate each of these processes are only now beginning to be dissected. For example, recent studies suggest that treatment of various epithelial and cancer cell types with SF induces resistance to DNA-damaging drugs and radiation by a process that involves the sequential activation of c-Met, phosphatidylinositol-3′-kinase, c- → Akt (protein kinase B). The latter is a serine/threonine kinase that functions to protect cells against apoptotic death.

The extracellular environment plays a major role in modulating the biologic responses to SF. Studies of SF-induced branching morphogenesis of MDCK epithelial cells provides clues as to how this modulation might occur. Thus, certain extracellular matrix molecules promote forward extension of tubules (collagen I, laminin), while others promote branching

(heparan sulfate proteoglycans, collagen IV). TGF-β, a component of the extracellular matrix, inhibits the entire process of branching morphogenesis. The binding of matrix proteins to integrins activates intracellular signaling processes, including tyrosine phosphorylation; and SF may induce the expression of a specific set of integrins that allows the extracellular matrix to modulate intracellular signaling. There is evidence that the extracellular matrix may modulate c-Met signaling by inducing the phosphorylation and dephosphorylation of different sites on c-Met and other signaling proteins.

SF and c-Met Participate in Various Physiologic and Pathologic Processes

- *Development.* An important role for SF in development was suggested by the finding that homozygous deletion of either *SF* or *c-Met* results in embryonic lethality in mice. Various studies implicate SF as a mediator of mesenchymal: epithelial signaling during embryogenesis. For example, during mouse development, the SF gene is expressed in mesenchymal cells, while the c-Met gene is expressed in adjacent epithelia. This pattern is observed in multiple developing organs, and appears to be regulated with great precision in space and time. Injection of SF into the developing chick embryo induces abnormalities of the neuraxis, indicating that inappropriate exposure to SF can interfere with normal development. In addition, several studies suggest that *SF* and *c-Met* can mediate the conversion of mesenchymal cells to an epithelial phenotype, as judged by its ability to induce morphologic alterations as well as the expression of epithelial specific markers (eg., cytokeratins and epithelial-specific junctional proteins). Mesenchymal:epithelial interconversion is commonly observed during embryogenesis, further supporting a role for the SF-c-Met ligand-receptor pair in development.
- *Oncogenesis.* Malignant cell transformation is mediated by the *Tpr-Met* oncogene, which encodes a truncated and constitutively active form of the c-Met receptor. This finding raises the possibility that SF-mediated over-stimulation of c-Met has similar consequences. The idea that SF could mediate tumorigenesis *in vivo* is suggested by several considerations. First, SF stimulates the motility, and invasiveness of a variety of carcinoma cell types *in vitro*. Secondly, SF is a potent inducer of angiogenesis (new blood vessel formation), a process considered to be essential for the continued growth of solid tumors. Finally, SF can overcome apoptosis (programmed cell death) of epithelial cells which is associated with detachment of cells from their substratum. Detachment of carcinoma cells from the underlying basement membrane is an early step in tumor invasion. Studies of experimental animal models and human clinical samples further support a role for SF in tumorigenesis. Over-expression of the *SF* and/or *c-Met* genes in a variety of cell types induces or further enhances the tumorigenic phenotype *in vivo*, by → autocrine and/or → paracrine mechanisms. In studies of human breast cancer, bladder cancer, gliomas, and other tumor types, significantly higher levels of SF and/or c-Met were observed in high grade invasive cancers than in low grade non-invasive cancers. And in a study of 258 primary invasive breast cancers, a high SF content in the tumor was strongly predictive of relapse and death. Finally, recent genetic-epidemiologic studies have strongly linked activating mutations of the *c-Met* gene to a specific type of kidney cancer: hereditary papillary renal carcinoma.
- *Angiogenesis.* The formation of new blood vessels from pre-existing vessels, occurs extensively during normal development and tissue remodeling, but occurs only to a limited degree in normal adults. Physiologic angiogenesis in adults is observed transiently during wound healing, ovulation, and placental implantation. However, persistant and inappropriate angiogenesis contributes to certain pathologic processes, including chronic inflammatory diseases (eg., rheumatoid arthritis) and cancer. SF induces an angiogenic phenotype in cultured vascular endothelial cells (ie., stimulates endothelial cell proliferation, chemotactic migration, and

capillary-like tube formation) and induces angiogenesis *in vivo* in several different experimental animal models. SF may contribute to angiogenesis in AIDS-related Kaposi sarcoma, a cytokine-dependent neoplasm associated with extensive endothelial cell proliferation and neovascularization. The observations that both SF content and tumor angiogenesis are powerful independent prognostic indicators for breast cancer suggest a role for SF as a tumor angiogenesis factor. However, a causal relationship between SF and tumor angiogenesis is not yet proven.

Clinical Relevance

The ability of SF (HGF) is to stimulate epithelial cell growth and morphogenesis, to induce angiogenesis, and to protect cells against toxins or environmental conditions that induce apoptosis suggests a variety of potential therapeutic applications for SF. In this regard there are a number of experimental animal (mouse and rat) studies suggesting that administration of the SF protein can block or ameliorate acute and chronic injury to the liver, kidney, or lung. For example, administration of SF prevents or reduces the loss of renal function caused by toxins such as $HgCl_2$ or cis-platinum in mice. In a rat model, infusion of SF blocked or slowed the development of liver fibrosis and cirrhosis; and SF blocked the development of pulmonary fibrosis induced by bleomycin in the mouse lung. These findings suggest that SF is potentially clinically useful as a hepatotrophic factor for repair of liver damage or as a renotrophic factor for repair of kidney damage. The use of SF in humans presents significant challenges, such as the delivery of sufficient quantities of the factor to the sites where it is needed, in view of its short biologic half-life. Nevertheless, if reliable methods of protein or gene delivery can be developed, there may be a variety of clinical applications for SF to promote organ repair and regeneration.

Several studies suggest that the administration of other angiogenic factors (VEGF and basic FGF) is potentially useful in restoring the blood supply and preventing tissue damage, in animal models in which coronary or peripheral blood vessels are ligated in order to produce acute ischemic injury. Because of its ability to induce angiogenesis as well as its ability to protect a variety of different cell types against apoptotic cell death, it is anticipated that SF may be particularly advantageous in these settings. There is also the potential for development of small molecule inhibitors of the c-Met receptor that could be used to treat pathologic processes driven by excessive production of SF, such as certain cancers. Such inhibitors have already been developed to inhibit the function of the EGF receptor. A criticism of this approach is that tumor growth is driven by a variety of cytokines, growth factors, and angiogenic factors, so that the specific inhibition of a single receptor type will be insufficient to halt tumor growth. Nonetheless, combinations of receptor inhibitors may be clinically useful, and there may be situations in which inhibition of a single receptor is sufficient to inhibit tumor growth due to synergistic interactions among growth factors and cytokines.

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SCC

Definition

SCC is a tumor marker for → cervical cancers, using a human cervical squamous cell carcinoma antigen.

SCE

Definition

Sister chromatids are the two chromatids present on a single metaphase chromosome that are joined by a centromere. Sister chromatid exchanges (SCEs) are regions where the sister chromatids have recombined or have broken and exchanged large segments. SCEs can be visualized on metaphase chromosomes following special staining prodedures. About 4-5 SCEs are seen under normal conditions in each human cell, but this number increases with exposure to many chemical mutagens. SCEs are believed to represent a poorly understood facet of DNA recombination and repair.

SCF

Definition

Stem cell factor (SCF); → Kit/stem cell factor receptor.

SCF Complex

Definition

Skp1 Cdc53 F-box complex is a multiunit ubiquitin-protein ligase [→ Ubiquitin pathway enzymes], E3, which generally includes a cullin protein, an → F-box protein and a → RING finger protein. Many distinct SCF complexes exist, with specific roles in the cell cycle, development and other biological processes. The SCF complex appears to be only involved in → ubiquitination.

Schistosoma Haematobium

Definition

Schistosoma haematobium is a trematode common to the Nile delta and Africa. Their eggs invade the bladder causing cystitis and hematuria and increasing the risk of squamous cell carcinoma of the bladder (also known as bilharzial → bladder cancer).

SCID

Definition

→ Severe combined immunodeficiency.

SCTAT

Definition

Sex cord tumor with annular tubes (SCTAT) is a rare tumor entity emerging from the ovaries, which can be found in benign and malignant forms. In Peutz-Jegher-syndrome patients, an accumulation of benign bilateral tumors is reported.

Second Messenger Molecules

Definition

Second messenger molecules is a term is used to describe molecules generated inside the cell (rapidly and transiently) in response to an extracel-

lular agonist, the so-called first messenger. Like the first messengers, which specifically recognize receptors at the cell surface, second messengers also specifically interact with proteins which are in this case present inside the cell. Examples include cyclicAMP, cyclicGMP, Ins(1,4,5)P3, DG and PtdIns(3,4,5)P3; → inositol lipids.

Selectins

Definition

Selectins are cell surface adhesion proteins that bind specific sugar oligomers.

Selex Aptamers

Definition

Systematic evolution of ligands by exponential (Selex) enrichment aptamers are DNA or RNA ligands which have been selected by a PCR-based approach for high affinity binding to different molecules.

Semanova II Syndrome

Definition

→ Nijmegen breakage syndrome.

Senescence

Definition

Senescence is the limited capacity of cells to divide, an irreversible growth arrest state that depends on the age or cell doublings of a cell, a stage in the life cycle of a cell at which it can no longer divide. This state is dependent on the number of cell divisions and is generally brought about through the gradual shortening of the telomeres (repeat sequences at the ends of chromosomes) with each successive population doubling. Senescence is a permanent growth arrest that occurs after cells have exhausted their proliferative capacity. In normal human fibroblast, senescence takes place after approximately 60 population doublings in culture.

Senescence and Immortalization

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Definition

Senescence is the permanent exit of a cell from the cell division cycle, accompanied by morphological and biochemical changes characteristic of ageing.

Immortalization is the ability of cell populations to undergo an unlimited number of cell divisions.

Characteristics

Senescence

Normal mammalian somatic cells can proliferate only a limited number of times *in vitro*, and the maximum number is often referred to as the 'Hayflick limit'. When this limit is reached, the cells undergo various morphological and biochemical changes suggestive of ageing, so the process is referred to as senescence. Senescent cells can remain metabolically active for a long period of time, even though they have permanently exited from the cell cycle. Senescence is thus distinct from cell death. It is also distinct from terminal differentiation, where cells also exit permanently from the cell cycle and undergo changes that allow them to perform specialized normal functions. Senescent cells have been extensively studied as an *in vitro* model of ageing. In humans, cellular senescence appears to be a major barrier to the development of cancer.

Immortalization

It is not practicable to test whether cells are truly capable of continuing to divide forever, so cells are usually regarded as being immortalized if they have undergone many cell divisions (typically 100) beyond the Hayflick limit. Many cancers contain immortalized cells and some cancer-derived cell lines have been proliferating *in vitro* for many decades.

Relevance of senescence and immortalization to cancer

Although the Hayflick number may be quite large (fibroblasts from an adult, for example, may divide up to 40 times before they become senescent), in most situations it is not large enough to permit tumor formation. A tumor containing 2^{40} cells would be big enough to be lethal, but there are two major reasons why 40 cell divisions does not result in a tumor of this size. The first is that cell death occurs at a very substantial rate within tumors, for reasons that include genetic instability and difficulties with blood supply. The second is that the genesis of a fully malignant tumor cell requires the accumulation of a number of critically important genetic changes. Most of these changes occur at random and provide a growth advantage to the nascent tumor cell. This process consumes many more cell divisions than a normal cell is able to undergo before it becomes senescent.

Consequently, senescence forms a major barrier to carcinogenesis in humans (1). A cell containing some of the genetic changes required for carcinogenesis will not usually be able to proliferate sufficiently to form a clinically significant tumor while the senescence barrier is intact. Human cells become immortalized at a very low frequency (no clear example has yet been found of a normal human cell undergoing immortalization spontaneously), so immortalization is a rate-limiting step in human carcinogenesis. In contrast, mouse cells become immortalized spontaneously at a measurable frequency and, accordingly, the probability of a mouse cell becoming malignant is many orders of magnitude higher than for human cells. The ability to suppress tumor formation is a major selective advantage for a long lived species such as *H. sapiens*.

A cell division counting mechanism

The existence of a limit to the number of times a cell can divide implies that there must be a cell division counting mechanism. According to the telomere hypothesis of senescence, the counting mechanism is based on the progressive shortening of the ends of chromosomes (→ telomeres) that occurs with cell division. Telomeres form protective caps that prevent the ends of chromosomes fusing with each other. They contain repetitive DNA (in all vertebrates the repeat unit is a hexanucleotide, TTAGGG), which ends in a single-stranded G-rich tail. Telomeric DNA is recognised by specific binding proteins. The reasons for telomere shortening include the following: First, DNA replication depends on small RNA primers, which get degraded and replaced by DNA. However, there is no mechanism for replacing the terminal RNA primer, and this results in the template for the next round of DNA synthesis being shorter. This is known as the 'end replication problem'. Second, there appears to be a 5′-3′ exonuclease that shortens the C-rich strand, which creates or increases the length of a single-stranded G-rich telomeric tail.

Regardless of the exact mechanism of telomere shortening, this may eventually result in the telomeres becoming so short that they trigger the cell to exit permanently from the cell cycle. In order for the cell to become immortalized, the cell must somehow prevent telomere shortening. In most cancers this is achieved by the activity of an enzyme, → telomerase, and in a minority it is achieved by another mechanism referred to as → alternative lengthening of telomeres (ALT). Every immortalized cell line examined to date has either telomerase or ALT activity (2).

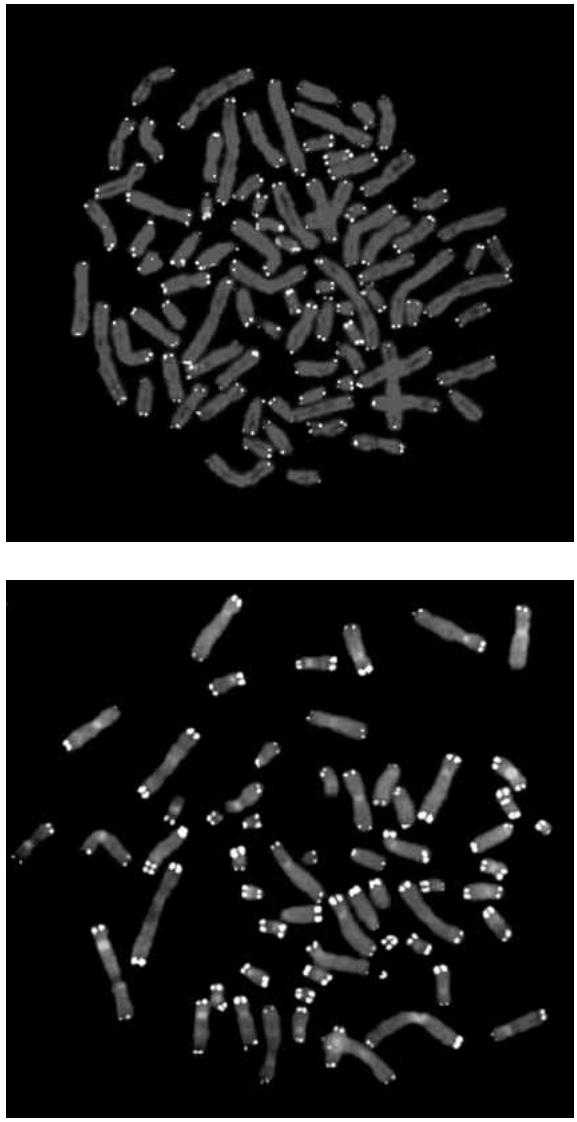
Telomerase

The telomerase holoenzyme complex is normally expressed in cells of the germ-line. It is also found in some normal somatic cells, espe-

cially those that are required to undergo extensive proliferation, but at levels that are insufficient to prevent telomere shortening. → Telomerase synthesizes telomeric DNA to replace the DNA lost during cell division. The essential subunits include an RNA molecule (**TE**lomerase **R**NA; TER) that acts as the template for synthesis of telomeric DNA and the reverse transcriptase catalytic subunit (**TE**lomerase **R**everse **T**ranscriptase; TERT) that carries out the synthesis. At least 85% of all cancers contain sufficient levels of telomerase to prevent telomere shortening (3). The factors controlling hTERT expression are not well understood but it is known that hTERT can be upregulated by → MYC. If TERT expression is artificially switched on by genetic manipulation in normal cells, it is able to induce telomerase enzyme activity. This prevents telomere shortening and in some types of human cells this results in immortalization.

Alternative lengthening of telomeres (ALT)

A minority of immortalized cell lines and cancers have no detectable telomerase activity and maintain their telomeres by an alternative mechanism (4). Although the details are not yet known, it is likely to be a recombinational mechanism in which one telomere uses another telomere (or itself via looping back) as a template for synthesis of new telomeric DNA. Cells that maintain their telomeres by ALT characteristically have very heterogeneous telomere lengths, ranging from undetectably short to extremely long (Fig.).



Senescence and Immortalization. Fig. – Telomeres in telomerase-positive and ALT immortalized cells. top Uniform telomere lengths in a telomerase-positive fibrosarcoma cells **bottom** Heterogeneous telomere lengths, ranging from undetectable to very long, in ALT immortalized cells.

Tumor suppressor genes

Immortalization is facilitated by loss-of-function of the → p16INK4a or → RB1 genes, and the → p53 gene. Loss of the normal function of these genes results in a significant, but finite, increase in cellular proliferative potential. This permits the accumulation of additional genetic changes and increases the probably that activation of a telomere maintenance mechanism will occur. Cells containing an inherited p53 mutation from individuals with → Li-Fraumeni syndrome are the only type of human cells known to undergo immortalization spontaneously.

Clinical relevance

Treatments that reverse the immortal phenotype may be a useful form of cancer therapy. An attractive target is telomerase, but inhibitors of telomerase may need to be combined with

inhibitors of ALT to prevent the emergence of drug resistance.

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Sentrin

Definition

→ UBL1.

SEREX

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Definition

A method for the identification and molecular analysis of antigens by recombinant cDNA expression cloning (serological analysis of antigens by recombinant cDNA expression cloning).

Description

For SEREX, a cDNA library is constructed from fresh tumor specimens, is cloned into phage expression vectors and the phages used to transfect *E. coli*. Recombinant proteins, which are expressed during the lytic infection of the bacteria, are transferred onto a nitrocellulose membrane. These membranes are incubated with diluted serum from autologous patients and screened for clones reactive with high-titered IgG antibodies. Positive clones are visualized by incubation with an enzyme-conjugated secondary antibody specific for human IgG. Positive clones are subcloned to monoclonality and the nucleotide sequence of the inserted cDNA is then determined.

The SEREX approach is technically characterized by several features:

- The use of fresh tissue obviates the need for culturing cells *in vitro* and therefore circumvents *in vitro* artifacts.
- The analysis is restricted to genes that are expressed by the tissue *in vivo*.
- The use of high-titered IgG antibodies in the initial screening procedure limits the analysis to such antigens that elicit a strong immune response in the host and imply a cognate T-cell help.
- The serological analysis covers the whole repertoire of proteins expressed by the respective tissue.
- SEREX uses polyspecific sera to scrutinize monoclonal antigens that are highly enriched in lytic plaques. This allows for a direct molecular definition of antigens, since the cDNA sequence of the antigen can be determined instantaneously.
- The specificity of the antigen, i.e. its expression spectrum, is determined by the analysis of the mRNA expression pattern by Northern blots and reverse transcriptase polymerase chain reaction (RT-PCR).
- If defined types of antigens are to be preselected, the original SEREX approach can be modified appropriately by using biased cDNA libraries (e.g. normal testis) or modified detection systems (e. g. for IgA, IgM).

High-titered IgG responses imply a cognate T-cell help. Therefore, in an approach of 'reverse T-cell immunology', antigens detected by SEREX can be used for the definition of epitopes that are presented in the context of MHC I and MHC II, respectively. Preferably, specific T-cell reactivities are searched for in patients

with high serum antibody reactivity to the respective antigen, and for many SEREX antigens both CD4- and CD8-stimulating epitopes have been identified.

SEREX allows for an unbiased search and the direct molecular definition of immunogenic proteins based on their reactivity with autologous and allogeneic patient sera. Hence, while SEREX was originally developed for the serological analysis of human tumor antigens, it can be used whenever antibody reactivities against tissue antigens are suspected and neither the antibody nor the antigen is known, e. g. for the identification and molecular characterization of autoantigens in autoimmune diseases. An international effort led by the Ludwig Institute for Cancer Research aims at defining the entire spectrum of antigens expressed by human tumors (the Human Cancer Immunome Project by Dr. L. J. Old). The aquired data (> 1800 entries by 12/00) include DNA sequences, expression spectra of the SEREX antigens, antibody reactivities and MHC-I and MHC-II binding motifs etc. All SEREX data are entered into the International SEREX Data Bank that is accessible to the public (http//www.licr.org/SEREX.HTML).

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Serine Proteinases

Definition

Serine proteinases are one of the four mechanistic classes of proteolytic enzymes. Serine proteinases contain a conserved Ser/His/Asp catalytic triad within the 3-dimensional structure of the enzyme active site. Positioning of these amino acids sets up a 'charge-relay' mechanism that polarizes the -OH side chain of the catalytically essential serine for nucleophilic attack on substrate peptide bonds. Examples of serine proteinases, believed to be important in cancer progression, are plasmin, urinary-type plasminogen activator (uPA, urokinase) and furin.

Serotype

Definition

Serotype is a virus type classified according to antibodies of the host.

Serum Dependence

Definition

Cultured cells need serum to provide essential growth factors, and therefore exhibit serum dependence. Tumor cells often grow under reduced serum conditions as they may be able to synthesize their own groth factors.

Severe Combined Immunodeficiency

Definition

Human severe combined immunodeficiency (SCID) is a set of primary immunodeficiency diseases characterized by profoundly impaired cell-mediated and humoral immunity. More

than 50% of SCID cases are X-linked SCID, which manifests complete or profound defect of T cells and NK cells, but carry normal or slightly increased numbers of B cells. This leads to death mostly within a year after birth if not treated with bone marrow transplantation. XSCID is commonly associated with mutations which chromosomally map to Xq13 in the gc receptor gene. Mutations occur throughout the entire gc gene, including the extracellular and cytoplasmic domains that impair cytokine ligand binding as well as signal transduction. These mutations are manifested as deletions, insertions, splice junction defects, point mutations and premature stop codons in the gc gene. The complete dysfunction of the gc subunit in ligand binding and signal transduction results in the typical phenotype associated with XSCID. Strikingly, a form of autosomal SCID exists with clinical symptoms identical to XSCID, in which the gene encoding JAK3 is affected.

SF

Definition

→ Scatter factor.

SH2

Definition

An Src homology 2 (SH2) domain is a structural motif contained in some signaling molecules that mediates high affinity interaction with phosphorylated tyrosine residues of another protein molecule. It is contained within many different signaling molecules that bears homology to the sequence and conformation of the corresponding domain found in Src. SH2 domains are protein-protein interaction domains that bind phosphotyrosine residues on partner proteins (→ SH2/SH3 domains).

SH3

Definition

Src homology 3 (SH3) is a domain contained within many different signaling molecules that bears homology to the sequence and conformation of the corresponding domain found in Src. SH3 domains mediate binding to other proteins via polyproline regions found on binding partners; → SH2/SH3 domains.

SH2/SH3 Domains

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Synonyms

- src homology 2 (SH2)/ src homology 3 (SH3) domains

Definition

Proteins containing SH2/SH3 domains play critical roles in regulating the formation of intracellular signal transduction complexes. SH2 and SH3 domains recognize amino acid motifs containing phosphotyrosine and polyproline, respectively. Signaling pathways activated by SH2/SH3 domains subsequently lead to cellular responses including differentiation, proliferation and migration.

Characteristics

SH2/SH3 domains were originally identified as conserved sequences found in the Src tyrosine kinase and a variety of other proteins. These domains are now known to reside in hundreds of functionally diverse signaling molecules, ranging from tyrosine kinases and phosphatases to phospholipases and transcription factors.

- SH2 domains are composed of approximately 100 amino acids organized into a modular structure that recognize phosphotyrosine-containing sequences on protein tyrosine kinases and their substrates. The specificity of interaction is generally determined by the 3-4 amino acids C-terminal to the phosphorylated tyrosine position on the target protein. SH2 domain-mediated signaling events lead to cellular responses ranging from DNA synthesis to transcriptional activation.
- SH3 domains are about 50 amino acids in size and bind to target proteins containing the proline-rich consensus sequence PXXP (where P is proline and X is any amino acid). Unlike SH2 domains, SH3 domains generally remain constitutively associated with their cognate ligands, making their function less dependent on tyrosine phosphorylation. Protein interactions mediated by SH3 domains have been implicated in cytoskeletal alterations necessary for changes in cell morphology and motility.

In response to extracellular cues, SH2/SH3 domains act both intramolecularly and intermolecularly to regulate signal transduction. Their functions are well illustrated by two classical signaling cascades exemplified by the Src protein tyrosine kinase pathway and the Ras GTPase pathway.

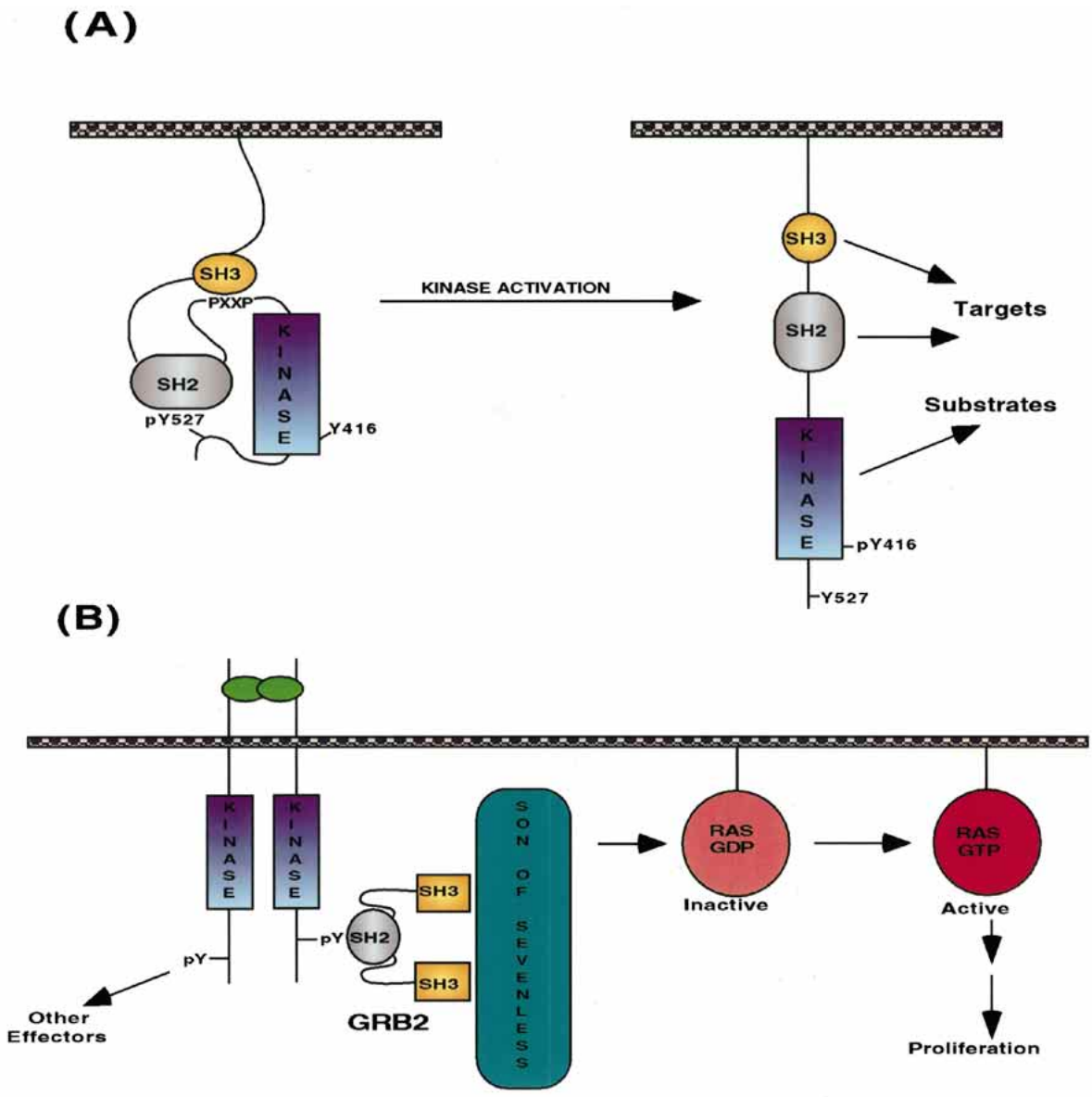
Src is a membrane bound intracellular tyrosine kinase that contains one SH2 and one SH3 domain (the kinase domain was originally termed the SH1 domain). The post-translational phosphorylation of Src on tyrosine 527 negatively regulates kinase activity. This inhibition is the result of complex intramolecular interactions between phosphotyrosine 527 and the SH2 domain, as well the SH3 domain and a proline-rich central region (see Fig.). Concomitant dephosphorylation of tyrosine 527 and phosphorylation of tyrosine 416 relieves this steric block. This allows for the association of the SH2 and SH3 domains with target proteins and enhancement of Src catalytic activity. Thus, Src SH2/SH3 domains both positively and negatively regulate protein tyrosine kinase activity. Various protein tyrosine kinases utilize a similar mechanism to regulate their function.

The Ras GTPase is a membrane associated growth regulator, and mutated forms are found in many human tumors. When in its GTP-bound state, Ras is considered 'on' and activates multiple downstream signaling pathways. In many cases growth factor stimulation of the Ras pathway occurs via the Grb2 SH2/SH3 protein (see Fig.). Grb2 is an SH2/SH3 'adaptor' protein, consisting of two SH3 domains flanking a central SH2 domain. The Grb2 SH3 domains constitutively associate with various proteins, including the son-of-sevenless (Sos) GTP exchange factor. Following growth factor stimulation, phosphotyrosine motifs on the activated receptors recruit the Grb2 SH2 domain. This event in part serves to re-localize the Grb2-Sos complex from the cytoplasm to the membrane, placing Sos in proximity to membrane-bound Ras and allowing the exchange of Ras-GDP for -GTP. Based on similar observations made for other SH2/SH3 adaptor proteins, intracellular re-localization may be a common mechanism used to activate a variety of enzymatic pathways.

These examples illustrate important aspects of SH2/SH3 domain function in response to a specific stimulus. It is clear, however, that most signal transduction pathways are interconnected, and that a single extracellular cue can elicit a response involving hundreds of effector proteins. Identifying how various extracellular cues individually and combinatorally affect signaling pathways will be an important challenge for fully understanding SH2/SH3 domain functions.

Clinical Relevance

Many tumor cells possess amplifications and/or mutations in genes encoding components of the tyrosine kinase machinery. Therefore, it follows that proteins regulating cell proliferation have become central targets for drug discovery. The crystallographic structures of many SH2 and SH3 domains in complex with their various ligands has allowed for the rational design of highly specific peptidomimetic molecules. The challenge is to utilize drugs that affect



SH2/SH3 Domains. Fig. – Pathways exemplifying SH2/SH3 domain functions. (**A**) Activation of the Src tyrosine kinase domain serves as an example of both intermolecular and intramolecular functions for SH2/SH3 domains. The Src SH2/SH3 domains serve to inhibit the kinase activity. Dephosphorylation of phosphotyrosine 527 and phosphorylation of tyrosine 416 results in a Src conformational change. The tyrosine kinase is activated and the protein associates with downstream substrates via its SH2 and SH3 domains. (**B**) The activation of the Ras GTPase by various receptor tyrosine kinases is a classic example of SH2/SH3 domains providing an intermolecular link between critical signal transduction components. Receptor autophosphorylation leads to re-localization of constitutively associated Grb2-Sos, leading to GTP exchange and Ras activation.

only the pathological action of the specific targeted molecule, while enabling normal signal events to proceed unperturbed. For example, one can envision using SH2 and/or SH3 domain antagonists to inhibit the proliferative capacity of cells transformed due to hyperactivated or aberrantly expressed protein tyrosine kinases.

Importantly, ongoing genomic analyses will undoubtedly identify new SH2/SH3 domain-containing proteins. Characterizing the func-

tions of these newly identified effectors and linking them to the pathogenesis of specific cancers and other disease states will be a worthwhile goal. The use of novel proteomic techniques in combination with classical cell biology will greatly aid this process.

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Shimada System

Definition

Shimada system is the clinical classification of → neuroblastoma.

Shotgun Experiment

Definition

Shotgun experiment is the cloning of an entire genome in the form of randomly generated fragments.

Shuttle Vector

Definition

A shuttle vector is a plasmid constructed to have origins of replication for two hosts (for example, *E. coli* and *S. cerevisiae)* so that it can be used to carry a foreign sequence in either prokaryotes or eukaryotes.

Sialoglycoconjugates

Definition

Sialoglycoconjugates are sugar chains terminally modified by an acidic sugar (sialic acid).

Sidestream Smoke

Definition

Sidestream smoke is the material released into the air from the burning tip of the cigarette plus the material which diffuses through the paper; → tobacco carcinogenesis.

Signal Sequence

Definition

A signal sequence is a protein region with which a protein can be directed to the appropriate cellular compartment within a cell, they initiate co-translational transfer through the membrane of the endoplasmic reticulum (ER). Proteins are often synthesized in an immature version (pre-protein) that is larger than the mature functional form. This is due to the presence of N-terminal amino acid stretches, referred to as leader sequences. The pre-protein is a transient precursor, since the leader sequence is cleaved off during protein processing. This signal sequence is a short stretch of 15-30 amino acids that mediates the transfer of any attached polypeptide to the endoplasmic reticulum. it provides the means for the ribosomes to attach to the ER membrane (ER regions with associated ribosomes are called 'rough ER'. As soon as the first few amino acids of the protein have been synthesized, the nascent protein chain can be co-translationally transferred to the membrane.

The signal hypothesis proposes that the N-terminus of a secreted protein has a signal sequence whose presence marks it for membrane

insertion. Once the protein chain is well inserted into the membrane, the signal sequence is cleaved off by a protease within the membrane and the protein can then enter or even pass through the membrane. This principle does not hold for nuclear proteins, which are synthesized in their mature form.

Signal-transducer Proteins

Definition

Proteins function as units in signalling pathways are described as signal-transducers. They have at least two 'working parts', one involved in recognition of an input signal (receptor part) and the other in generation of an output signal (generator) recognized by the downstream components. Some signal transducer molecules can respond to additional input signals that modulate their function (modulator) or could, after a short period, terminate generation of the output signal (timer).

Signal Transducers and Activators of Transcription in Oncogenesis

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Synonyms

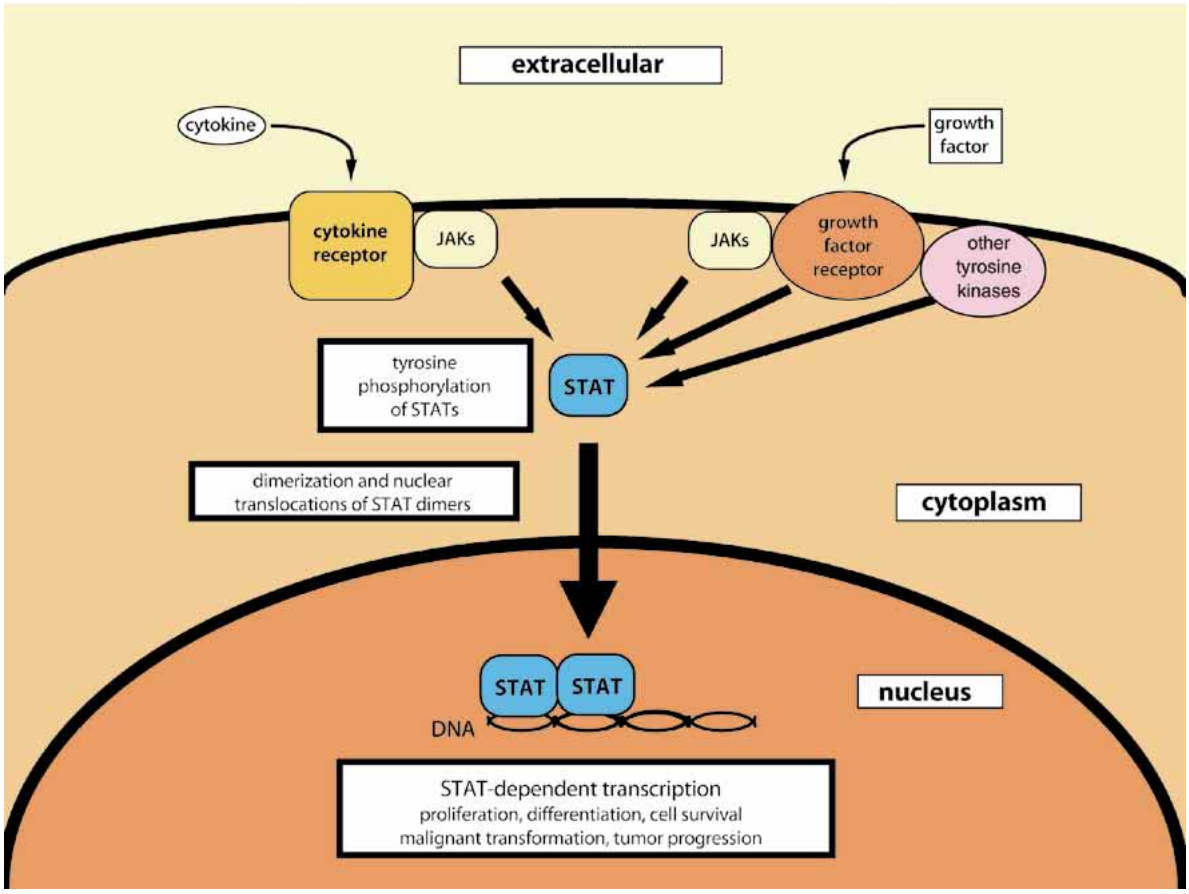
- STAT (signal transducers and activators of transcription)

Definition

Signal transducers and activators of transcription (STATs) comprise a family of latent → transcription factors that reside in the cytoplasm and have been shown to control normal → cytokine and → growth factor-induced responses. In response to extracellular signals, such as cytokine or growth factor receptor activation, STATs are phosphorylated, dimerize and translocate [→ translocation] into the nucleus where they regulate the transcription of specific cellular genes. Thus, STATs perform dual roles in → ligand-induced signaling, direct transmission of receptor-generated signals from the cytoplasm to the nucleus and direct regulation of cellular genes necessary for ligand-induced biological responses.

Characteristics

All multicellular organisms possess complex networks of chemical messengers that coordinate vital organ functions. Among the most common mechanisms used by multicellular organisms to ensure appropriate timing and duration of essential biological processes are production and secretion of cytokines and growth factors. Cytokines and growth factors control a wide variety of biological processes in diverse cell types, including immune responses, cellular differentiation, proliferation and → programmed cell death. Although differences exist between the biological processes and cell types regulated by cytokines and growth factors, these ligands possess some overlapping functions and share remarkably similar mechanisms of signal transmission. Cytokines and growth factors generally elicit a biological response by binding to receptor proteins located on the outer cell surface. Binding of these ligands to their specific receptors induces a change in the ability of the receptor to recruit and activate cytoplasmic signaling molecules that participate in signal transmission. Modulation of the activity of these cytoplasmic signaling proteins by the receptors initiates a cascade of biochemical signaling events, which ultimately lead to changes in nuclear gene expression that mediate the biological response. Cytokine and growth factor-induced processes are normally tightly controlled to ensure proper functioning. Aberrant functioning of these pathways results in unregulated signaling and is associated with development of a variety of pathological conditions including cancer.



Signal Transducers and Activators of Transcription in Oncogenesis. Fig. – Model for STAT function in oncogenic signaling. Schematic representation of oncogenic signaling pathways involving STAT proteins. Activation of cytokine signaling pathways, due to aberrant production of cytokines or activation of receptor-associated JAKs, leads to constitutive tyrosine phosphorylation of STATs, nuclear translocation of STAT dimers and increased transcription of growth regulatory genes, involved in oncogenic growth. Similarly, activation of growth factor signaling pathways, due to aberrant production of growth factors, overexpression of growth factor receptors or activation of receptor-associated tyrosine kinases such as Src and/or JAKs, leads to constitutive activation of STATs and oncogenesis. These individual pathways are not mutually exclusive and may cooperate to fully transform a variety of cell types.

STATs in cytokine and growth factor induced signaling

Recently, a family of proteins termed 'signal transducers and activators of transcription' (STATs) has been shown to participate in normal cytokine and growth factor signaling in a variety of cell types. In response to cytokine or growth factor-induced activation of signaling, cytoplasmic STATs become phosphorylated on specific → tyrosine residues by receptors or receptor-associated proteins possessing tyrosine → kinase activity. Tyrosine phosphorylation of one STAT protein induces its interaction with another STAT protein through → SH2-mediated coupling. The phosphorylated STATs assemble into dimers that rapidly translocate into the nucleus. Once in the nucleus, STAT dimers bind to DNA and control the transcription of specific cellular genes. Under normal circumstances, the biological responses elicited by cytokines and growth factors are transient, and transmission of signal is terminated through activation of a variety of tyrosine → phosphatases that dephosphorylate STATs and inactivate STAT-dependent gene transcription.

While tyrosine phosphorylation of STAT proteins has been shown to be essential for cytokine-induced signals, activated cytokine receptors generally do not possess kinase activity and are not capable of directly phosphorylating STAT proteins on tyrosine. Cytokines induce activation of STATs indirectly through activation of receptor-associated tyrosine kinases of the → Janus kinase (JAK) family. After cytokine binding, STATs are phophorylated by the receptor-associated JAKs, allowing signal transmission to proceed. Activation of STAT proteins has also been shown to be required for growth factor-induced cellular responses. However, the majority of growth factors that induce STAT activation bind to receptors that possess intrinsic tyrosine kinase activity, and tyrosine phosphorylation of STATs can be directly mediated by the activated receptor protein, receptor-associated JAK proteins or by additional receptor-associated tyrosine kinases, such as Src and Abl.

Bioactivity

Significantly, constitutive tyrosine phosphorylation and activation of STATs has been detected in cells that have undergone malignant transformation in response to expression of a variety of viral oncoproteins, which are often themselves constitutively activated tyrosine kinases. Consistent with a role for STATs in oncogenesis, regulation of gene expression by one STAT family member, Stat3, has been shown to be required for induction of malignant transformation by the oncogenic Src tyrosine kinase. Furthermore, expression of a constitutively activated mutant of Stat3 can induce malignant transformation of specific cell types. These results demonstrate that, in addition to signals important to normal cellular functions, activated STATs can also transmit signals critical to oncogenic transformation.

STAT activation during progression of human cancer

Constitutive tyrosine phosphorylation and activation of STATs has been shown to occur frequently in a variety of human tumor types including leukemias, lymphomas, multiple myeloma, head and neck cancer, lung cancer, prostate cancer, renal cell carcinoma, colon carcinoma and breast cancer. Frequent activation of specific tyrosine kinase signaling pathways, including activation of growth factor receptor tyrosine kinases, has also been detected in many of these tumor types. These findings suggest that constitutive activation of STAT proteins may result from the constitutive activation of these tyrosine kinases. Moreover, because STAT proteins control the transcription of nuclear genes involved in growth control, constitutive activation of STATs may transmit signals essential for oncogenic signaling and contribute to → tumor progression. Among the genes that are regulated by STATs are genes involved in controlling cell cycle progression and programmed cell death, including *cyclin D1*, *MYC* and *BCL-x*. Thus, constitutive activation of STATs in human tumors may contribute to progression of cancer by stimulating cellular proliferation and/or preventing apoptosis.

Summary

Because STATs play essential roles in regulation of cellular growth and death, the frequent activation of these proteins in human tumors suggests that they may play essential roles in the progression of human cancers. In addition, detection of constitutively activated STATs could provide an important marker for activation of oncogenic tyrosine kinase signaling pathways during tumor progression. Currently, characterization of the full complement of STAT-regulated genes that participate in growth regulation and transformation remains a very active and important area of investigation. In the near future, these studies have the potential to provide new information critical to understanding how the fundamental processes of cell proliferation and cell death are achieved. Finally, activated STATs in human tumors provide promising targets for the design of novel therapeutics that block STAT functions involved in stimulation of proliferation, prevention of cell death, and induction of oncogenic transformation and cancer progression. Early results in this area suggest that tar-

geted inactivation of STAT proteins may be an effective approach to halting the growth of various types of human tumor cells.

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Signal Transduction

Definition

Signal transduction is the biochemical pathway by which external stimuli are received at the cell surface, transmitted through the cytoplasm and then enter the nucleus to alter patterns of gene expression. It is the transmission of a chemical message from a specific receptor on the plasma membrane to the nucleus through a chain of intermediate molecules (e.g. → PDGF; STAT [→ signal transducers and activators of transcription in oncogenesis]).

Signal Transduction Cross-talk

Definition

Signal transduction cross-talk is the mechanism by which activated signaling molecules in a primary signal transduction pathway can regulate signaling molecules in another primary signal transduction pathway.

Simian Virus 40

Definition

→ SV40.

Single Letter Code

Definition

→ Amino acid single letter code.

Single Nucleotide Polymorphism

Definition

SNP is the most common type of genetic variation, consisting of a single base change within a DNA molecule.

Single-strand Annealing

Definition

Single-strand annealing (SSA) is a subpathway of → DSB-repair by homologous recombination, requiring only limited sequence homology of the ends joined and without the formation of recombination junctions. There is probably some overlap between SSA, → NHEJ and DSB-repair by homologous recombination.

SIOP

Definition

The Societe Internationale Oncologie Pediatrique (International Society of Paediatric Oncology) is an international organisation of clinicians involved in the treatment of childhood cancer; they have co-ordinated clinical trials

for the treatment of Wilms tumour since the 1970s. They also have data on several thousand children treated for Wilms tumour.

Sister Chromatid Exchange

Definition

Sister chromatid exchange (SCE) is the mitotic recombination between the 2 newly replicated sister chromatids, leading to exchange of genetic information.

Sister Chromatids

Definition

Sister chromatids are the copies of a chromosome that are produced by its replication.

SKI

Definition

Ski was first identified as a viral oncogene from the avian Sloan-Kettering → retrovirus, which transforms chicken embryo fibroblasts. Elevated levels of c-ski has been detected in several human tumor cell lines derived from neuroblastoma, melanoma and prostate cancer. Ski appears to bind to DNA and be part of the → histone deacetylase complex.

SLMVs

Definition

Synaptic-like microvesicles; → synaptic vesicle-like microvesicles.

Smad Proteins in TGFβ Signalling

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Definition

The Smad proteins are a family of structurally related molecules which perform a pivotal function in the transforming growth factor-β (→ TGF-β) superfamily intracellular cascade. This cytokine superfamily includes TGFβ, activins and bone morphogenetic proteins (BMP) and regulate a broad scale of biological responses, including cell fate and extracellular matrix production. TGFβ superfamily members signal through heteromeric complexes of transmembrane type I and type II serine/threonine kinase receptors. Upon ligand binding, type II receptor phosphorylates type I receptor, thus activating its kinase. The activated type I receptor then propagates signals to downstream targets such as the Smad proteins. Smads (for Sma and Mad proteins from *Caenorhabditis elegans* and *Drosophila*, respectively) are currently divided into three classes:

- The receptor-activated Smads (R-Smads) transiently interact and become phosphorylated by specific activated type I receptor. In mammals, Smad1, Smad5 and Smad8 are specifically involved in BMP signalling, and Smad2 and Smad3 are restricted to TGFβ/activin pathway.
- The common-mediator Smad4 proteins (Co-Smad) form heteromeric complexes with either BMP or TGFβ/activin pathway-restricted Smad. These complexes then translocate to the nucleus where they control expression of target genes.
- The inhibitory Smads (I-Smads), namely Smad6 and Smad7, prevent the activation of the R- and Co-Smads through competition with R-Smads for binding to the activated type I receptor. Another mechanism has also been proposed for Smad6; this pro-

Smad Proteins in TGFβ Signalling. Table 1 – Smad synonyms.

Smad protein	other names				*Xenopus*	*C. elegans*	*Drosophila*
Smad1	MADR1	Bsp-1	DWF-A	hMAD1	Xmad1		Mad
Smad2	MADR2	JV18-1		hMAD2	Xmad2		Sma 2
Smad3				hMAD3			Sma 3
Smad4		DPC4		hMAD4	Xmad4	Sma 4/DAF-3	Medea
Smad5			DWF-C				
Smad6							Dad
Smad7							
Smad8	MADH6						
Smad10	Smad4β						

tein can compete with Smad4 for interacting with receptor-activated Smad1, yielding an apparent inactive Smad1-Smad6 complex.

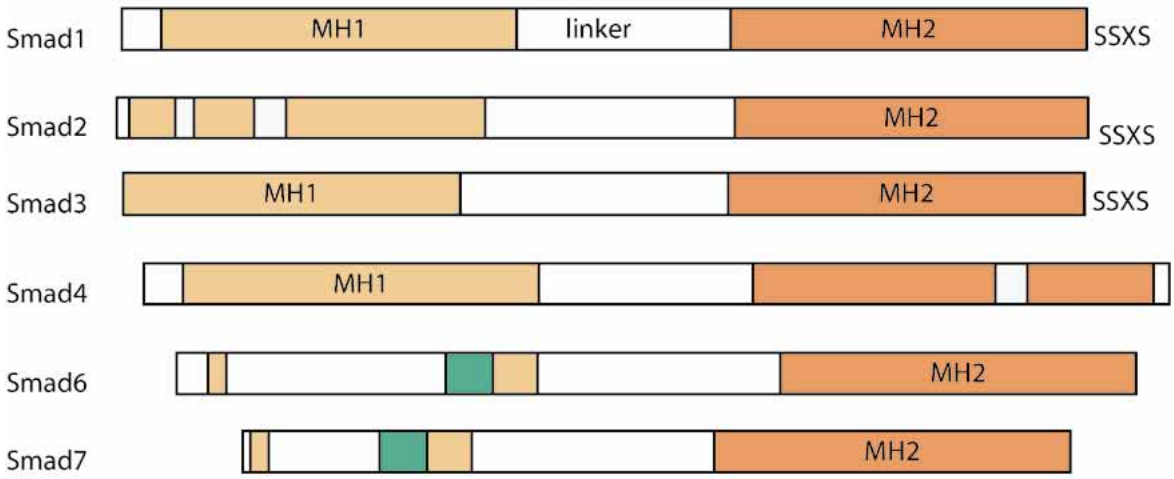
Characteristics

Smad proteins share two highly conserved domains named → Mad-Homology domain 1 and 2 (MH1 and MH2) on the N- and C-terminal part of the proteins, respectively. The crystal structure of both domains has been determined. The MH1 and MH2 domains are adjoined by a divergent proline-rich linker region.

Smad proteins do not appear to contain any intrinsic enzymatic activity but rather exert their function through protein-protein or protein-DNA interactions. The MH2 domain mediates the association with other Smads, interaction with activated type I receptors (for R- and I-Smads) and various transcription factors, for example forkhead activin signal transducer (FAST). Furthermore, the MH2 domain enables the interaction of Smad proteins with various transcription co-activators or co-repressors. The MH1 domain also mediates protein-protein interactions with transcription factors, for example c-Jun. Importantly, the MH1 domains of Smad3 and Smad4, but not of Smad2, are able to bind directly to a 5′ GTCT DNA sequence through a β hairpin motif. As Smads bind to DNA with rather low affinity and specificity, these proteins appear to need the interaction with other DNA binding partners to regulate TGFβ target gene expression. In the basal state, MH1 and MH2 domains mutually inhibit each other functions, probably because of a physical interaction. In R-Smads, cytokine-triggered C-terminal serine phosphorylation relieves this auto-inhibition.The non-conserved linker region contains several peptide motifs that participate in Smad activity regulation.

Cellular and Molecular Regulation

In the absence of cytokine stimulation, R- and Co-Smad monomers are mainly localized in the cytoplasm, whereas I-Smads are predominantly nuclear. Smad anchor for receptor activation (SARA), a protein anchored to membranes, presents the unphosphorylated R-Smads to the TGFβ-activated receptor complexes. This SARA/R-Smad interaction targets the Smad proteins to the plasma membrane and promotes the cytokine intracellular cascade. The type I receptor mediated R-Smad phosphorylation triggers homo- and heteromerization with Smad4 and induces a nuclear accumulation of these proteins. Thus the R-Smad localization prior to or after activation of the pathway is an important feature of TGFβ superfamily signalling. For example, activated → Ras induces the phosphorylation of R-Smads in their linker region through MAP kinase activation, thus preventing Smad translocation to the nucleus.



Smad Proteins in TGFβ Signalling. Fig. 1 – Structure of Smad proteins. Pathway-restricted Smads (R-Smads) are phosphorylated by the activated type I receptor on the two most C-terminal serine residues in the SSXS motif. The common-mediator Smad (Co-Smads) contains various small insertions in the MH1 and MH2 domains. The antagonistic Smads (I-Smads) lack most of the conserved MH1domain. Boxes in brown indicate regions that are highly conserved between Smad proteins. Green boxes are regions of similarity in I-Smads only.

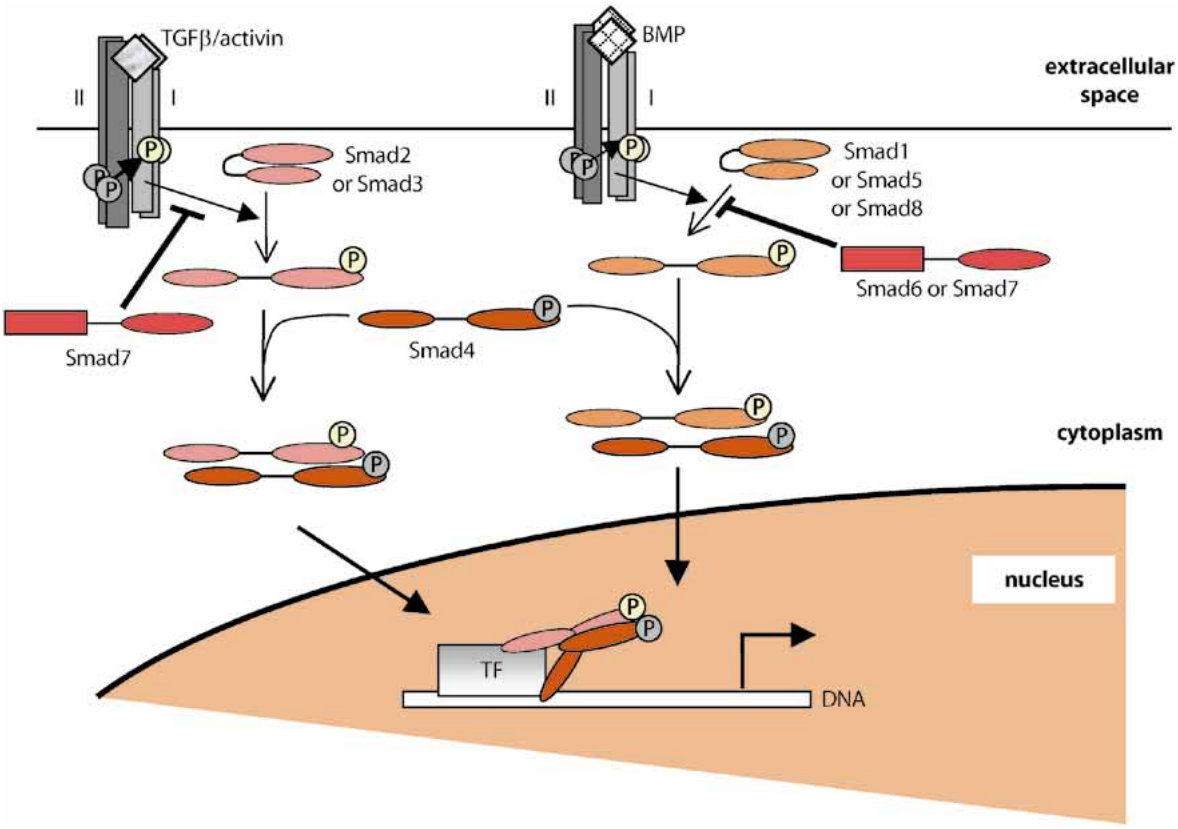
As a result oncogenic Ras inhibits TGFβ signalling. Interferon γ (IFNγ) also inhibits TGFβ signalling by abrogating R-Smads nuclear translocation. In this case IFNγ promotes the expression of Smad7, an inhibitory Smad that prevents TGFβ-restricted Smad activation.

Smad transcriptional activity is also regulated in the nucleus where Smad interacts with several proteins that promote or repress their activity. For example the oncogenic → Evi-1 protein interacts with Smad3 through its MH2 domain and abrogates Smad3 binding to DNA, thus reducing its transcriptional activity. Smads also recruit co-activators like → CBP/p300 which promote Smad transcriptional activity or co-repressors as the oncoprotein → Ski. These repressors recruit histone deacetylases to Smad complexes.

R-Smad are activated by phosphorylation, however, no phosphatase has yet been implicated in turning off TGFβ signalling. When R-Smad proteins enter the nucleus, they appear to activate their own degradation through ubiquitin-mediated proteolysis.

Smad Proteins in TGFβ Signalling. Table 2 – Smad gene characteristics.

Smad protein	human chromosome	number of exons	mutation in human cancer
Smad1	4q28-31		
Smad5	5q31	8	
Smad8	13q12-14		
Smad2	18q21.1	11	colorectal, lung
Smad3	15q21-22	9	
Smad4	18q21.1	11	pancreas, colorectal, lung, ovary
Smad6	15q21-22		
Smad7	18q21.1		



Smad Proteins in TGFβ Signalling. Fig. 2 – Schematic representation of the TGFβ/Smad pathway. Cytokine binding leads to the formation of a heteromeric receptor complex in which type II receptor phosphorylates and activates type I receptor. Pathway-restricted Smads are then phosphorylated by the type I receptor and form complexes with the common-mediator Smad4. These heteromeric complexes enter the nucleus where they participate, in combination with other transcription factors (TF), in the regulation of target genes. Inhibitory Smads bind to the activated type I receptor thus preventing R-Smads activation.

Clinical Relevance

The gene encoding Smad4 was originally cloned as a tumor suppressor gene and called deleted in pancreatic cancers 4 (→ DPC4). Smad4 appears to have a role in the late stages of a subgroup of colorectal cancers and many pancreatic cancers. In heterozygous mice carrying mutations of Smad4 and adenomatous polyposis coli (→ APC) genes on the same chromosome, loss of heterozygosity and reduplication of the gene carrying the mutations results in intestinal polyposis with more malignant phenotypes than the simple APC heterozygotes.

The Smad2 gene is located on the same chromosome region as Smad4 and is also frequently mutated or deleted in colon cancer. The inactivating mutations found in Smad2 and Smad4 mostly affect the MH2 domain in regions important for protein-protein interactions. However, the real role of these proteins in the cancer process has not yet been clearly defined. Mutations in other Smad proteins have so far not been found in human tumors. Nevertheless, one of the mouse Smad3-deficient strains develop metastatic colorectal cancer.

TGFβ is involved in several pathologies, however, the implication of Smad proteins in these disorders remain to be eluciated.

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SMRT Co-repressor

Definition

The POZ domain of → Bcl-6 is associated with the silencing mediator of retinoid and thyroid hormone receptor (SMRT), which was originally isolated as a corepressor of some nuclear receptors without a ligand. SMRT is a component of a larger multiprotein complex including mSin3A and histone deacetylase (HDAC). The recruitment of an HDAC-containing complex is a common transcriptional repression mechanism used by transcription factors belonging to various functional classes.

SMRT/N-CoR

Definition

Silencing-mediator-repressor-transcription (SMRT) and nuclear-hormone-co-repressor (N-CoR) are related co-repressors that link DNA binding proteins with → histone deacetylases.

SMT3C

Definition

→ UBL1.

SMT3H3

Definition

→ UBL1.

SNAP Receptors

Definition

SNAP receptors (→ SNAREs) are required for synaptic vesicle exocytosis. According to the SNARE hypothesis, synaptic vesicles dock to the target membrane through the interactions of N-ethylmaleimide-sensitive fusion (NSF) protein (an ATPase), NSF-attachment proteins, α/γSNAP (synaptosomal-associated proteins) and vesicular and target membrane proteins (v- and t-SNARE). In the case of synaptic vesicle exocytosis two synaptic vesicle proteins (synaptotagmin and synaptobrevin) represent the v-SNAREs, while syntaxin 1 and SNAP 25 are the t-SNAREs; → neuroendocrine tumors.

SNARE

Definition

SNAREs (soluble NSF attachment protein receptors, also known as → SNAP receptors), SM (sec/munc18 homologous) and Rab proteins belong to the framework of molecules that appear to be involved in all intracellular fusion reactions. SNAREs are classified into Q- and R-SNAREs because the central residue in the SNARE motif is either an arginine or a glutamine. These central arginine and glutamines form an ionic layer, in which three Q-SNAREs bind to one R-SNARE. The latter are associated with transport vesicles and shuttle between trafficking compartments. SNAREs were first discribed in yeast cells and neurons, but are now recognized to be widely distributed in cells. SNAREs are implicated in the regulated release of neurotransmitters, hormones, en-

zymes and cytokines. The SNAREs synaptobrevin1,2 (also known as VAMP1,2) syntaxin 1a, and SNAP-25 are predominantly localized in the synaptic vesicles and plasma membranes of neurons and neuroendocrine cells and are thus considered as neuronal SNAREs. They assemble in a stable ternary core complex, bind to each other exclusively via their SNARE motifs and are crucial for the specialized pathway of synaptic-vesicle exocytosis. The expression of a neuronal subset of SNAREs can be considered as a hallmark of a neural or neuroendocrine differentiation of normal or neoplastic tissues.

SNP

Definition

→ Single nucleotide polymorphism.

Somatic Cross-over Point Mapping

Definition

Somatic cross-over point mapping is a method exploiting the fact that certain genetically unstable cells, e.g. in → Bloom syndrome, show excessive DNA cross-overs within the BLM gene itself. This leads to restoration of BLM function in cells inheriting 2 different mutations in BLM; → genetic disorders associated with cancer predisposition.

Somatic Mutation

Definition

A somatic mutation occurs in somatic cells and therefore affects only its descendants and is not inherited.

Somatostatin

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Synonyms

- Somatotropin Release Inhibiting Factor (SRIF)

Definition

Somatostatin is a bioactive peptide that exists in the two isoforms SST-14 and SST-28, of 14 and 28 amino acids, (SST-14) and SST-28, respectively. It acts as a → neuropeptide, (neurotransmitter), is produced by neurons and endocrine-like cells and is distributed throughout the central and peripheral nervous system, endocrine pancreas, gut, thyroid, adrenals, submandibular glands, kidneys, prostate and placenta. Many tumor cells, immune cells and inflammatory cells produce somatostatin.

Characteristics

Somatostatin was initially isolated as an inhibitor of growth hormone (GH) release, but is now best described as a multifunctional peptide, capable of inhibiting secretory processes and cell proliferation. Hypothalamic somatostatin inhibits the release of pituitary growth hormone (GH), thyroid-stimulating hormone (TSH) and corticotropin releasing hormone (CRH). As a neurotransmitter it affects several functions such as autonomous, sensory, locomotive and cognitive. Locally produced somatostatin generally inhibits gut exocrine secretion (e.g. on insulin, glucagon, gastrin), suppresses stomach, small intestine and gall bladder motility, and mediates vasoconstriction, especially of splanchnic vessels. In the adrenals somatostatin inhibits angiotensin II stimulated aldosterone secretion and in the kidneys hypovolemia stimulated renin release. In the immune system it blocks the release of cytokines, including IFN-γ and IL-6, and limits the prolif-

eration of lymphocytes, intestinal mucosal and inflammatory precursor cells. Furthermore, it blocks the action of growth factors such as → IGF1, → EGF and → PDGF. The plasma half life of somatostatin is less than three minutes.

Somatostatin secretion and gene expression

The human somatostatin gene is encoded on chromosome 3q28 in a prehormone form (→ preprosomatostatin) with a mRNA of 351bp. The two bioactive forms SST-14 and the N-terminally extended SST-28 are produced by proteolytic cleavage of prosomatostatin. SST-14 is the peptid that is found predominantly. However, up to 30% of immunoreactive SST in the brain is SST-28. Somatostatin secretion is triggered by membrane depolarization, certain ions, nutrients and neurohormones/neuropeptides. Potent stimulators are glucagon, growth hormone releasing hormone (GHRH), neurotensin, corticotropin releasing hormone (CRH), calcitonin gene related peptide (CGRP) and bombesin. Somatostatin gene expression is stimulated by multiple cytokines and growth factors including IGF1 and 2, GH, IL-1, -6,-10, TNF-α, IFN-γ, NMDA receptor ligands and by steroid hormones such as testosterone, glucocorticoids and estradiol. Insulin, leptin, → TGF-β and some glucocorticoids inhibit somatostatin expression. Transcription of the somatostatin gene is regulated by the intracellular second messengers cAMP, cGMP, NO and Ca^{2+} and the associated pathways involving → CREB and → CBP (cyclic AMP response element binding protein and CREB binding protein).

Somatostatin receptors (sst)

Somatostatin binds to five currently known G-protein coupled seven transmembrane receptor subtypes ($sst_{1\text{-}5}$) that were initially classified according to their differential binding of somatostatin analogues. The genes for $sst_{1,3,4,5}$ lack introns, while sst_2 contains at least three potential transcriptional start sites, one of which is located in exon 1, 50 kb upstream of the start site in exon 3. All cloned receptor subtypes contain recognition motifs for glycosylation and phosphorylation. Homo- and heterodimerization of the receptors as well as receptor internalization have been described. Upon ligand binding ssts induce a multitude of intracellular effects mediated by varying G-proteins coupled to second messengers. All receptors block the formation of cAMP by inhibiting → adenylyl cyclase and activate tyrosine phosphatases.

Molecular basis of somatostatins antiproliferative effects

Somatostatin limits the proliferation of tumor cells *in vitro* and *in vivo* directly and indirectly. Direct regulation is by somatostatin receptors (ssts) that are localized on neoplastic cells; indirect regulation is exerted via ssts on non-neoplastic cells. Mechanistically, this is done by inhibiting the secretion of growth promoting hormones and growth factors (e.g. IGF-1), by promoting vasoconstriction (which leads to a reduced blood flow to tumor tissue), by inhibiting angiogenesis and by influencing the function of immune cells. The block of secretion is due to inhibition of the second messengers cAMP and Ca^{2+} and the inhibition of exocytosis in a G-protein dependent manner.

The direct antiproliferative effects of somatostatin appear to be largely due to the activation of protein phosphatases. Somatostatin induced protein tyrosine phosphatases (PTP) dephosphorylate tyrosine kinases of receptors for growth promoters such as insulin and possibly EGF and IGF-1. Furthermore, somatostatin inactivates MAPK activity *via* PTP dependent dephosphorylation (sst_2), PTP-dependent Raf-1 inactivation (sst_3) and inhibition of cGMP formation (sst_5). Activation of PTP is also involved in somatostatin induced apoptosis. While in CHO-K1 cells transfected with each individual sst, sst_3 appears to induce apoptosis by activation of *TP53*, independent of G_1 arrest, all other ssts prompt G_1 arrest and induction of *Rb*.

A base pair change in the sst_2 gene, found in a lung cancer cell line (COR-L103), lead some authors to the assumption of a tumor suppressor role for somatostatin receptors.

Somatostatin. Table – The somatostatin receptor family (sst) (modified after Patel). Characteristics of the five cloned human ssts and information about their distribution in human cancer. Methods that rely on tissue homogenates are unreliable since ssts are found in immune cells and veins surrounding the tumor tissue. Subtype selectivity of ligands is indicated by bold italics for IC50. Subtype expression has only been studied in a limited number of tumor types.

	sst_1	sst_{2A}	sst_3	sst_4	sst_5
chromosome	14q13	17q24	22q13.1	20p11.2	16p13.3
mRNA (kb)	4.8	8.5 (?)	5.0	4.0	4.0
amino acids	391	369	418	388	363
mol weight (kD)	53-72	71-95	65-85	45	52-66
ligand affinity (IC_{50} nM)					
SST-14	0.1-2.26	0.3-1.3	0.3-1.6	0.3-1.8	0.2-0.9
SST-28	0.1-2.2	0.2-4.1	0.3-6.1	0.3-7.9	0.05-0.4
Octreotide	290-1140	0.4-2.1	4.4-34.5	>1000	5.6-32
RC-160	>1000	5.4	31	45	0.7
Seglitide	>1000	0.1-1.5	27-36	127->1000	2-23
CH275	3.2-4.3	>1000	>1000	4.3-874	>1000
L-797,591	1.4	1,875	2240	170	3600
L-779,976	2760	0.05	729	310	4260
L-796,778	1255	>10,000	24	8650	1200
L-803,087	199	4720	1280	0.7	3880
L-817,818	3.3	52	64	82	0.4
signal transduction					
adenylyl cyclase	↓	↓	↓	↓	↓
tyrosine phosphatase	↑	↑	↑	↑	↑
MAP kinase	↑	↓	↑↓	↑	↓
Ca^{2+}-influx	↓	↓			
Na^+/H^+ exchange	↑				
phospholipase C activity		↑			↑↓
phospholipase A2 activity				↑	
tumor expression	• gastro-entero-pan-creatic tumors • medullary thyroid carcinoma • ovarian cancer • prostate cancer • pheo-chromo-cytoma	• gastro-entero-pan-creatic tumors • growth hormone (GH) and thyroid stimulating hormone (TSH) producing pituitary adenomas • breast carcinoma • neuro-blastoma • pheo-chromo-cytoma • medullo-blastoma • meningioma • small cell lung cancer (SCLC) • Hodgkin lymphoma • peritumoral vessels	• gastro-entero-pan-creatic tumors • medullary thyroid carcinoma • ovarian cancer	• meningioma lioma	• pituitary adenoma ***not in*** non-functioning pituitary adenoma

Clinical Relevance

Somatostatin analogue therapy

The demonstration of receptor subtype expression in malignancies has paralleled the creation of subtype selective receptor ligands. While the best characterized and oldest analogue, the octapeptide octreotide (plasma half life 2 hours), exhibits a preference for sst_2 with lower affinities for sst_5 and sst_3, highly specific non-peptide agonists for each of the five subtypes have been developed.

The longest clinical experience exists for the treatment of hormone secreting tumors with octreotide and its microencapsulated longacting release form (LAR). The classic indication for somatostatin analogue therapy is a growth hormone secreting pituitary adenoma in → acromegaly. Furthermore octreotide and other somatostatin analogues have been used in the treatment of carcinoids, insulinomas, gastrinomas, → VIPomas, glucagonomas and somatostatinomas, producing symptomatic or subjective responses in 30 – 75%, and significant reduction in tumor size in 10 – 15% of patients. In clinical studies octreotide and other analogues have been used as single agents or in combination with conventional cytostatic drugs with varying results in carcinomas of the breast, prostate, pancreas, colorectum, thyroid and lung, in meningiomas, neuroblastomas and Non-Hodgkin-lymphomas.

Somatostatin receptor imaging and radiotherapy

The *in vitro* detection of somatostatin receptors on a multitude of tumor tissues has led to the development of → somatostatin receptor scintigraphy (SRS). Apart from the already mentioned neuroendocrine tumors expressing ssts, SRS has successfully been used in the imaging of pheochromocytomas, non small cell lung cancers (NSCLC), meningiomas, → breast cancer, → gliomas, → medulloblastomas, Non-Hodgkin and Hodgkin lymphomas [Hodgkin disease [→ Hodgkin disease]], granulomatous disease, Sjögren syndrome and rheumatoid arthritis. Binding of radioactive somatostatin analogues has been used in radio-receptor-guided surgery as an asset in the surgery of neuroendocrine gastroenteropancreatic tumors and → neuroblastomas with occult metastases.

After ligand binding a fraction of receptors are internalized. This phenomenon has been used for clinical studies of *in situ* radiotherapy using ^{111}Indium or ^{90}Yttrium labeled somatostatin analogues in terminally ill patients with neuroendocrine tumors.

General clinical applications

In carcinoids and neuroblastomas the level of somatostatin or somatostatin receptor expression (e.g. by SRS) has been reported to correlate with tumor differentiation and therapeutic outcome of the disease.

As a therapeutic adjuvans octreotide has been used in the treatment of infectious and secretory diarrhea, L-asparaginase induced pancreatitis, symptomatic treatment of fistulas and in the management of severe pain due to neoplasia.

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Somatostatin Receptor Scintigraphy

Definition

Somatistatin receptor scintigraphy is the nuclear medicine based imaging of somatostatin receptor bearing tissues with radiolabeled analogues of somatostatin. Most commonly used is the radioligand 111In-pentetreotide with high affinity for the somatostatin receptor subtype 2 (sst2).

SOS

Definition

Sos is the mammalian homologue of the *Drosophila* son of sevenless (Sos) gene product; → Ras activation.

SOS Response

Definition

The SOS response in *E. coli* describes the coordinate induction of many enzymes, including repair activities, in response to irradiation or other damage to DNA. It results from activation of protease activity by RecA to cleave LexA repressor.

Southern Blotting

Definition

Southern blotting describes the procedure for transferring denatured DNA from an agarose gel to a nitrocullulose filter, where it can be hybridized with a complementary nucleic acid.

SP1

Definition

SP1 (often misspelled Sp-1) is a transcription factor that is ubiquitously expressed in mammalian cells. SP1 was discovered as a protein capable of binding specifically to the early promoter of the simian virus 40 (SV40) DNA. It binds to GC-box promoter elements and selectively activates transcription from genes that contain functional recognition sites. Sp1 is a nuclear protein of 696 aa and 72 kD; the gene maps to 12q13.

SP-A

Definition

Surfactant pulmonary-associated protein A1 (SP-A), also known as SFTPA1, SFTP1 or SP-A1, is a member of the collectin family. The human SFTPA1 gene locus maps at 10q22.2-q23.1 and the mouse sftpa1 gene locus at chromosome 14 (14.00 cM). SP-A plays an important role in innate immunity, is involved in host defense and inflammatory processes of the lung, and also involved in the surface tension lowering ability of pulmonary surfactant.

SPF

Definition

→ S-phase promoting factor (SPF); a S-phase promoting factor, cyclin-dependent kinase activity that is capable of inducing the initiation of replication.

S Phase

Definition

S phase is the restricted part of the eukaryotic cell cycle during which synthesis of DNA occurs.

S-phase Damage-sensing Checkpoints

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Definition

Cell-cycle checkpoints function by sensing DNA damage and transmitting a signal that results in arrest at defined stages of the cycle. The S-phase 'checkpoint' halts cells during the DNA synthetic period in response to DNA damage, and is somewhat different from other checkpoints in two ways: First, in the classic paradigm, cell cycle progression is halted at various discrete points in the cycle (e.g., in G1, at the → G2/M transition and during mitosis), and cells are usually arrested for many hours prior to reentry into the cycle. In contrast, the S-phase damage-sensing pathway arrests cell cycle progression for only an hour or so and seemingly at any position within the S-phase; thus, it is difficult to think of it as a checkpoint *per se*. However, as more is learned about the molecular basis of all of checkpoints, it is becoming clear that each may have multiple targets. Thus, the underlying strategies for mobilizing these vital damage-sensing pathways may turn out to have many similarities. A second possible difference is that the classic G1, G2/M, and mitotic checkpoints are thought to serve an anticipatory or surveillance function by arresting cells prior to critical processes such as DNA replication or mitosis, both of which are capable of converting potentially repairable damage into catastrophic lesions, such as double-strand breaks or chromosome non-disjunction. However, there is little evidence to suggest that the classic S-phase damage-sensing pathway has an anticipatory function, and it is formally possible that the arrest of DNA synthesis in response to damage results from a competition for trans-acting factors between multiple DNA-templated responses (repair, transcription, replication).

Characteristics

At what point(s) in S-phase does the S-phase checkpoint operate?

It has been known for decades that when exponentially growing cells are subjected to ionizing radiation, DNA synthesis in rapidly and significantly inhibited. Compelling evidence has been obtained that damage-induced inhibition of DNA synthesis represents a bona fide damage-sensing signal transduction pathway, as opposed to a simple arrest of replication forks by damage in the template (i.e., a cis-acting mechanism). For example, a dose of 5 Gy of γ-radiation results in a single strand break every 25 replicons on average, yet inhibits overall DNA synthesis by ~50%. Therefore, it has been presumed that the S-phase checkpoint is a global cellular response that functions in *trans* and inhibits initiation of DNA replication as opposed to chain elongation. There is direct experimental evidence from studies on mammalian viruses that doses of ionizing radiation too small to damage individual viral templates nevertheless down-regulate viral replication, suggesting a transregulatory mechanism. Results from three different experimental systems also provide direct evidence that the S-phase checkpoint downregulates DNA synthesis by inhibiting initiation. Alkaline sucrose gradient analysis of pulse-labeled DNA showed that ionizing radiation inhibits the appearance and maturation of the smallest nascent fragments to a much greater extent than the maturation of larger fragments. Secondly, DNA fiber autoradiographic analysis of the changes in the pattern of replication after irradiation sug-

gested that initiation is preferentially inhibited. Thirdly, a two-dimensional gel replicon mapping approach, which can distinguish between initiation and elongation of DNA synthesis, showed that ionizing irradiation preferentially downregulates initiation in a defined chromosomal replicon (the amplified, early-firing dihydrofolate reductase domain). To determine whether damage-induced inhibition of initiation also occurs at origins that fire later in the S period, advantage was taken of the naturally amplified ribosomal gene cluster, in which a restriction site polymorphism distinguishes between early- and late-firing rDNA replicons. The 2-D gel replicon mapping approach showed that both the early- and late-firing origins were inhibited by ionizing radiation. By extension, it is likely that all origins in mammalian chromosomes will be inhibited in response to DNA damage, regardless of when they are activated.

An interesting recent observation suggests that there may be more than one checkpoint or pathway responsible for the overall inhibition of the rate of DNA synthesis that occurs when ionizing radiation is delivered to an asynchronous culture of growing cells. When cells are synchronized and replicate cultures are irradiated at hourly intervals during S-phase, the subsequent inhibition of DNA synthesis never exceeds more than about 25% at any time in the S period. Whereas DNA damage delivered to an asynchronous population results in 50% or greater inhibition of the overall rate of DNA replication. By careful fluorescence-activated flow sorter analysis of γ-irradiated log cultures, it was possible to demonstrate the existence of a population of late G1 cells whose entry into S-phase was prevented for several hours. Since this potentially new checkpoint was uncovered in CHO (Chinese hamster ovary) cells, which are deficient in p53 activity, it is distinct from the well known p53-mediated G1 checkpoint. This pathway is therefore analogous to a G1/S checkpoint that has been described in *Saccharomyces cerevisiae*, which acts between the cell cycle steps effected by the DBF4 and CDC7 gene products.

It is possible that downregulation of entry into the S-phase in mammalian cells (i.e., at the G1/S transition) functions by inhibiting the earliest S-phase origins, and is therefore mechanistically related to damage-induced inhibition of later-firing origins (i.e., does not differ from the S-phase checkpoint *per se*). Since irradiation delays the entire S period for several hours (i.e., effectively repositions the S-phase along the cell cycle time axis), it would have to be argued that later-firing origins cannot fire until early-firing origins have done so. Interestingly, however, cells arrested near the G1/S boundary in either mimosine or aphidicolin (both effective inhibitors of chain elongation) are resistant to radiation-induced inhibition of DNA synthesis, lending weight to the argument that there is a unique damage sensing pathway that operates at the G/1S transition prior to origin firing. Since the events preceding initiation of DNA synthesis in mammalian cells are largely uncharacterized, it will be some time before the molecular nature of this potential checkpoint will be uncovered.

What proteins are involved in the S-phase damage-sensing checkpoint?

Cells derived from patients with → ataxia telangiectasia (AT) fail to inhibit DNA synthesis in response to irradiation. This phenomenon was originally termed radiation resistant DNA synthesis (RDS), but today is known as the S-phase checkpoint. DNA sequence analyses showed that ATM encodes a protein in the family of PI3-like kinases, since its C-terminal domain contains homology to the lipid phosphatidylinositol 3 kinase (PI3K). It is now clear that ATM functions as a protein kinase and several interesting *in vivo* substrates have been identified, including C-abl, p53 and p95/nbs. Although the mechanism by which ATM is activated by ionizing radiation and radiomimmetic drugs is not known, ATM is thought to function as a sensor of DNA damage as well as other forms of genotoxic stress. ATM somehow transduces the damage signal to elicit several different checkpoints, including the G1 checkpoint mediated by p53, the G2 checkpoint possibly mediated by → Chk2, as well as the S-phase checkpoint.

It was initially assumed that p21, a protein activated in the G1 checkpoint signal transduction cascade, is also an essential intermediary in the S-phase checkpoint, based on the observations that:

- induction of both p53 and p21 is delayed in AT cells;
- p21 interacts with proliferating cell nuclear antigen (→ PCNA) and inhibits its ability to activate the replicative DNA polymerase δ in an *in vitro* SV40-based replication system;
- overexpression of p21 inhibits S-phase progression *in vivo*, and the Cdk binding domain of p21 (which is essential for its activity in the p53-mediated G1 checkpoint) had a much stronger effect on retarding S-phase progression than the PCNA binding domain. However, it has been shown that both the S-phase checkpoint and the newly discovered G1/S checkpoint are p53-independent. Furthermore, the possibility that p21 is involved in the S-phase checkpoint independent of p53 induction was ruled out by showing that the S-phase checkpoint is intact in an HCT-116 cell line that lacks both copies of p21.

If the S-phase checkpoint is independent of the p53-p21 axis, what are the relevant proteins(s) that function downstream from ATM in this pathway? Recently, it has been reported that the product of the → Nijmegen breakage syndrome gene (p95/nbs) functions downstream from ATM and regulates S-phase progression following ionizing radiation. Many of the cellular and clinical features of this syndrome overlap with ataxia telangectasia, including radiation-resistant DNA synthesis. Furthermore, p95/nbs was recently cloned and found to be part of a protein complex that co-localizes to sites of DNA damage (i.e., radiation-induced foci) with hmre11/hRad50. Sequence analysis of p95/nbs revealed a forkhead associated domain (FHA) at the N-terminus and a breast cancer carboxyl-terminal domain (BRCT). Since both of these domains have been associated with DNA damage-responsive checkpoints, it is likely that p95/nbs functions in DNA repair. The finding that ATM phosphorylates p95/nbs on serine 343, and that mutation of this residue results in an S-phase checkpoint defect following ionizing radiation, argues strongly that ATM regulates S-phase progression following DNA damage through p95/nbs. However, the exact mechanism is presently unclear.

What are the target(s) of the S-phase checkpoint?

Precise regulation of the initiation of DNA synthesis is critical since it ensures that the genome is replicated once per cell cycle. Much of what we know about the regulation of initiation at origins comes from studies in yeast. Autonomously replicating sequence (ARS) elements were first identified in a functional assay by virtue of their ability to support plasmid replication. In *Saccharomyces cerevisiae*, it is now known that a step-wise assembly of proteins onto origins precedes origin firing. In yeast, a multi-protein complex (the origin recognition complex or ORC) has been isolated and shown to interact with ARS elements. ORC is comprised of six proteins and remains bound to yeast origins throughout the cell cycle. During M-phase, the cdc6 protein is recruited to ORC, which, in turn, recruits six mini-chromosome maintenance (MCM) proteins to form the pre-replicative complex (pre-RC) on origins. An S-phase cyclin is then thought to be necessary for the association of the cdc45p protein just prior to the onset of DNA synthesis. Origin activation in *Saccharomyces cerevisiae* is a two-step process regulated by the cdc28 kinase together with B type cyclins (Clbs). Clb/cdc28 is thought to trigger initiation as well as prevent re-replication by blocking the assembly of new pre-replication complexes.

Very little is known about origin firing in mammalian cells. However, mammalian homologues of many of these yeast proteins exist and it is likely that in both systems they will be targets for the S-phase checkpoint(s). Indeed, it has been shown that mutation in the *S. cerevisiae* orc2, a component of the origin recognition complex, abrogates inhibitory effects on initiation induced by adozelesin (a DNA-alkylating antitumor agent). In addition to specific

chromatin-associated proteins that could serve as targets for damage-sensing pathways, global chromatin structure may also be affected. Recent studies suggest that Gadd45, which is a downstream player in the p53-mediated pathway in mammals, effects chromatin remodeling of templates concurrent with DNA repair. Additionally, low doses of ionizing irradiation have been shown to lead to dephosphorylation of histone H1 in an ATM-dependent manner as well as phosphorylation of the histone 2A variant, H2AX. Thus, global damage-induced changes in chromatin, including post-translational modifications of histones as well as specific changes in origin-binding proteins and protein complexes, may prove to be important for the S-phase checkpoint.

What is the biological significance of the S-phase checkpoint?

Cell-cycle checkpoints by definition provide an adaptive cellular advantage following genotoxic stress. In the case of the S-phase checkpoint, radiation sensitivity has been uncoupled from checkpoint function, arguing that the S-phase checkpoint may not always function to enhance survival following DNA damage. However, these experiments were performed on AT or AT-like cells in which it is not possible to exclude other mutations that are epistatic to the checkpoint defect. Furthermore, ATM may directly influence DNA repair, in which case, checkpoint-related changes in radiation sensitivity may be obscured because of the underlying repair defect. Another possibility is that the S-phase checkpoint functions to maintain genomic stability. Irradiation produces single-strand DNA breaks, and replication through single-strand breaks has the potential to produce double-strand lesions that, if not correctly repaired, can lead to chromosomal rearrangements and carcinogenesis. An interesting further possibility is that the S-phase checkpoint functions in normal cell division to cope with endogenous oxidative or other genotoxic stresses.

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S-phase Promoting Factor

Definition

S-phase promoting factor (SPF) has cyclin-dependent kinase activity that is capable of inducing the initiation of replication.

Spheroplast

Definition

A spheroplast is a bacterial or yeast cell whose wall has been largely or entirely removed.

Spindle Pole Apparatus

Definition

The spindle apparatus is a highly organized structure that consists of kinetochore microtubules attached to segregating chromatids, and polar microtubules moving apart the spindle poles immediately prior to cell division. Independent changes during G_2/M culminate in the formation of the spindle pole apparatus (→ G_2/M transition). It consists of two opposed poles each arising from one of the two duplicated centrosomes. The bisecting line at which microtubules emanating from each pole of the spindle meet is known as the metaphase plate. Pairs of chromosomes align and attach themselves along the metaphase plate and are then pulled apart from one another by motors that push and pull the separated chromosomes towards opposite poles of the cell. Formation of a spindle pole apparatus requires a change in micro-

tubule dynamics. This is brought about by changes in the phosphorylation states of structural and motor microtubule associated proteins (MAPs); (not to be confused with mitogen activated protein kinase, →MAP kinase!). Cyclin-dependent kinase 1 (CDK1) is known to phosphorylate many MAPs.

Splicing

Definition

Splicing describes the removal of introns and joining of exons in RNA. Thus, introns are spliced out while exons are spliced together.

Sporadic Tumor

Definition

A sporadic tumor is a term frequently used as a synonym for non-hereditary tumors.

Squamous Cell Carcinoma

Definition

Squamous cell carcinoma is a solid tumor originating from an epithelial cell (keratinocyte).

SRC

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Synonyms

- c-Src
- v-Src
- $pp60^{c\text{-}Src}$
- $pp60^{v\text{-}Src}$

Definition

v-Src (or viral Src) is a 60 kD protein encoded by the oncogenic retrovirus, Rous sarcoma virus. The protein derives its name from its ability to induce sarcomas in experimental animals and malignantly transform cells in tissue culture. c-Src, or cellular Src, is the normal cellular progenitor of v-Src. c-Src is non- or weakly transforming when overexpressed in tissue culture cells. Both v- and c-Src are cytoplasmic tyrosine kinases that transfer phosphate from ATP to tyrosine residues within specific protein substrates. The resulting phosphotyrosine acts as a docking site for other molecules that transmit growth signals to the nucleus in a chain of events involving multiple phosphorylation and binding reactions. c-Src contains a carboxy-terminal region that maintains the molecule in a mostly inactive state. In v-Src, this 12 amino acid region is deleted, rendering the molecule constitutively active.

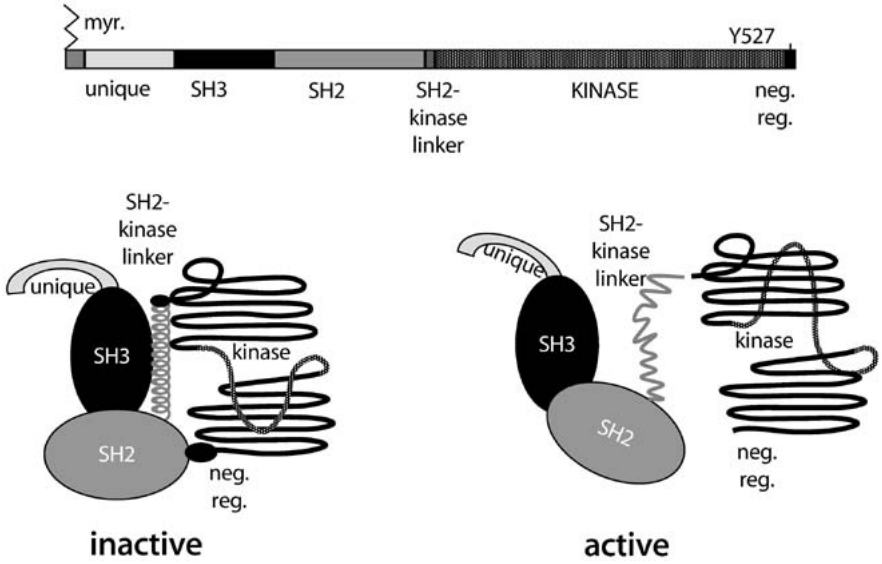
Characteristics

Domain structure

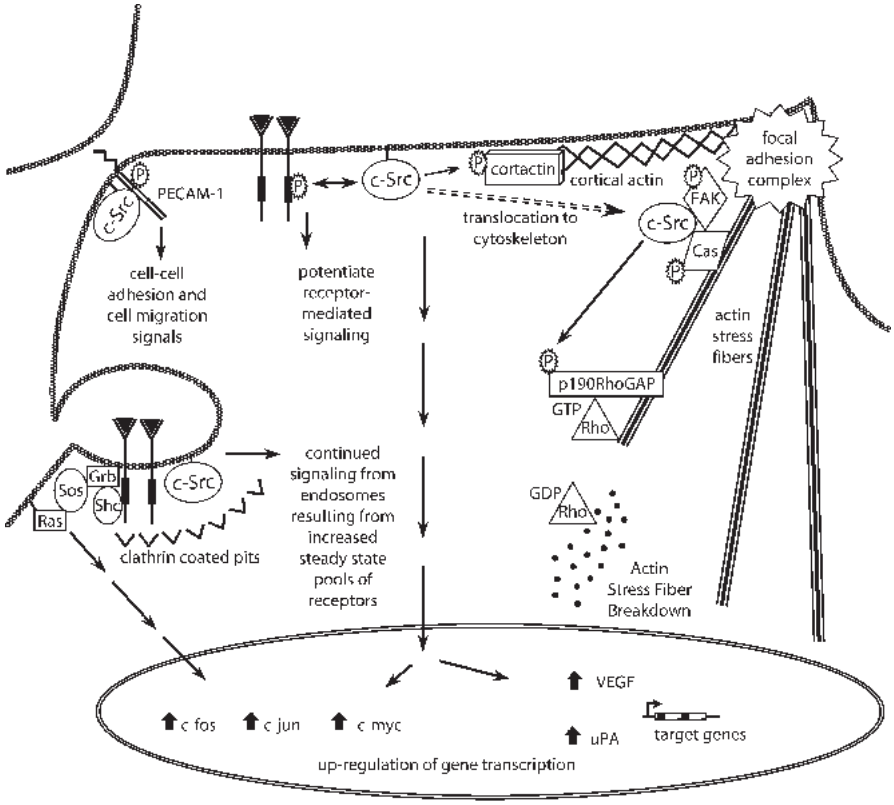
Each molecule of Src contains seven domains that are involved in targeting the protein to cellular membranes (the myristylation domain), in binding other proteins (the Unique, SH3 and SH2 domains) and in regulating the catalytic activity (Fig. 1). Src is one of a family of at least nine proteins that have a similar overall structure. Some of the family members are present only in certain cell types, such as cells of the hematopoetic lineage, while others are ubiquitously expressed. c-Src is one of the latter family members.

Subcellular localization

The myristate fatty acid modification on the amino terminus of Src targets it to intracellular membranes, including the plasma membrane and membranes of intracellular organelles, especially those of the endocytic pathway. c-Src has also been found to associate with centrosomes in interphase cells.



SRC. Fig. 1 – Structure of c-Src. As a linear molecule, c-Src consists of an N-terminal membrane association domain that contains the site of myrisylation, a Unique domain that exhibits the widest sequence divergence among family members, an SH3 domain, an SH2 domain, an SH2/kinase linker, the catalytic domain, and a negative regulatory domain that contains Tyr 527 (531 in human c-Src). How these domains relate to one another in a three-dimensional context to generate the inactive and active states of the enzyme is also shown and explained in the text.



SRC. Fig. 2 – Examples of c-Src targets and their potential roles in transformation.

Interacting proteins

Src forms complexes with a variety of intracellular signaling molecules via its unique SH2 and SH3 domains (Fig. 2). These proteins include, but are not limited to, polypeptide growth factor receptors such as the EGF receptor, intercellular adhesion molecules such as PECAM and E cadherin, gap junction proteins such as connexin 43, and several proteins found in focal adhesions such as focal adhesion kinase (FAK) and p130CAS. While most of these binding proteins are also substrates of Src, Src can phosphorylate proteins that do not form complexes stable enough to extract from the cell, such as the cortical actin binding protein, cortactin, p190RhoGAP (a GTPase activating protein for Rho family GTPases) and clathrin, a component of endocytic vesicles.

Cellular and molecular aspects

The extreme C-terminal domain of c-Src contains a tyrosine residue (Tyr 527 in chicken c-Src and Tyr 531 in human c-Src) that when phosphorylated binds its own SH2 domain in an intramolecular fashion (Fig. 1). This binding, together with the coupling of the SH3 domain to a polyproline sequence in the SH2 kinase linker, renders the protein inactive. In this regard, v-Src is constitutively activated by loss of the C-terminal phosphotyrosine 527 or 531. c-Src becomes activated when these intramolecular interactions are disrupted by competition with other signaling molecules that contain either phosphotyrosine or polyproline regions that bind the SH2 and SH3 domains, respectively, of c-Src. Such events occur when the c-Src SH2 domain binds phosphotyrosine 397 of FAK or phosphotyrosines of activated and tyrosine phosphorylated polypeptide growth factor receptors. c-Src activity has been reported to increase following integrin engagement of extracellular matrix (and subsequent activation of FAK) or upon stimulation of cells with growth factors, such as EGF, PDGF and FGF.

Clinical Relevance

c-Src is overexpressed or activated in multiple human tumors, particularly in glioblastomas and carcinomas of the breast, lung, colon, prostate, cervix, stomach and ovary. In breast cancers, the frequency of tumors overexpressing c-Src approaches 70%. Although analyses of other tumor types are not as extensive as those of breast cancers, existing data suggest the frequency of c-Src overexpression in lung and colon tumors may be similar to that in breast malignancies. Members of the epidermal growth factor (EGF) receptor family are also overexpressed in many of the same types of tumors that overexpress c-Src. Recent studies indicate that c-Src and EGFR synergistically promote tumor growth. This enhanced growth is accompanied by an EGF-induced association between c-Src and the EGFR, phosphorylation of EGFR by c-Src on several novel sites, and activation of signaling pathways that are required for EGF-induced mitogenesis. These findings are providing the impetus for discovering novel therapeutics that disrupt both the physical and functional interactions between c-Src and EGFR family members.

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Src Homology Domains

Definition

→ SH2/SH3 domains.

SRE

Definition

The serum response element (SRE) was first identified in the *c-fos* promoter to mediate serum induced *c-fos* expression in fibroblasts. The SRE core element was identified as the binding site for the serum response factor → SRF. The SRE also mediates gene regulation by peptide growth factors such as → EGF and → PDGF.

SRE-1

Definition

Sterol regulatory element 1 (sre 1) is a sequence (5′ATCACCCCAC-3′) found in the flanking region of different genes responsive to sterols. Sterol regulatory element binding transcription factor 1 (→ Srebp1) has dual sequence specificity, binding to sre-1 and the → E-box motif.

SREBP1

Definition

Sterol regulatory element binding transcription factor 1 (Srebp1), also known as Srebf1 os a → bHLH protein member of the Srebp/Add family of 1147 amino acids and 121 kD. The human SREBF1 or SREBP1 gene locus maps at 17p11.2 and the mouse srebf1 gene locus at chromosome 8 (33.00 cM). Srebp1 is a transcriptional activator that binds to the sterol regulatory element 1 (sre-1) (5′-ATCACCCCAC-3′) which is found in the flanking region of the Ldrl gene, as well as other genes. Add1/Srebp1 heterodimer has dual sequence specificity, binding to both an → E-box motif (ATCCGTGA) and to sre-1.

SREBP2

Definition

Sterol regulatory element binding protein-2 (Srebp2), also known as srebf2, is a → bHLH protein member of the Srebp/Add family of 1141 amino acids and 123 kD. The human SREBP2 or SREBF2 gene locus maps at 22q13 and the mouse srebf2 gene locus at chromosome 15 (44.80 cM). Srebp2 is a transcriptional activator that binds to the sterol regulatory element 1 (sre-1) (5′-ATCACCCCAC-3′) which is found in the flanking regions of the Ldrl and Hmg-CoA synthase genes.

SREBP/ADD Family

Definition

Srebp/Add family is a group of → bHLH poteins, comprising Srebp1, Srebp2, Add1, which are involved in cholesterol homeostasis, sterol synthesis and adipocyte determination. Under sterol-depleted conditions, Srebps are proteolytically cleaved to produce N-terminal fragments that enter the nucleus and activate transcription. Similar cleavage by the cysteine proteases, apopain and caspase-7, is induced during → apoptosis and is independent of sterol levels.

SRF

Definition

Serum response factor (SRF) forms a ternary complex together with a ternary complex factor (TCF) or Fli-1 and the serum response DNA element (SRE). SREs are found in many immediate-early-genes and mediate responses to a variety of extracellular stimuli.

SSA

Definition

→ Single-strand annealing.

Staging

Definition

Staging is a term frequently used to describe the extent of spread of a cancer; typically, stages include stage I, stage II, stage III, and stage IV. In general, stage I means the cancer is confined to the organ of origin (e.g., the breast) and is associated with a better prognosis, while a stage IV indicates that cancer has spread to distant places (metastasized) in the body and is associated with the worst prognosis.

STAT1

Definition

Signal transducer and activator of transcription 1 (STAT1). Two isoforms of the protein arise by alternative splicing of the same gene (α-isoform (p91) and the β-isoform (p84)); the gene maps to 2q32. The protein is a transcription factor that binds to the interferon-stimulated response element. It translocates into the nucleus in response to its phosphorylation that is triggered by interferon α and β. In the nucleus it assembles into a transcription complex; → signal transducers and activators of transcription.

STAT2

Definition

Signal transducer and activator of transcription 2 (STAT2), also known as STAT113, is a protein of 851 aa and 97 kD. Phosphorylated in response to interferon-α, it migrates into the nucleus and assembles into a protein complex where it acts as transcription factor; → signal transducers and activators of transcription.

STAT5

Definition

→ STAT5A.

STAT5A

Definition

Signal transducer and activator of transcription 5A (STAT5A), also known as STAT5 or MGF, is a protein of 794 aa and 90 kD which carries out dual functions of signal transduction and activation of transcription. It is translocated into the nucleus following phosphorylation; → signal transducers and activators of transcription.

STAT6

Definition

Signal transducer and activator of transcription 6 (STAT6), also known as IL-4-STAT, is a protein of 847 aa and 94 kD which is induced by interleukin-4 induced. It carries out dual functions in signal transduction and activation of transcription in interleukin-4 signalling and is translocated into the nucleus in response to phosphorylation. The gene locus maps to 12q13; → signal transducers and activators of transcription.

STAT91

Definition

→ STAT1.

STD

Definition

Sexually transmitted diseases (STD) include chlamydia trachomatis genital infection, lymphogranuloma venereum, gonorrhea, herpes simplex virus genital infection, trichomoniasis, condylomata acuminata (human papilloma virus infection), syphilis, chancroid soft chancer and HIV.

Stem Cell

Definition

A stem cell is a primordial cell that can differentiate into different cell lineages. It is any precursor cell that has the capacity for both replication and differentiation. Evidence suggests that most, if not all, tumors originate from stem cells.

Stem Cells and Cancer

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Definition

Morphological criteria do not exist to identify stem cells in most tissues and therefore, they are defined by their properties. Stemness is not a single property but a number of properties that a cell has the capability to perform, depending upon circumstances. In adult steady state renewing tissues, a stem cell is a relatively undifferentiated cell, capable of proliferation and self-maintenance, producing a variety of cell lineages and capable of tissue regeneration following injury. A stem cell retains the ability to switch between these options when appropriate.

Characteristics

General characteristics of stem cells

Many tissues in the adult undergo self renewal, and accordingly, establish a life-long population of relatively pliable stem cells - the adult stem cell. While examples of properties appropriate to embryogenic stem cells may be applied to adult stem cells, the latter is the focus of this chapter. The general characteristics of stem cells are shown in Table 1.

Tissue renewal and models of cellular hierarchy

The different capacities of tissues to proliferate is the basis for conventionally categorizing tissues in adulthood into:

- those that are constantly renewing (e.g. bone marrow, intestine);
- those that proliferate slowly but may renew their population in response to injury (e.g. lung, liver);
- those that are more static (e.g. nerve, muscle).

Tissues are renewed by cell division and differentiation from a small number of stem cells, which have a high capacity for cell proliferation, but their actual rate of cell division is usually slow in the absence of injury or demand. Between the stem cell and the mature cell of a particular tissue, a number of different stages of differentiation may be recognized, of which some retain a degree of stemness. Cells have been classified into three types on the basis of replicative potential as actual stem cells, potential stem cells or committed cells. Actual stem cells are defined as undifferentiated cells capable of:

- proliferation
- self-maintenance
- production of large numbers of differentiated progeny
- regeneration of the tissue after injury
- flexibility in the use of their options.

Stem Cells and Cancer. Table 1 – General characteristics of stem cells.
Table modified from Miller, Lavker and Sun. In: *Stem Cells*. Ed Potten, Academic Press Ltd, London 1997.
[a] This is controversial. Some investigators believe that stem cells are slow growing and hence chemoresistant. In practice, however, stem cells are chemosensitive and probably have a baseline turnover rate similar to that of the progenitor cells.

1	Stem cells exist at specific locations within a given tissue (*niches*) and are permanently resident at these locations.
2	Stem cells comprise a small percentage of total cell population.
3	Stem cells are ultrastructurally unspecialized, with a large nuclear-to-cytoplasmic ratio and few organelles.
4	Stem cells are pluripotent in terms of the differentiation option of their offspring.
5	Stem cells are slow-cycling,[a] but may be induced to proliferate more rapidly in response to certain stimuli.
6	Stem cells have a proliferative potential that exceeds an individual's lifetime.
7	Because stem cells cycle slowly, and represent only a small percentage of a cellular population, an intermediate group of more rapidly proliferating cells exist, that form clonal expansions resulting in the final, differentiated cell population.
8	A stem cell's micro-environment plays an important role in its homeostasis and in the differentiation of it progeny.
9	Most cancers arise from stem cells or early progenitor cells.

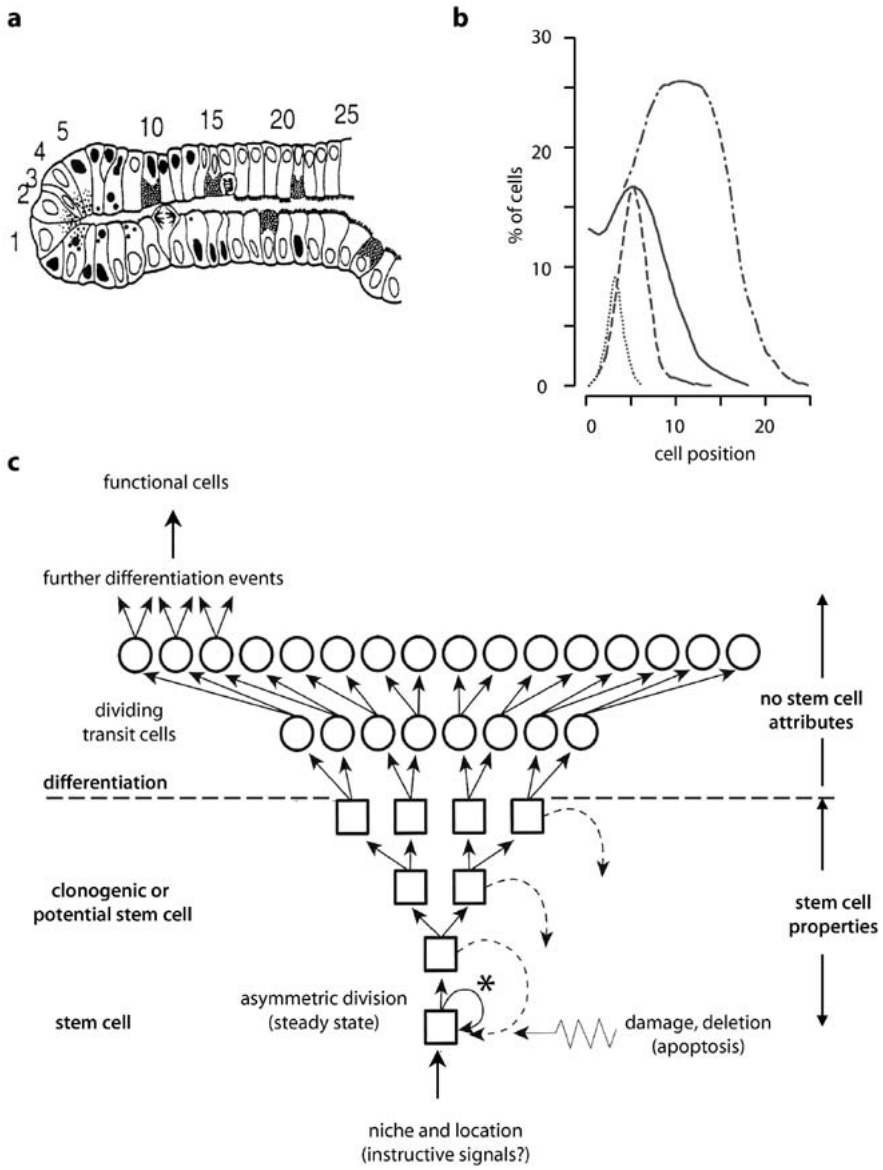
The potential stem cells are latent or reserve counterparts of actual stem cells, which may be reactivated to become functioning stem cells. The essential properties of stemness may be retained by some proliferating cells located distally in a lineage. This is referred to as the compartment model of cellular hierarchy and is illustrated for the intestinal crypt in Fig.

Stem cell division

Seeking to understand the control of cell division in the first few generations of the stem cell daughters is an area of intense research. It has been proposed that a specific type of mitosis occurs in the functioning stem cells that is responsible for the self-maintenance of this cell type. In general, normal stem cell mitosis will proceed by asymmetrical division to produce one daughter stem cell and one daughter that continues to divide, mature and differentiate. However, mathematical modeling suggests that about five percent of the time a stem cell may undergo symmetrical division to produce either two stem cells or two maturing cells. In the former case, a stem cell is lost from its niche by differentiation, displacement or apoptosis to ensure constancy in numbers. Stem cell competition is one consequence of this variable outcome of stem cell division and is thought to occur in many systems. Thus, occasional (possibly random) symmetrical divisions by a single stem cell may gradually populate a system (e.g. an intestinal crypt) with its own stem cell daughter cells. Even in steady state conditions, a single stem cell may gradually replace the other stem cells in a system with its own progeny, ultimately producing a monoclonal population of cells.

Problems in measuring stem cells

Proving that such forms of specialized division occur and a stem cell hierarchy exists has been extremely difficult because there are no definitive stem cell markers. Various transgenic approaches and polymorphisms (e.g. expression of glucose-6-phospahte dehydrogenase, G6PD) have provided results that are consistent with stem cell competition, but the definitive experiments on stem cell characterization have proven elusive.



Stem Cells and Cancer. Fig. – **a)** Diagram of a longitudinal crypt (large intestine) section showing the cell positions. **b)** The apoptosis frequency plot (radiation-induced: solid line) can be compared with the theoretical distribution of actual stem cells (dotted line), clonogenic or potential stem cells (dashed line) based on mathematical modeling. All of these are centered around cell position four. The broken line represents the distribution of rapidly proliferating cells which are predominantly determined by the committed dividing transit cell (modified from Potten, 1998). **c)** The current model for a three-tiered hierarchical stem cell compartment is also illustrated. There are from 4 to 6 actual stem cells per crypt, but many more cells (potential stem cells) that are capable of stem cell function. When a stem cell undergoes a commitment to differentiation, it often first enters a transient state of rapid proliferation. Upon exhaustion of its proliferative potential, the transiently amplifying cell withdraws from the cell cycle, and executes its terminal differentiation.

* Approximately 5% are symmetrical divisions.

In attempting to measure stem cells, one may find oneself in a circular argument. In order to answer the question whether a cell is a stem cell we have to alter its circumstances, and in doing so inevitably lose the original cell. In addition, only a limited spectrum of responses may been seen. This situation has a marked analogy with Heisenberg's uncertainty principle in quantum physics - this states that the very act of measuring the properties of a certain body inevitably alters the characteristics of that body, hence giving rise to a degree of uncertainty in the evaluation of it properties.

Stem cell location

Adult stem cells are often localized to specific environments or niches. For a number of systems, these niches correspond to specific histologically identifiable locations, but these have not been determined for all tissues (Table 2). Within each niche, the stem cells are influenced by neighboring cells and extracellular matrix. For each tissue, the stem cells in specific niches are pluripotent for the cell population of that tissue. Thus, for example, five different cell types (Paneth cell, goblet cell, entero-endocrine cell, M cell, enterocyte) are derived from the intestinal stem cell. Some stem cells give rise to a particular phenotype, but there is heterogeneity in the cellular characteristics depending on the micro-environment. Thus, for example, the blood vessels present in renal carcinoma metastases to the brain may display the typical fenestrated morphology of renal vessels and not the continuous morphology of endothelial cells in invading brain vessels. Moreover, recent observations in humans have shown that transplanted bone-marrow cells (in sex-discordant transplant patients) may give rise to new hepatocytes in the livers of the recipients. These observations raise the possibility that human stem cells may be reprogrammed to express dormant areas of the genetic code and thereby regenerate physically distinct phenotypes.

What remains unclear is whether stem cells are intrinsically different from daughter cells or whether they are instructed to be different by their microenvironment. In other words, could any cell behave as a stem cell if given the appropriate niche and signals? This has implications for tumorigenesis and clinical situations such as tumour seeding and implantation. It is conceptually possible to manipulate any cell *in vitro* to behave like a stem cell, but this is an unlikely scenario *in vivo*. The very early mammalian embryo (less than 4 cells) - the ultimate stem cells - is strong evidence favoring the argument that stem cells are intrinsically different.

Regulation of stem cell numbers

The conditions at the stem cell position within the niche must be optimum for stem cell maintenance and homeostasis. These factors include a cocktail of growth factors, cytokines and extracellular matrix (Table 3). The exact mechanisms whereby these factors regulate stem cell numbers is not clearly understood. To-date, no factors have been identified that are specific to stem cells and not expressed in daughter progenitor cells. It is likely, however, that stem cells themselves may express specific combinations of growth factors and matrix macromolecules in response to the local extracellular environment.

In addition to these factors, regulation by → apoptosis is important. One could speculate that an extra stem cell might result from the occasional symmetrical cell division. For example, in the intestine this could have dramatic consequences for the crypt as each extra stem cell is capable of producing an entire lineage of up to 64-128 cells, which clearly does not occur since the size of the crypt is remarkably constant. To prevent such fluxes in the enterocyte population, these supernumerary stem cells therefore require deletion or apoptosis. → Bcl-2 (an anti-apoptotic gene) and $p21^{Waf1/CIP1}$ are important in maintaining this integrity, but at least in the intestine are independent of → p53.

The importance of apoptosis and stem cell regulation are further illustrated by comparisons between the small and large intestine. In both human and mouse normal healthy small intestinal crypts, a 'spontaneous apoptosis' incidence of 1 – 10% is observed. In the mouse, this is easily recognized and quantified, and

Stem Cells and Cancer. Table 2 – Adult stem cells, locations, and derived cells for different sites of origin.
[a] 'Intermediate' between epithelial and myoepithelial cells
[b] see Figure for description of cell positions in intestinal crypt.

Site of origin	Stem cell	Location	Derived cells
Skeletal muscle	Satellite cells	Between muscle fiber plasma membrane and basal lamina	Mononuclear myoblast Multinucleated muscle fiber cell
Nervous tissue	Neural cell precursors	Ependymal cells Subventicular zone	Mesectodermal derivations Melanocytes Neuroendocrine cells Glial cell, Schwann cell Neurons
Blood vessels	Not known	Not known	Endothelial cell Pericyte Smooth muscle cell
Breast	Intermediate stem cell[a]	Terminal end duct	Ductal cell Myoepithelial cell Alveolar cell
Stomach	Gastric stem cell	Neck of gastric glands (mid-crypt region)	Parietal cell Chief cell Entero-endocrine cell Gastrocyte
Small intestine	Intestinal stem cell	Base of crypt; above Paneth cells (cell position 4-5)[b]	Enterocyte Paneth cell Entero-endocrine cell Goblet cell M cells
Large intestine	Intestinal stem cell	Base of crypt: (cell position 1-2)[b]	Enterocyte Entero-endocrine cell Goblet cell
Liver	Oval cells	Terminal bile ductules (Canal of Hering)	Hepatocyte Biliary duct epithelium
Testis	Primordial germ cell (Epiblast cells)	Peripheral layer of testicular tubules	Gonocyte A1 spermatogonia A2 spermatogonia Undifferentiated spermatogonia
Blood	Bone marrow stem cell	Niche on bone surface	Erythrocyte Granulocyte Megakaryocyte Marcophage Osteoblast Lymphocyte Mast cell
Hair follicle	Hair follicle stem cell	The 'bulge' of the hair bulb and/or the matrix of the growing follicle	Inner root sheath cells Hair
Skin (non-hair)	Interfollicular epidermis stem cell	Tip of rete pegs within basal layer Center of epidermal proliferative unit	Keratinocyte Keratinocyte
Eye	Corneal stem cell	Basal layer of limbus	Corneal keratinocyte

Stem Cells and Cancer. Table 3 – Important growth factors/ cytokines and regulation of stem cells. EGF = epidermal growth factor; IGF = insulin-like growth factor; TGF = transforming growth factor; FGF = fibroblast growth factor; IL = interleukin; TNF = tumour necrosis factor; SCF = stem cell factor; LIF = leukemia inhibitory factor; GCF = glial cell factor; IL = interleukin; GM-CSF = granulocyte-macrophage colony stimulating factor; M-CSF = macrophage colony stimulating factor; G-CSF = granulocyte colony stimulating factor; MIP = macrophage inhibitory protein.

Site of origin	Stimulators	Repressors
Skeletal muscle	IGF-I, bFGF	TFGβ
Nervous tissue		GCF2
Breast	EGF, TGFα, IGF-I, IGF-II	IL-1, IL-6, TFGβ
Intestine	EGF, IGF-I, IGF-II, TGFα, bombesin, trefoil peptides	TGFβ1
Liver	IGF-I, TGFβ	
Testis	SCF, LIF, TNFα, bFGF	TGFβ1
Skin (non-hair)		fibronectin
Bone marrow	IL-3, GM-CSF, M-CSF, G-CSF, erythropoietin, thrombopoietin	MIP-1α, TGFβ

shows that maximum apoptosis is associated with the stem cell position (position 4-6). In contrast, in the large intestine, spontaneous apoptosis is rare and when it occurs it does not appear to have any particular association with the stem cell position. These observations, together with studies delineating the relationships of apoptosis-related genes with stem cells, have led to the conclusion that spontaneous apoptosis in the small intestine is part of the stem cell homeostatic process. This is particularly relevant to the interesting, but incompletely understood, clinical observation that carcinomas are very common in the large intestine but very rare in the small intestine in humans. It has been hypothesized that the apoptosis homeostasis process is less efficient in the large intestine, and as a consequence, stem cells may gradually drift upwards with time along the crypt producing more cells at risk of carcinogenesis.

Cells at the stem cell position in the small intestine of the mouse exhibit an exquisite sensitivity to DNA damage by activating apoptosis or a process of altruistic cell suicide. This effectively protects this tissue from genetic (carcinogenic) damage. This protective mechanism is compromised in the large intestine by the presence of the anti-apoptotic 'survival' gene Bcl-2, which allows damaged cells to survive and attempt repair processes. Low levels of damage may be inefficiently detected or misrepaired, thus providing another possible explanation for the increased cancer risk in the large bowel compared with the small intestine.

The stem cell concept applied to tumorigenesis

Tissues with cell-renewal capacity may undergo expansion either with (metaplasia) or without (hyperplasia) changes in the proportion of differentiated cells. The reversibility of the proliferative response in hyperplasia and metaplasia distinguishes such proliferation from neoplastic growth, in which the response is permanent and inherited by subsequent generations of cells. Tumor growth therefore is related to heritable changes in the control of cell proliferation and differentiation. Several features of the tissue of origin may remain, and the rate of cell proliferation may show only a small increase over the rate of cell loss. Tumors have therefore been described as 'caricatures' of normal tissue renewal.

Evidence that human tumors arise from stem cells

Monoclonal origin of human tumors. For the most part, human tumors are monoclonal, sug-

gesting that they arise from a single transformed cell. This concept is supported by evidence that a unique identifying feature (a clonal marker) can be found in all the cells of many types of tumors. Classical examples of clonal markers are immunoglobulins and their component heavy and light chains secreted by malignant plasma cells in multiple myeloma and some B-cell lymphomas. Another example is the expression of a single isoenzyme of G-6-PD (a X chromosome gene) in tumors, supporting the hypothesis of monoclonality.

Lack of de-differentiation. Given the evidence for monoclonal origin of most human tumors, there are two candidate target cells in the process of carcinogenesis, proliferating differentiated cells or stem cells. The acquisition of proliferative features in a differentiated cell necessitates the concept of de-differentiation, a cessation of migration and a resistence to the pressures exerted by moving neighboring cells. These scenarios are unlikely. Taking the intestinal crypt as an example, the proliferating cells or transit cells are short lived (5-7 days) and migrating, whereas the natural history of carcinoma development is many years. Stem cells are the only cells with such a life span. Transit cells could conceivably be transformed but would be lost with the upward drift of cells in the crypt - the so called 'escalator' effect.

Tissue-specific differentiation. Most human tumors contain cell types consistent with an origin from the stem cells of that tissue. Thus, for example, a cloned colorectal adenocarcinoma cell line subcutaneously xenografted in immunodeficient mice gives rise to a tumor consisting of mucous, absorptive and endocrine cells.

Changes in stem cell regulation mechanisms during tumorigenesis. In the small intestine there is no expression of the anti-apoptotic or cell survival gene *Bcl-2*. However, in the large intestine it is expressed at the base of the crypt, co-existent with the stem cell position. It has been shown in a number of studies that Bcl-2 expression is increased in adenomas (precursors of carcinomas) and in the base of crypts adjacent to adenocarcinomas, but is generally low in adenocarcinomas, while another survival gene *Bcl-w* is upregulated. This may indicate that altered expression of the *Bcl-2* gene initially confers an advantage upon the (stem) cell that is later superseded by more potent factors. Interestingly, persistently high levels of Bcl-2 in adenocarcinomas confers chemoresistence.

Caveats to the monoclonal origin theory of human tumors

Pathologists have long been puzzled by tumors such as the salivary pleomorphic adenoma and uterine carcinosacroma, which appear to have at least two distinct neoplastic phenotypes, epithelial and mesenchymal, in the same tumor. Attempts to explain the pathogenesis of these tumors has led to hypotheses of a bicellular origin. However, despite many experiments there is no clear evidence to support this theory.

It remains possible that single tumors may arise from several cells possessing a range of genetic mutations that confer differing growth advantages. Through a process of clonal evolution the cell type that is optimally adapted to proliferate under the prevailing conditions emerges to form the tumor. A seminal case study of a patient found to possess both an XO/XY mosaic and familial adenomatosis polyposis demonstrated that 76% of microadenomas, but not larger adenomas, expressed XO and XY cells and thus were polyclonal. Polyclonality of adenomas (79%) has also been shown in a chimeric Min/ ROSA mouse model. It is not known how these polyclonal tumors arise and what, if any, interaction occurs between the genetically distinct cell types during the early stages of tumor evolution.

Another interesting example is provided by a specific type of B-cell lymphoma termed MALToma (**m**ucosa **a**ssociated **l**ymphoid **t**issue). This is believed to arise from an antigen-driven (→ *Helicobacter pylori*) lymphoproliferation of B-cell populations and is frequently proceeded by a benign lymphoepithelial lesion (e.g. in the salivary glands). Recent studies, using polymerase chain reaction (PCR) and Southern blot techniques, have demonstrated the presence of monoclonal B cell populations in up to 90% of histologically benign lesions, often many years before the evidence of malig-

nant transformation. Furthermore, metachronous or synchronous histologically benign lesions may contain two or more distinct dominant clones, and multiple clones (oligoclonality) may be present in established lymphomas. As malignant lymphoma is conventionally defined by the demonstration of monoclonality (using immunoglobulin detection techniques), these observations suggest that the monoclonal origin theory of human tumors may be complex.

Clinical implications and future directions

Some of the benefits from our understanding of stem cell biology are already realized in certain clinical settings, for instance in stem cell (bone marrow) transplantation. In the future, it is hoped that stem cell research will lead us into an era where cancer therapies may be aided by newly synthesized genetically identical equivalents, negating the need for organ donation and prosthetic implants. Stem cells from several tissue types can currently be grown in culture, but contamination with other rapidly proliferating cell types is problematic. The development of markers to aid the identification and purification of stem cells remains a major scientific challenge.

One of the main area of potential therapeutic benefit could arise from the determination of mechanisms underlying ordered cellular growth and differentiation. Questions about stem cell regulation are at the very heart of the origin of human cancers. Once the activities of genes mutated in cancers are identified, this will facilitate the development of novel approaches to cancer therapy aimed at limiting the proliferative capacity of mutant clones either through gene therapy, the administration of antagonistic growth factors or by immunotherapy directed towards unique/overexpressed cellular markers.

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Steroid Sex Hormones

Definition

Steroid sex hormones are compounds with a characteristic basic structure, secreted chiefly by the ovary in the female (estradiol, progesterone) and the testis in the male (testosterone). Among other actions, they regulate reproductive function.

Sterol Regulatory Element Binding Transcription Factor 1

Definition

Srebp1

Sterol Regulatory Element Binding Transcription Factor 2

Definition

→ Srebp2.

STICKS

Definition

Substrates that interact with C-kinase; → protein kinase C (PKC) substrates. STICKS belong to a group of cytoskeletal proteins that are referred to as adducins and which facilitate interactions between spectrin and actin to form the subcortical membrane skeleton. The term STICKS is a collective term of the earlier identified proteins, MARCKS (myristoylated-alanine-rich-C-kinase-substrates) and MacMARCKS (MARCKS homologue in macrophages). The increased PKC activity in tumor cells directly influences cytoskeletal remodeling by phosphorylating regulatory proteins such as the adducins. Additional PKC interacting proteins include PICKs, which were identified by yeast two-hybrid screening [→ yeast two-hybrid assay] and RACKs (receptors for activated C-kinase), which are non-substrate proteins that bind catalytically to PKC thus targeting active PKC to the vicinity of appropriate substrate proteins.

STK

Definition

Stem cell-derived tyrosine kinase (STK) is a member of the hepatocyte growth factor (HGF) receptor family. The STK ligand, → macrophage-stimulating protein (MSP), is a serum protein activated by members of the coagulation cascade in response to tissue damage. The *RON* (Recepteur d'Origine Nantais) gene (synonyme: macrophage stimulating receptor, MST1R; c-met-related tyrosine kinase) is a human homolog of the murine Stk. The gene maps to 3p21. The protein (1400 aa, 152 kD) has a tyrosine-protein kinase activity and is closely related to → Met.

STK11/LKB1

Definition

STK11/LKB1 is a gene found on chromosome 19p13.3 that shares 82% homology to the serine-threonine protein kinase XEEK1 from *Xenopus*.

Streptolysin

Definition

Streptolysin is an agent that permeabilizes membranes to permit cellular uptake of large or charged molecules.

Stress Response

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Synonyms

- immediate early stress response
- damage response

Definition

Stress response is a process that occurs in response to an altered balance of endogenous homeostasis following altered external and internal stimuli.

Characteristics

A cellular stress response consists of cellular changes required to accommodate internal or external insult. It can be induced following exposure to DNA damage, inflammatory cytokines, growth factors and irradiation, as well as osmotic or heat shock. Exposure to anti-cancer drugs or deprivation of survival factors also results in the activation of a stress response. A

large set of cellular sensors, which include redox sensitive proteins, cell surface receptors, and proteins that recognize DNA damage regulate the activity of stress kinases and their respective substrates. Depending on the type and degree of stress, the combined activation of stress kinases and their corresponding substrates dictate the cellular fate (survival or death) in response to the stress administered. The activation of one or more stress activated kinase pathways allows the cell to determine whether DNA repair and growth arrest will prevail over the initiation of programmed cell death or cellular differentiation. The nature of the cellular stress response is therefore dependent on cell type, expression pattern of cell surface receptors and stress kinases in concordance with respective phosphatases and kinase inhibitors.

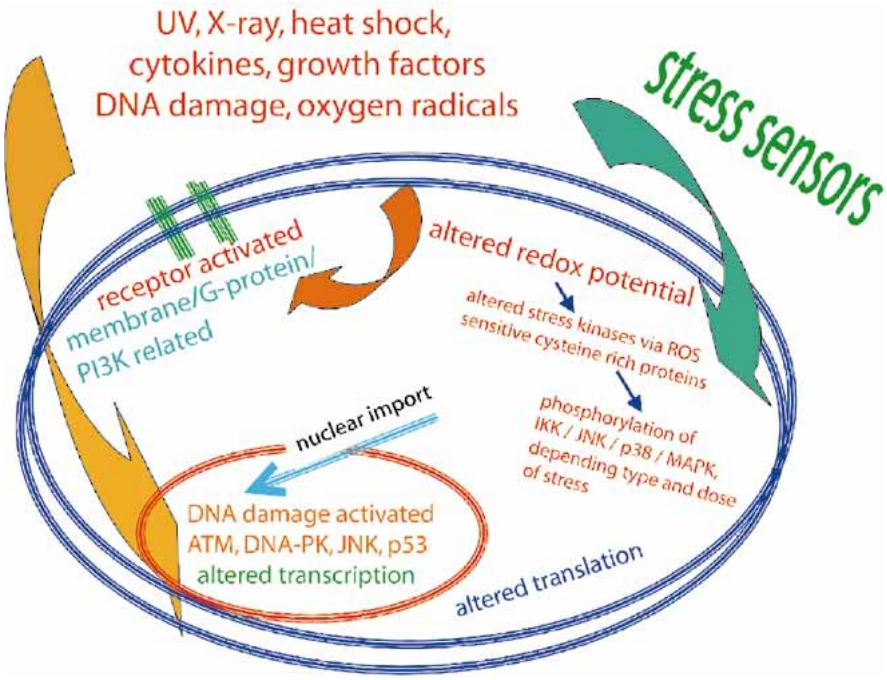
Cellular & Molecular Regulation

Recognition of cellular stress can be attributed to one of the following (as well as to any of their combinations):

- Altered organization of cell surface receptors, including changes in the oligomerization of EGFR, PDGFR and IGFR as well as altered localization and conformation of membrane anchored proteins including phosphoinositol 3 kinase (PI3K).
- Change in the redox potential within the cell, primarily due to an altered balance of reactive oxygen radicals (as a result of impaired activity of → detoxification and homeostasis maintaining enzymes among which are thioredoxin and glutathione S-transferase π which were shown to inhibit, under non-stressed growth conditions, the activities of ASK1 and JNK, respectively).
- DNA damage which results in the activation of DNA-PK, JNK, and other kinases that sense changes in the presence of DNA lesions (Fig. 1). Each change, or the combination of these changes, has been implicated in the activation of a selective subset of stress kinases and in the subsequent activation of their respective substrates.

Among key stress signaling pathways are the IKK, MAPK, JNK and p38 cascades. Interestingly, the upstream regulatory components that include TRAF2, MEKK1, ASK(1-2), TAK(1-2), MEK(1-4) often share regulation of downstream components of the stress kinase pathways (Fig. 2). The interplay between signaling pathways is controlled through organization *via* scaffold proteins, which facilitate contact between selective members of a given stress-signaling pathway. Requirements for scaffold proteins were shown for IKK, JNK and MAPK family members. The activation of stress kinases leads to the phosphorylation and/or stabilization of their substrates and to their transcriptional activities. Among the four major stress signaling pathways are:

- IKK, a family of kinases implicated in the regulation of IκB phosphorylation and subsequent ubiquitination and degradation. Consequently, NFκB is free to enter the nuclei to mediate its transcriptional activities. NFκB has been associated with the cellular ability to cope with stress through its positive effect on proteins that antagonize the apoptotic cascade, including IAPs, TNFα, Fas and Bcl2.
- MAPK, which is responsive to Ras and PI3K signaling, has been implicated in the cellular response to ROS and elicits the activation of p42/p44 ERKs.
- JNK and p38 which are activated by the upstream kinases MKK4/7 and MKK3/6, respectively. The latter are tightly regulated by ASK1 and MEKK1 signals. c-Jun/ATF2 are phosphorylated by JNK and p38, which represents divergent signaling cascades that result in transcriptional output that is often shared by the heterodimer ATF2/Jun. Other members of the p38 families are expected to play a role in the phosphorylation of substrates that are not modified by JNK and *vice versa*, thereby conferring selectivity for the stress response.
- ATM and DNA-PK represents enzymes that recognizes DNA damage and contributes to cellular stress response *via* phosphorylation of the tumor suppressor protein p53.



Stress Response. Fig. 1 – Outline of major stress sensors. Cell exposure to stress, as exemplified in the list of DNA damage and stress inducing treatments listed, affects one or more of the major cellular stress sensors. Those sensors include cell surface receptors, membrane anchored proteins and cysteine rich proteins which are sensitive to the formation of reactive oxygen radicals, and DNA lesions which are sensed by nuclear-residing protein kinases. The activation of one or multiple sensors depends on the type of stress, its dose and duration. In turn, respective changes in the activities of stress kinases (Fig. 2) will determine cell fate.

Activation of p53 is a good paradigm for stress response as this tumor suppressor stress inducible protein is phosphorylated by multiple stress kinases resulting in the dissociation of proteins that otherwise target its → ubiquitination and degradation. Phosphorylation of p53 depends on the nature and degree of damage and stress and is carried out by ATM, DNA-PK, p38, and JNK. Phosphorylation on multiple residues (including 15, 20, 37, 46, 81, 389) depending on the type and dose of damage results in p53 that is able to elicit growth arrest or a signal triggering → apoptosis.

Clinical Relevance

The interplay between stress-activated kinases appears to play a key role in the type of cellular response to stress. Changes in the expression, activation or in the duration of stress kinase output are expected to result in an altered cellular response to stress. Tumors were found to harbor such changes, which conferred greater resistance to radiation or chemotherapy, with the example of TRAF2/GCK in human melanoma. Mutations in stress kinases are rarely found, although cases reported an MKK4 mutation in certain human tumors. Changes in the activation of stress kinases were also reported in heart and neurological disorders.

Our greater understanding of mechanisms underlying the regulation of stress kinases and their substrates is expected to allow the design and use of reagents that would specifically target the selective kinase to alter its activities, which will determine the ability of the cell to cope better (or worse) with the type of stress and DNA damage.

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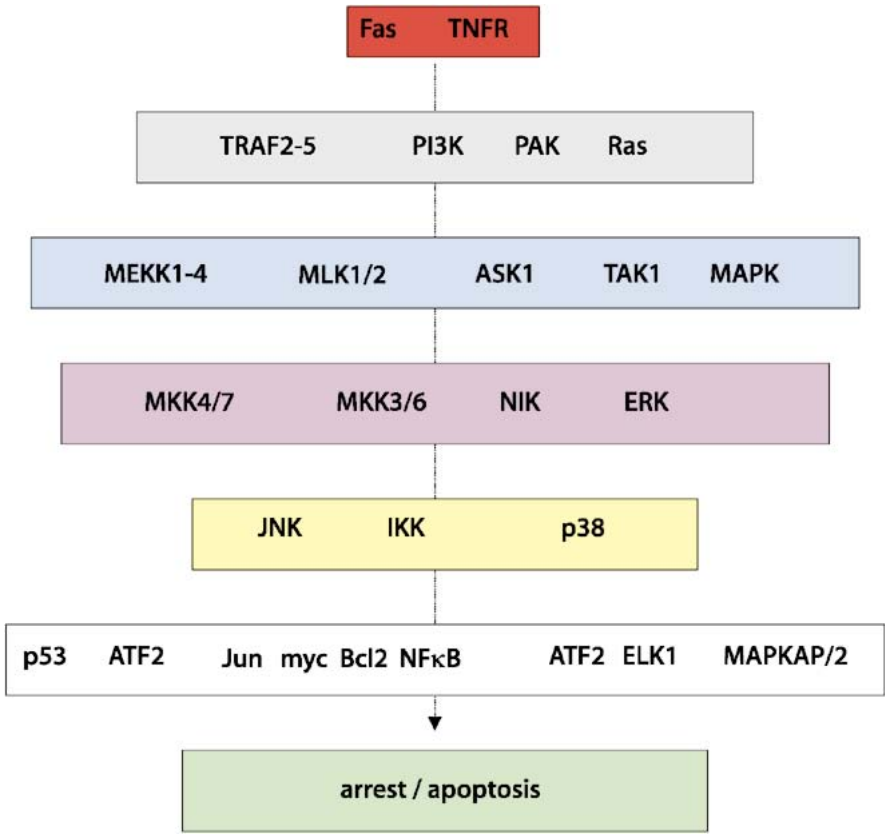

Stress Response. Fig. 2 – Stress kinases. Several stress signaling cascades are based on their affinity to upstream and downstream components. Among the major stress kinases are ERKs (extracellular signal regulated kinases), IKK (inhibitory κ kinase), JNK (Jun kinase) and p38. Each of these signaling cascades is activated at different kinetics and with diverse affinities in response to stress. Thus, various types of stress can activate different subsets of stress signaling cascades. Activation of the upstream stress components results in the phosphorylation of respective downstream targets. For example, TRAF2 (tumor receptor associated factor 2) can elicit the activation of NIK (nuclear factor κ B inducing kinase), MEKK1 (MAP/ERK kinase 1), ASK1 (apoptosis signal-regulating kinase 1), mitogen activated protein kinase (MAPK), which in turn phosphorylates IKK, MKK4/7 (mitogen activated kinase kinase 4/7), p38 or ERKs, respectively. Organization of these signaling cascades via 'scaffold' proteins maintains their close contact and the high affinity for related family members. These kinases will in turn phosphorylate their transcription factors substrates. Depending on the combination of activated transcription factors and apoptosis regulatory proteins, the cell can undergo growth arrest, allows proper damage repair or can undergo programmed cell death.

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Stroma

Definition

The stroma is the tissue that surrounds tumor cells consisting of connective tissue cells, blood vessels and extracellular matrix.

Subendothelial Matrix

Definition

Subendothelial matrix is a continuous thin layer of extracellular matrix (ECM) produced by vascular endothelial cells.

Suicide Gene Therapy

Definition

Suicide gene therapy is the transfer of a gene which, when expressed in cells, activates a normally non-toxic pro-drug to a toxic form; → HSV-TK/Ganciclovir mediated toxicity.

Sulphotransferase

Definition

Sulphotransferase is a family of enzymes that catalyze the sulphate conjugation of aliphatic alcohols and phenols using 3′-phosphoadenosine-5′-phosphosulphate as co-factor; → detoxification.

SULT

Definition

→ Sulphotransferase.

SUMO-1

Synonyms

- → UBL1

Definition

Small ubiquitin-related modifier-1.

Surrogate Tissue

Definition

Surrogate tissues or cells are used in biomonitoring because, in many exposure situations, target tissues or cells for carcinogenesis in humans are not available. Therefore, alternative sources of DNA and proteins are utilized, e.g. peripheral blood cells, bronchoalveolar lavage cells and DNA base adducts excreted in urine. In order to be used as valid biomarkers, the proportionality between DNA adduct levels in the target organ and those in the surrogate tissue or body fluid has to be examined individually for each carcinogen exposure.

Survivin

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Definition

As a structurally unique member of the → inhibitor of apoptosis protein (IAP) family, survivin is highly expressed in fetal tissues, but not in most adult tissues. Most human cancers return to the fetal pattern of survivin overexpression, therefore suggesting a pivotal role of survivin for tumor cell survival.

Characteristics

The human survivin gene is located on chromosome 17 (band q25) and encompasses 14.796 base pairs that comprise five exons. The coding strand is preceded by a TATA-less promoter and a GC-rich region corresponding to a → CpG island. The coding sequence of survivin is largely complementary to the coding strand of the → effector cell protease receptor-1 (EPR-1) gene. This suggests that survivin and EPR-1 transcripts originate from duplicated genes that were arranged in opposite orientations. The human survivin gene encodes three different splice variants, which may contribute to the fine-tuning of survivin actions (Fig. 1):

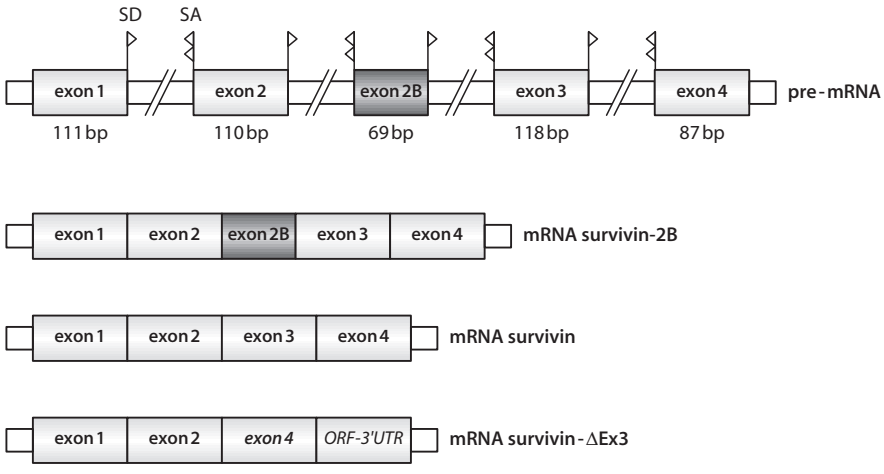
- survivin is the transcript identified first and consists of exon 1 (111 bp), exon 2 (110 bp), exon 3 (118 bp) and exon 4 (87 bp);
- survivin-2B is characterized by the insertion of an additional exon 2B (69 bp) between exon 2 und 3;
- survivin-Δ Ex3 shows a loss of exon 3 as well as a frame shift with extension of the open reading frame into the 3′ untranslated region.

The structure of the corresponding survivin proteins is unique when compared to other → IAPs. Thus, most mammalian IAPs contain a carboxy-terminal → RING finger domain, a → caspase recruitment domain (CARD) and, most importantly, two or three copies of a → baculovirus IAP repeat (BIR), a zinc finger domain essential for the inhibition of apoptosis by IAPs. In contrast, survivin proteins exhibit only a single BIR domain and lack both the RING finger and the CARD region. The splice variants survivin-2B and survivin-DEx3 exhibit pronounced structural alterations that also affect their single BIR domain (Fig. 2). To date, attention has been focussed on the functional properties of the survivin protein, whereas little is known about survivin-DEx3 and survivin-2B.

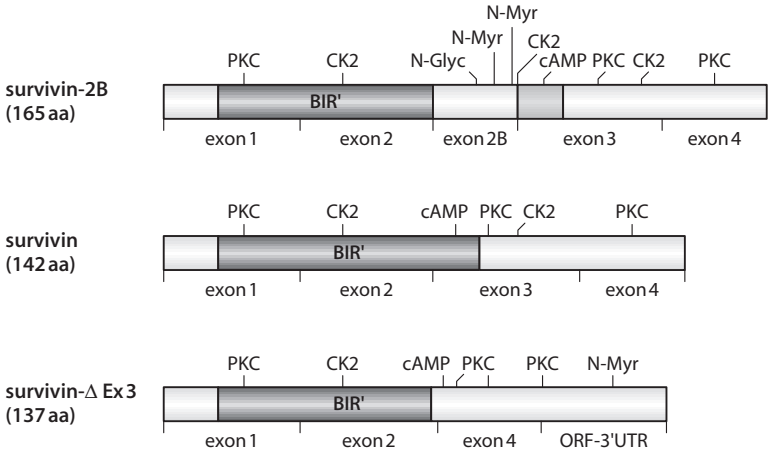
Transcription of survivin shows a marked cell cycle-dependent pattern with a pronounced up-regulation in the G2/M phase. This cell cycle periodicity has been related to the presence of two Sp1 sites in the proximal promoter region. They might interact with zinc finger transcription factors of the Sp1 family that are also implicated in the control of other cell cycle-related genes. Suppression of survivin transcription in the G1 phase may be further regulated by a cell cycle homology region (CHR) and by three cell cycle-dependent elements (CDE) of the promoter that are also known from other G2/M-expressed genes.

Functional and cellular characteristics

In accordance with other mammalian IAPs, survivin antagonizes a broad range of apoptotic



Survivin. Fig. 1 – Structural organization of the alternatively spliced transcripts of the survivin gene flags: splice donor (SD) and splice acceptor (SA) sites.



Survivin. Fig. 2 – Protein domains in the different survivin splice variants. Note the truncation of the BIR domain and the introduction of new potential sites for N-glycosylation and N-myristoylation in survivin-2B and -Δ Ex3. (PKC, proteinkinase C phosphorylation site; N-Myr, N-myristoylation site; N-Glyc, N-glycosylation site; CK 2, caseinkinase 2 phosphorylation site; cAMP, cyclic AMP dependent kinase phosphorylation site)

stimuli by inhibiting caspase-3 and -7. To date, survivin-dependent inhibition of caspases has only been observed in survivin-overexpressing transfectants. This, however, does not exclude other mechanisms of action in a more physiological cellular context. Survivin is abundantly expressed in fetal tissues as revealed by immunohisto-chemistry in lung alveolar epithelium, proximal tubule epithelium of the kidney, pancreatic islets, endometrial glands, intestinal crypt epithelium, thymic medulla and neurons of the spinal cord. In contrast to other mammalian IAPs, however, survivin expression is not detectable by Northern blot, *in situ* hybridisation and immunhistochemistry in normal adult tissues, with the exception of thymus and placenta. The restriction of survivin expression to fetal tissues suggests a key role of this IAP protein for the regulation of developmental apoptosis.

Survivin actions have been located to the microtubules of the mitotic spindle apparatus [→ spindle pole apparatus]. Survivin binding to the polymerized microtubules is mediated by a carboxy-terminal → coiled-coil domain. The increased levels of survivin expression during the G2/M phase of the cell cycle might protect the mitotic apparatus from proteolytic degradation by caspases. Therefore, survivin has been suggested to be an active component of the → G2/M checkpoint control that preserves chromosomal ploidy and genetic stability by induction of apoptosis in aberrant cells. The down-regulation of survivin expression in adult tissues may lower the threshold for apoptosis in replicating cells harbouring genetic defects.

Clinical Relevance

Many types of human cancer, such as carcinomas of the lung, stomach, colon, breast, prostate, skin as well as non-Hodgkin lymphomas, neuroblastomas and melanomas, return to the fetal pattern of survivin expression. Survivin re-expression may be an early step of malignant transformation, as evident from its presence in precancerous lesions such as colorectal adenomas and Bowen's disease of the skin. The exact molecular mechanisms, involved in the reactivation of the survivin gene in human cancers are currently unknown. Nevertheless, the overexpression of survivin in many histogenetically distinct tumor types indicates a strong selection advantage from survivin-related resistance to apoptosis. This selection advantage may result from the loss of an effective G2/M checkpoint control that permits progression of genetically unstable tumor cells through mi-

tosis. Moreover tumor cells may profit from the increased resistance to many different pro-apoptotic stimuli, including - inter alia - hypoxia and death signals from immunocompetent cells.

The clinical implications of survivin-related resistance to apoptosis are profound. First retrospective studies on gastric, colorectal and bladder carcinomas as well as neuroblastomas suggest that survivin may be a prognostic factor, helping to identify patients with an increased risk of rapidly progressive disease. Because survivin also confers increased resistance to certain anticancer drugs, e.g. paclitaxel and methotrexate, the level of survivin overexpression may be used as a predictive parameter for anticancer-drug sensitivity. Finally, the disruption of survivin-related antiapoptosis may become an attractive therapeutic target, selectively increasing the susceptibility of cancer cells to apoptosis-based treatment strategies without affecting the viability of non-neoplastic tissues that do not express survivin.

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Susceptibility Marker

Definition

A susceptibility marker is a measurable indicator of genetic or acquired host factors, existing before, and independent of, exposure that influences the disease outcome caused by external carcinogen exposure; → adducts to DNA.

SV40

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Synonyms

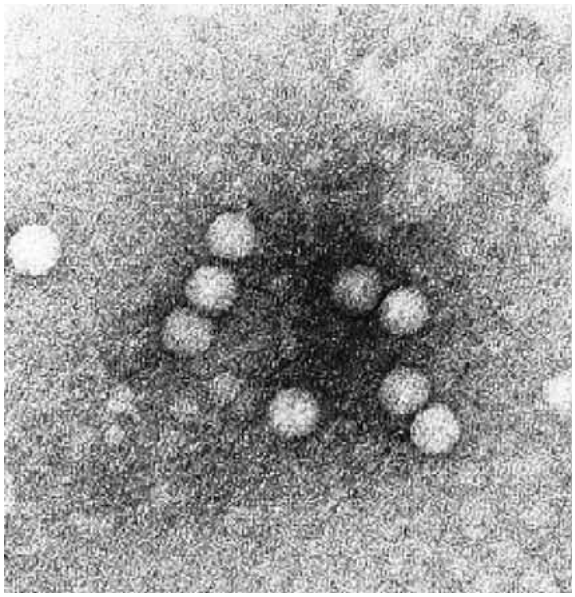
- simian virus 40

Definition

A DNA tumor virus (genus Polyomavirus) found to be a contaminant of Salk and Sabin poliovaccines (1955-1961) that propagates naturally in kidney cell lines of Asian macaque species, specifically the rhesus and African green monkey. SV40 in these species, and related primates, produces no cytopathic effects upon the animals, but the virus injected into hamsters and other rodents causes ependymomas, lymphomas, osteosarcomas, sarcomas and mesotheliomas. Subsequent research has shown a possible correlation between SV40 and human mesotheliomas.

Characteristics

SV40 particles lack a lipid envelope and have a diameter of approximately 40 to 50 nm with spherical icosahedral symmetry. The molecular mass of the SV40 virion has been estimated to be 270 kD. The icosahedral capsid contains three viral proteins (VP1, VP2, VP3), with VP1 being the major protein and VP2/VP3



SV40. Fig. 1 – Electron micrograph (250,000×) of SV40 virions (kindly provided by Dr. R. Fresco).

being minor proteins. Along with the viral proteins, the SV40 virion contains cellular histones (H2A, H2B, H3, H4) that aid in condensing the viral DNA. The SV40 genome comprises of a closed circular dsDNA (5243 bp) that associates with the various histones to achieve condensation, similar to that of cellular DNA in the form of chromatin. Nucleosomes that number between 24-26 on the viral DNA are made from the assembly of the histone-DNA complexes.

The genome of SV40 is numbered in a clockwise direction beginning at the origin (Ori) and continuing around until the site of Ori is reached again, marking the end of the genome. SV40 genome is responsible for coding six genes, synthesizing VP1, VP2, VP3, LP1, and the large (T-ag) and small (t-ag) t-antigens. SV40 large T-ag is comprised of 708 amino acids, while the small t-ag contains 174 residues. The initiation of viral DNA synthesis is mediated by the essential replication protein large T-ag, which in turn is regulated by phosphorylation. The transformation of cells and induction of tumor formation by SV40 is another function of the large T-ag, which causes the inactivation of products made from several tumor suppressor genes, including p53, pRb, p107, p130/Rb2, p300 and p400. One of the crucial consequences of binding large T-ag to p53 is the inactivation of an essential checkpoint that halts the process of mitosis if DNA damage is present, causing the cell to continue cycling. The small t-ag protein is not an essential mediator in SV40 replication, but it does play a significant role by causing an increase in the production of large T-ag and aiding in inactivating p53.

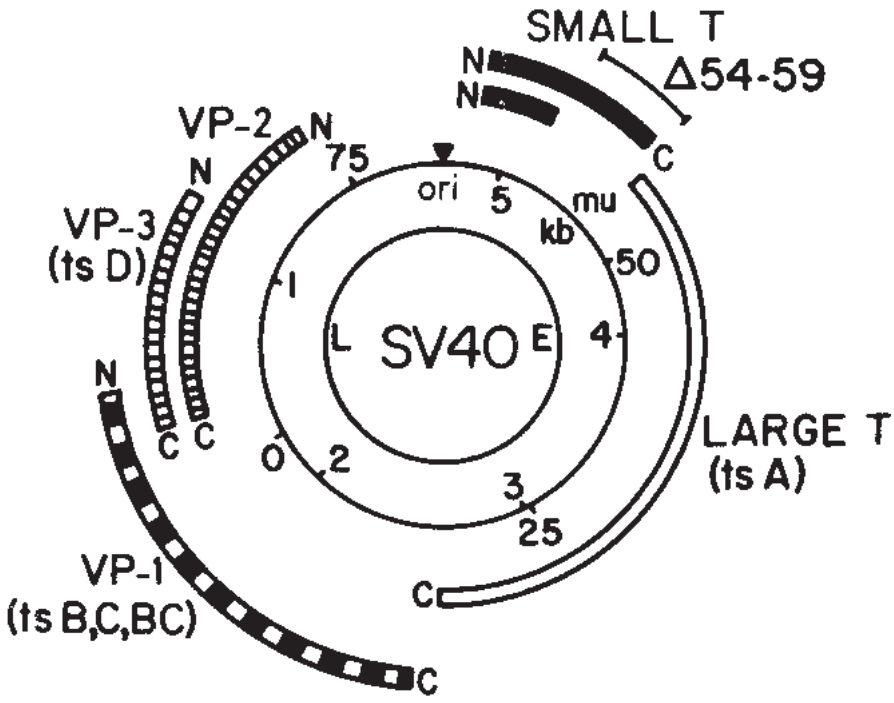
Different regions of the SV40 genome, designated 'early' and 'late', are expressed at different times during the stages of infection. The early region codes for the large T-ag, the small t-ag, which are the SV40 oncogenes, and for a 17 kD protein of uncertain biological significance. The late region codes for the capsid proteins VP1, VP2, VP3, and for LP1, a protein involved in the process of SV40 particle assembly late in infection.

Replication

The early and late regions of the SV40 genome distinctly separate the replication process into two events: Similar to other viruses, SV40 virions come into contact with the outer membrane surface of the host cell and attach to receptors located throughout the outer cell surface. These receptors are thought to represent the major histocompatibility complex class I molecules (MHC class I). Upon being transported into the cell, SV40 virions are moved to the cell nucleus. Once inside the nucleus, the virion capsid disassembles and the viral DNA is released.

At this point in replication, the early region of the SV40 genome is transcribed first, synthesizing the small t-ag/large T-ag proteins. This causes the cells to enter S phase. The SV40 72-bp enhancer elements help positively regulate transcription in the early region of the genome.

It has been identified that there are SV40 strains with either one 72-bp enhancer ('archetypal') or two 72-bp enhancers ('non-archetypal'). Nonarchetypal SV40 replicates more rapidly compared to archetypal SV40. Three G + C-rich domains, also referred to as 21-bp repeat regions, are binding sites for cellular factors. Upon the initiation of S phase, the viral



SV40. Fig. 2 – Genetic map of SV40. Outer circular segments represent specific proteins (Tag/tag, VP1, VP2 and VP3). Inner circle represent the direction of transcription and region of origin.

DNA replication and transcription can now begin from the late region, causing the production of necessary structural proteins (VP1, VP2, VP3, LP1). After the necessary proteins and DNA replication is complete, the various viral particles assemble together forming the next generation of SV40 virions. When a high number of virions accumulates in the cells, the cell is lysed and infectious SV40 is released. Infected mesothelial cells, however, can release infectious SV40 without undergoing lysis.

Clinical Relevance

Poliomyelitis was a devastating disease that swept throughout the western world until 1955 when the Salk vaccine began to be used against this crippling disease. Poliovaccines made from monkey kidney cell cultures between 1955 and 1961 and sold until 1963 were found to have been contaminated with numerous supposedly harmless viruses. Knowledge of this contamination was well known, but there was no evidence to suggest any tumorigenic properties of any viruses present in the vaccines. In 1960, Sweet and Hilleman established that an unknown percentage of poliovaccines produced from monkey kidney cell lines was contaminated with SV40 (6). In 1962, Eddy and colleagues produced investigations showing that newborn hamsters injected with rhesus monkey kidney cell cultures developed sarcomas (7). The resultant sarcomas were attributed to the DNA tumor virus SV40.

It was shown that SV40 was able to successfully replicate, produce infection, and spread throughout humans by oral and respiratory routes. In 1964, when SV40-transformed human cells were injected subcutaneously into volunteer terminally ill patients, those cells were found capable of growth. Vaccines that were produced after 1961 were required by federal law to be tested for SV40, but by that time it has been estimated that approximately 98 million people, both adults and children, had already been exposed to SV40 through a contaminated poliovaccine. SV40 was able to transform both human and rodent cells in tissue culture. However, epidemiologic studies suggested that SV40 was not oncogenic in humans, because

the overall incidence of cancer in cohorts injected with contaminated poliovaccines was similar to that of cohorts who had not been exposed to SV40 contaminated poliovaccines.

In more recent investigations done by Carbone (reviewed in 2) dealing with the association of SV40 and mesotheliomas, the majority of hamsters injected intracardially with SV40 developed pleural mesotheliomas. When hamsters were injected with SV40 in the pleural space, all of the animals developed mesotheliomas within 3-6 months. Other investigations demonstrated that only the SV40 wild type, not small t-ag by itself, injected intracardially or intrapleurally, caused the transformation and subsequent formation of mesotheliomas. Since asbestos is a known carcinogen that induces mesotheliomas, animals that had asbestos inhaled or injected into them also developed mesotheliomas, but to a lesser extent then the injection of just SV40.

These results prompted investigations into the possibility that mesotheliomas in humans could be attributed to SV40 infection directly or with SV40 acting as a co-carcinogen with asbestos. Mesothelioma samples studies in 1994 showed that 60% of the samples expressed SV40-like DNA sequences in human pleural mesotheliomas. Additional studies conducted by Testa (reviewed in 2) showed that 83% of mesothelioma samples tested positive for SV40. The results of both investigations were confirmed by a total of thirty independent laboratories. The presence of SV40 in mesotheliomas and SV40-like DNA sequences does not necessarily confirm SV40 as the sole direct cause of mesotheliomas. The large T-ag protein was investigated to determine what effect it had specifically on the tumor suppressing gene p53. Polymerase chain reaction (PCR), immunohistochemistry, RNA *in situ* hybridization and Western blot experiments demonstrated that the large T-ag protein was able to bind successfully to the p53 and inactivate it in the mesothelioma samples. In addition, later tests showed that large T-ag was able to bind to other tumor suppressor genes and cause a similar result to the p53 inactivation. This was a key finding since it demonstrated that the large T-ag interactions with tumor suppressor genes could have an effect in promoting the development of mesothelioma tumors by allowing mesothelial cells to become more susceptible to other carcinogens, specifically asbestos. If taking into consideration whether or not exposure to asbestos is correlated with higher rates of SV40 aided mesotheliomas, 60% of patients that had been exposed to asbestos had SV40-like DNA sequences present in their tumors.

As of yet, no definitive and final conclusion can be extrapolated from the results that would suggest or refute a direct correlation between SV40 contaminated poliovaccines and its direct causal role in the development and manifestation in human mesothelioma and other tumors. Mesothelioma studies that tested Finnish samples for presence of SV40-like DNA sequences failed for SV40 detection. This raises the question of whether or not the Finnish poliovaccines were contaminated with SV40. Using epidemiologic data in connection with laboratory investigational results, a strong connection between SV40 and mesotheliomas exists. Prior to 1970, the occurrences of mesotheliomas was uncommon, but since then the incidence rate has increased significantly. The degree of contamination with SV40 of the poliovaccine that a person received is also a factor to consider in forming a correlation between SV40 and mesothelioma; it has been shown that SV40 carcinogenesis is proportional to the dosage amount given to the animal. More recently, a modified large T-ag was used as a vaccine to protect animals when a SV40 active large T-ag was injected into the same animal, causing a potential for development of therapeutic drugs to combat human cancers associated with SV40.

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Swordtail

Definition

→ Xiphophorus.

Synaptic Vesicle Recycling

Definition

Synaptic vesicle recycling is a specialized class of small vesicles (synaptic vesicles, ~ 50 nm diameter) in nerve cells store neurotransmitters and releases them upon the arrival of an action potential at the nerve terminal. Neurons can fire in excess of a thousand times per second, which would rapidly lead to depletion of synaptic vesicles. Thus, synaptic vesicles are efficiently internalized after their fusion with the plasma membrane by a molecular machinery that is largely overlapping that of → endocytosis.

Synaptic Vesicle-like Microvesicles

Definition

Synaptic-like microvesicles (SLMVs) of neurons and neuroendocrine cells have a diameter of 40 to 80 nm. They are characterized by a clear content and contain the membrane proteins synaptophysin, protein SV2, synaptotagmins, synaptobrevins/VAMPs and other proteins including heterotrimeric G-proteins. They are good tools to study neuroendocrine differentiation, even in poorly-differentiated → NETs.

Syntenic

Definition

Syntenic is genes or genetic loci that lie on the same chromosome, i.e. are genetically linked.

T

TAAT Sequence

Definition

TAAT is a DNA consensus sequence that is bound by regulatory proteins, for instance → homeobox genes.

TAG12

Definition

Tumor-associated antigen 12 (TAG12); → micrometastasis.

TAL-1

Definition

T-cell acute lymphocytic leukemia 1 (Tal-1), also known as Scl1 or Tcl-5, is a bHLH protein member of the Lyl family of 331 amino acids and 34 kD. The human TAL1, SCL or TCL5 gene locus maps to 1p32 and the mouse tal1 gene locus at chromosome 4 (49.50 cM). Tal-1 is implicated in the genesis of hemopoietic malignancies. A form of stem cell leukemia is characterized by a chromosomal translocation t(1;14)(p32;q11) that involves Tal1 and T-cell receptor a chain genes. Tal-1 may play an important role in hemopoietic differentiation, serving as a positive regulator of erythroid differentiation.

Tandem Repeats

Definition

Tandem repeats are multiple copies of the same sequence lying in series.

Target Validation

Definition

Target validation is the demonstration that a protein is involved in or required for a disease-relevant cellular process. Target validation is an early rate limiting step in drug discovery and hence is very important in developing new therapies.

TAT Protein of HIV

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Definition

Tat is a small viral protein that is encoded by the spliced two-exon *tat* gene in the HIV genome, responsible for transactivation of the HIV genome.

Characteristics

The HIV Tat protein gets its name from its principal activity, Tat stands for Transactivator,

which means that it binds to DNA and activates the transcription of DNA into RNA. The Tat protein has an important role in controlling the transcription of the lentivirus HIV genome from its built-in 'promoter', known as the long terminal repeat (which refers to its structure) or LTR, to make the RNA that forms new HIV virus particles. In addition to this major role, Tat has also been implicated in a wide variety of pathologies encountered in persons infected with HIV. How does this small (about 101 amino acids) Tat protein do this? Tat has a capacity to bind a striking number of different proteins, nucleic acids and even polysaccharides. It is this combination of binding to different partners that has linked Tat to numerous events in AIDS and intrigued many researchers, making Tat one of the most extensively studied HIV proteins.

Transcriptional regulation: control of HIV replication

The HIV → LTR acts as a gene promoter. The gene promoter is a portion of DNA that mediates the binding of RNA polymerase (usually through a series of other proteins that bind to the promoter DNA as well) at the beginning of the gene to be transcribed into RNA. This interaction of proteins with DNA controls the transcription of each gene so that it occurs at a certain moment. In the absence of Tat, very little RNA is transcribed from the HIV LTR promoter. When Tat is present, the rate of transcription shoots up several hundred fold, making the transcription of the HIV genome efficient.

After HIV infects a cell, it is reverse transcribed into DNA, which is then integrated into the genome of the cell (similar to → retroviruses). The viral DNA is then packaged along with the rest of the cellular DNA by winding onto the histone proteins. In this state, the HIV LTR promoter is rather inactive. In fact, in order to be transcribed, the promoter region of any gene must be unwound from these histones. The HIV DNA is bound to the histones in a very specific manner, with one histone group just prior to, and another just after, a site which binds DNA binding proteins of the cell known as SP1 and NFκB. The activity of these proteins may be enough to allow the binding of an RNA polymerase, known as RNA polymerase II or RNAP II, to the HIV LTR.

The binding of RNAP II to the LTR alone is not enough for efficient transcription. In the absence of Tat it does not appear to be able to advance forward to synthesize RNA beyond the first 44 nucleic acid base pairs. The major role for Tat is to unlease this machinery and send it to work. When Tat is present, it binds to a segment of this short initially polymerized RNA, which forms a peculiar loop known as the TAR (TransActivation Responsive) element. The TAR-bound Tat then brings in a series of other proteins that allow the transcription process to proceed.

Tat has been demonstrated to play a key role in the unwinding of HIV DNA from histones, which is one key for allowing RNA transcription. To do this, Tat binds to a group of proteins known collectively as Tat-associated histone acetyltransferase (TAH). The TAH complex can be formed by different cellular proteins such as p300 or is close relative CBP, along with P/CAF and/or TAF250. These TAH proteins have an enzymatic activity that transfers an acetyl group to histones and are known as histone acetyltransfeases. The recruitment of the TAH complex to the HIV LTR region by TAR-bound Tat leads to acetylation of the histones that bind to the LTR, causing changes in their conformation that facilitate RNA transcription. In fact, in cells where p300 and P/CAF are limiting, addition of Tat increases transcription only about 7-fold, while addition of both Tat and p300-P/CAF allows increases transcription about 80-fold.

The TAH complex also appears to acetylate the Tat protein itself. This appears to lower the binding of Tat for the TAR, but increases the binding of Tat to another complex of proteins known as the Tat-Associated Kinase (TAK). This complex of proteins consists of cyclin-dependent kinase 9 (cdk9) and one of the cyclin T isoforms (T1, T2a, T2b). This protein complex can directly bind Tat. More importantly, the kinase activity of the TAK complex phosphorylates (adds a phosphate group to) the RNA polymerase RNAP II. This phosphorylation ap-

pears to alter the activity of the RNAP II, improving its ability to transcribe the HIV genome. Together, the TAH and TAK complexes brought in by Tat unleash the RNAP II to finish the job it started, giving the 100-fold improvement of transcription, and therefore HIV replication, observed when Tat is added to HIV infected cells.

Tat also transactivates several cellular genes in addition to the HIV LTR. The activation of these genes is also thought to contribute to the pathogenesis of HIV. The genes include the cytokines IL-6, TNF and IL-1, which are known to be increased in AIDS patients and may have detrimental effects on the overall function of the immune system.

AIDS-associated pathologies: a direct contribution by Tat

One of the most striking properties of Tat is its ability to exit from cells, where it is released into the extracellular environment. Several studies have shown that the HIV-1 Tat protein can exit from cells, including HIV infected cells. As the Tat gene does not encode a signal peptide, the release of HIV-Tat has been suggested to occur via an alternative secretion pathway, like that demonstrated for some cytokines. It may also come from cells dying due to HIV. The Tat that is found extracellularly appears to be intact and active, and substantial levels of Tat protein have been observed found in the serum of many HIV patients. Antibodies can be made against Tat in AIDS patients, indicating that it is released, and interestingly an inverse correlation between anti-Tat antibodies and patient survival has been reported in some studies. These data suggest that extracellular Tat may favor HIV replication, and have spawned tests on the possibility of using Tat as part of an AIDS vaccine.

A wide range of activities have been attributed to the Tat protein released extracellularly. Several studies have demonstrated that the HIV Tat protein, or peptides based on Tat, are capable of entering cells cultured *in vitro*. The Tat that enters cells is capable of transactivating the HIV LTR. Tat and Tat peptides have even been used to deliver other proteins into cells, and a peptide based on Tat may find use as a signal to move drugs into cells.

In addition to getting into other cells, the Tat that is released also appears to bind to several cell surface proteins, including specific receptors. These activities of Tat have been linked to many of the pathological alterations found in HIV infection. For example, some groups of AIDS patients frequently have → Kaposi sarcoma, an otherwise rare, benign vascular tumor. Unlike most Kaposi, the Kaposi sarcoma associated with AIDS is very malignant and could be life-threatening for many of these patients. Tat was first linked to Kaposi sarcoma when Kaposi-like lesions were found on mice genetically engineered to express Tat. Soon after, Tat was shown to be a growth factor for Kaposi cells, but the reason for this was not known. Later studies showed that Tat could bind to KDR, a receptor for the growth factor → VEGF, on the surfaces of endothelial and Kaposi sarcoma cells. VEGF (vascular endothelial growth factor) is important in the formation of new blood vessels, as is its receptor KDR. The ability of Tat to bind and activate KDR means that Tat could stimulate the formation of vessels found in Kaposi tumors, as well as the growth of cells of the tumor itself. We now know that Kaposi sarcoma is probably due to an infection with a herpesvirus, HHV8, that occurs when the immune system is unable to control this virus. All Kaposi cells, whether from aggressive AIDS or benign sporadic of iatrogenic (post-transplant), have KDR, however the Tat stimulation of KDR and perhaps other receptors appears to make AIDS Kaposi potentially lethal.

Many Tat proteins have an RGD sequence, 3 amino acids found in many proteins of the extracellular matrix. Through this RGD sequence Tat can bind to cell surface integins, proteins that are normally involved in binding to extracellular matrix molecules. Studies have shown that several integrins bind the Tat protein. Tat-integrin binding has been shown to trigger events typical of integrin-extracellular matrix ligand interactions, including activation of p125 Focal Adhesion Kinase. The binding of Tat to these receptors has also been linked to Kaposi sarcoma and other activities of HIV Tat.

The immune suppression seen with AIDS appears to affect cells that are not infected with HIV aside from those harboring the virus. Several studies have shown that there is immune suppression of non-HIV infected cells from AIDS patients, and that the number of immune suppressed cells seems to exceed that of the potentially HIV-infected cells. Proteins released from HIV-infected cells are clearly potential candidates for mediating this immune suppression. Tat has been linked to induction of T-cell anergy (lack of activity), T-cell → apoptosis (programmed death), but also to a T-cell hyper-activation that appears to prime cells for infection by HIV. These events are probably all closely linked to the same phenomenon. The potential receptor system(s) involved in these activities of Tat include CD26. CD26 is a dipeptidyl peptidase that is known to cleave and alter the activities of chemokines, molecules whose receptors are very important cell surface receptors for HIV and that can regulate HIV infection. Tat has been shown to significantly increase the expression of two key chemokine-HIV receptors, CXCR4 and CCR5, by monocytes and T-lymphocytes, potentially increasing HIV infection.

The Tat protein has been reported to act as a growth factor and protect transfected cell lines from apoptosis. Tat has been consistently found to up-regulate the expression of CD95- → Fas, a protein that signals cells to die. The increase in apoptosis is typical for partially activated T-cells, as is entry into anergy resulting from an incomplete stimulation of T-cells. Tat may be capable of partial, but incomplete, T-cell activation. HIV does not readily infect resting T-cells, T-cell activation is a key requisite for HIV infection of these cells. A partial T-cell activation may be sufficient for HIV infection yet detrimental to the host immune response, a potential role that Tat may fulfill.

Extracellular HIV Tat has been shown have wide ranging effects on lymphatic cells such as monocytes, macrophages, dendritic cells and even natural killer cells. Tat has been reported by several groups to be a strong chemoattractant for monocytes. This activity could contribute directly to the recruitment of potentially 'infectable' cells toward an HIV-infected cell producing and releasing Tat protein, an activity which may have a direct affect on establishment and spread of HIV infection in the host. Tat has been shown to bind to some chemokine receptors (including CCR3 and CKCR4) and partially mimic chemokines. Tat can even inhibit HIV infection in high doses by binding chemokine recpetors, although the physiological relevance of this observation is not yet clear.

The Tat protein appears to inhibit dendritic cell phagocytosis and natural killer cell function, apparently by blockage of certain calcium channels. Finally, Tat has been linked to AIDS associated dementia. Tat has been shown to excite neurons, which is associated with neurotoxicity. The molecular identity of the Tat receptor(s) on neural cells is not yet known. However, the neuroexcitory properties of Tat were blocked by lowering extracellular calcium, suggesting that interference with calcium channel function may be involved.

Conclusion

Tat is known to have a major role in HIV replication through a complex series of interactions with nuclear proteins. In addition, Tat outside the cell appears to be able to stimulate through, or interfere with, several cell surface receptors, sending signals that may be a root cause of many pathologies found in HIV-infected patients.

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TATA

Definition

A 5′-TATAAA-3′ sequence (=TATA box) in the promoter region of most genes, located about 25 nucleotides before the initiation site of transcription.

TATI

Definition

Tumor-associated trypsin inhibitor (TATI) is a low molecular weight protein used as a tumor marker, e.g. → ovarian cancer.

Taxol

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Definition

Taxol (Paclitaxel) was first isolated in 1971 from a crude extract of *Taxus brevifolia*, a scarce, slow growing yew plant found in the forests of the Pacific Northwest. It is a diterpenoid containing the characteristic taxane ring (Fig. 1). Total chemical synthesis of this compound was achieved in 1994, opening the way for the production of various analogues. Currently, taxol is commercially prepared by hemisynthesis, in which a synthetic side chain is attached to natural products isolated from the needles of *Taxus* plants. Enzymatic conversion of various taxanes to 10-deacetylbaccatin III (a precursor for taxol hemisynthesis) has been recently reported.

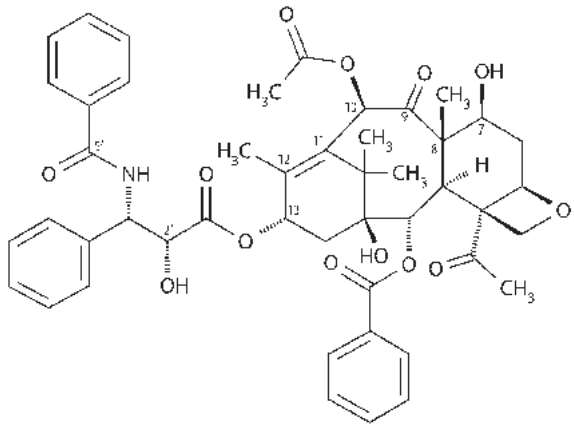
Characteristics

Mode of action

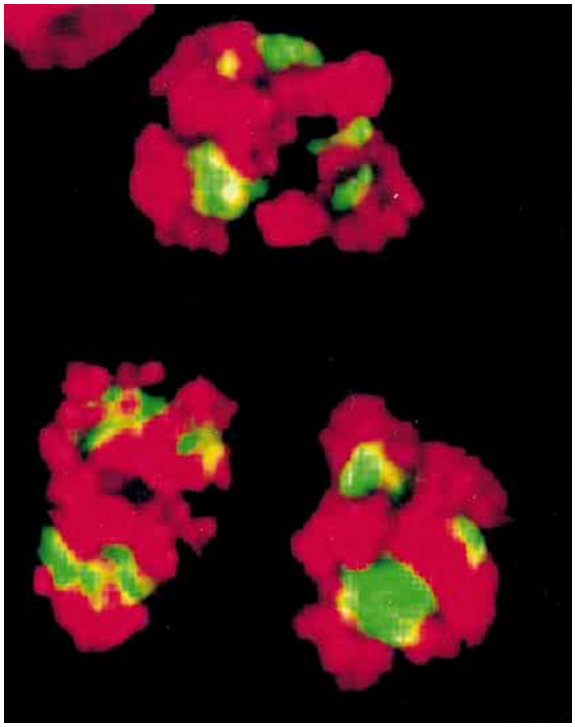
Due to its hydrophobic character, taxol readily crosses the plasma membrane. Once in the cytoplasm, the drug binds with high affinity to the β-subunit of tubulin, modifying and stabilizing microtubules. When modified by taxol, these cytoskeletal structures exhibit decreased dynamic instability and increased rigidity. As a result, the function of the microtubule-based machines (e.g., mitotic spindle) is compromised and the cells cannot divide properly. Recently, it has been proposed that taxol also binds to → Bcl-2, a protein involved in the process of programmed cell death (→ apoptosis).

The cellular effects of taxol vary depending on dose and treatment scheme. In the range of nanomolar concentrations it induces sustained mitotic arrest (Fig. 2), inhibits protein prenylation and triggers apoptosis. At micromolar doses it promotes synthesis and release of cytokines, such as tumor necrosis factor (TNF) and interleukins (IL1 and IL8), increases tyrosine phosphorylation by MAP kinases, induces early response genes and stimulates production of nitric oxide.

How taxol exerts its cytotoxic action remains elucive. Structure-activity studies differentiate



Taxol. Fig. 1 – Chemical structure of taxol.

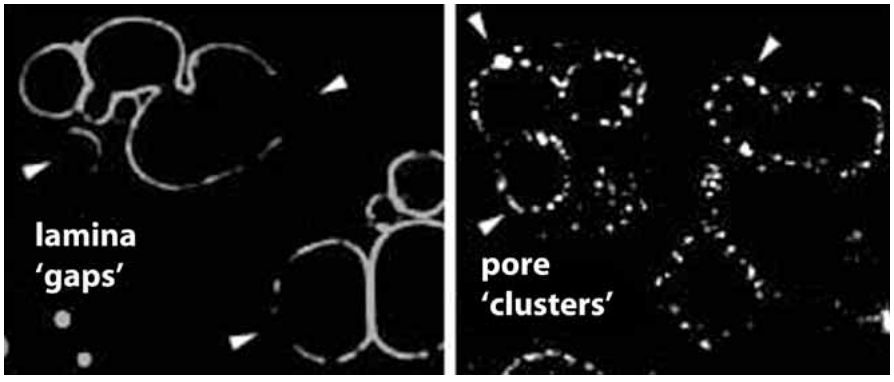


Taxol. Fig. 2 – Confocal microscopy image, showing human cervical carcinoma cells in mitosis. Cells were treated with 10 nM taxol for 20 hours. Immunostaining was done with anti-tubulin antibodies and counter-staining was done with propidium iodide. Chromosomes are shown in red, the mitotic spindle in green.

microtubule stabilisation from other effects, supporting a direct effect on the genetic and signal transduction machinery. However, other studies suggest that the drug acts by inducing cytoskeletal damage.

Recent observations show that taxol activates Raf-1 kinase and induces phosphorylation of Bcl-2. Phosphorylation of the latter may, in turn, lead to dissociation of Bcl-2/Bax complexes, unleashing → Bax into the cell and thus triggering apoptosis. Although these hypotheses are intuitively attractive, neither of them could fully account for the cell killing action of taxol: programmed cell death is also induced by other microtubule-stabilising drugs which are free of genomic side-effects (e.g., axotere), while apoptosis can still occur independently of Raf-1 phosphorylation, or after Bcl-2 is dephosphorylated by cellular phosphatases.

Taxol and other microtubule-acting agents affect dramatically the architecture of the cell nucleus. It is widely known that cells treated with nanomolar amounts of taxanes or vinca alkaloids develop lobulated nuclei or multiple micronuclei and missort key cellular constituents. Recent studies also show that taxol affects the nuclear envelope. Even at nanomolar concentrations, the drug induces focal unraveling of the nuclear lamina and extensive clustering or ectopic localization of nuclear pore complexes (Fig. 3). Cells that possess a defective nuclear envelope and which are treated with taxol remain alive for at least 24 hours after the end of the treatment, but are unable to import karyophilic proteins, such as the transcription factor NFκB, into the nucleus. It



Taxol. Fig. 3 – Nuclear lamina and pore complex lesions after treatment of human endometrial carcinoma cells with 10 nM taxol. Left: indirect immunofluorescence using anti-lamin B antibodies. Right: indirect immunofluorescence using anti-nucleoporin antibodies. The interruption of the nuclear lamina and the formation of large pore clusters is evident in these images.

has been proposed that inhibition of NFκB import may render the cells prone to programmed cell death.

Clinical pharmacology

Antitumor activity. Taxol as a single chemotherapeutic agent has been proven effective against a variety of tumors, including ovarian, breast, head and neck, esophageal, bladder and lung carcinomas. In addition, several schedules of combination therapy have been developed as alternatives for patients with advanced cancer. Pilot studies show that taxol can enhance radiation sensitivity of tumor cells, potentiate tumor response and increase the therapeutic ratio of radiotherapy.

Absorption and excretion. The drug is administered as a 3-hour or 24-hour infusion. It undergoes cytochrome P450-mediated metabolism to 6-OH derivatives and other products. Less than 10% of a dose is excreted intact from the urine.

*Toxicity.*The principal toxic effect of taxol is neutropenia. Several other toxic effects, such as myalgias, mucositis, hypersensitivity reactions, stocking-glove sensory neuropathy and disturbances of the cardiac rhythm, have also been encountered.

Structurally and functionally-related compounds

Structurally-related.

- Docetaxel (taxotere), produced by semisynthesis (1986) from 10-deacetyl baccatin III, a taxoid precursor. It is the second member of the taxane class to reach clinical use.
- Several chemically synthesized taxoids bearing substitutions or modifications.

Nontaxane, microtubule-stabilising agents.

- Estramustine, a conjugate of estradiol and nor-nitrogen mustard.
- Epothilones A and B, two macrolides isolated from myxobacterium, *Sorangium cellulosum*.
- A familly of marine-derived compounds extracted from sponges (discodermolide and laulimalide) or corals (sarcodictyins A - F and eleutherobin).

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T-cell Leukemia/lymphoma 1 (TCL1)

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Definition

The *TCL 1* gene is involved in the generation and/or manifestation of the T-Prolymphocytic leukemia (T-PLL).

Characteristics

Clinical characteristics of the T-PLL

The T-PLL is a disease that represents 20% of prolymphocytic leukemias. It occurs at an advanced age of 70 to 80 years, with a slight male predominance. It is, however, quite frequent in patients with the immunodeficiency syndrome → ataxia telangiectasia (AT) (1-5% of these pa-

tients develop it). Clinically it is accompanied by splenomegaly (75%), hepatomegaly (42%), lymphoadenopathy (55%), a high blood count ($>200x10^9$/L), with a very bad survival rate (< 8 months). It involves mature T-cells ($CD4^+$ in the 65%; but also $CD8^+$ and $CD4^+CD8^+$); T-cell prolymphocytes usually express CD3, CD5 and CD7. Morphologically the T- prolymphocyte is smaller than its B-counterpart, usually with a single, sometimes irregular, nucleolus. Chromsomal aberrations are quite peculiar and involve either inv 14 (q11;q32); or t(14;14)(q11;q32) and t(X;14)(q28;q11)translocations. Other recurrent changes involve chromosome 8 either as i(8)(q10) or as der(8) t(8;8). Molecular characterization of these breakpoints has been achieved and have brought to the identification of a new class of oncogene son chromosome 14q32.1 (TCL1) and Xq28 (MTCP1) that are dysregulated (overexpressed) in this disease.

Cytogenetic and molecular characteristic of the *TCL1* locus

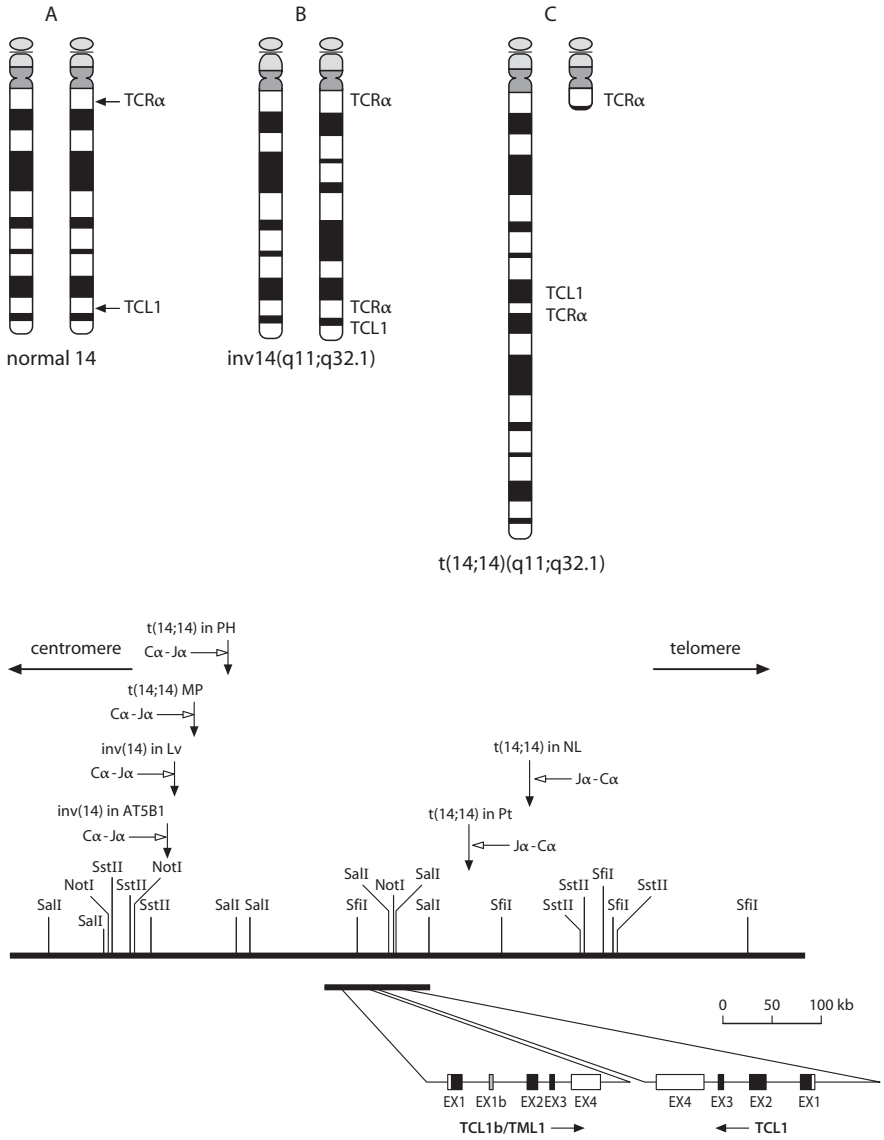
Intracellular overexpression of the *TCL1* gene, is usually the result of a chromosome translocation or inversion juxtaposing the human region of chromosome14q32.1 (*TCL1*) to the region 14q11 of the alpha/delta locus of the T-cell receptor gene (*TCR*) (Fig. 1, top). Less frequently the translocation involves the *TCL1* gene and the β locus of the *TCR* thus giving origin to a t(7;14)(q35;q32.1) translocation. The *TCL1* gene was first identified inside a 80 kb area comprised between the group of cloned inversions and translocations. This area has been recently fully characterized and completely sequenced (Fig. 1, bottom): other than *TCL1*, it comprises three other genes: *TCL1b* (also named *TML1*), that is highly homologous to *TCL1* and lies just 15Kb on the centromeric side of *TCL1*; *TNG1* and *TNG2* (also named *TCL6*), with no homology of *TCL1*. All of these genes are overexpressed in T-PLL, probably as a consequence of the juxtaposition of the *TCR* regulatory elements. The chromosomal aberrations described above are present in approximately 85% of T-PLL while in the remaining 10 – 15% it is observed another chromosome translocation between region Xq21 (*MTCP1* gene) and region 14q11 (TCR α/δ). The *TCL1* and *MTCP1* genes share more than 41% of identity and 61% of homology. As mentioned above T-PLLs are rare in the normal population but quite frequent in the patients affected by the autosomic immunodeficiency ataxia-telangiectasia (AT), a syndrome that results from the disruption of the *ATM* gene [ATM protein [→ ATM protein]]. The disruption of the both alleles of the *ATM* gene is also present in those cancer cells derived from patients with T-PLL but without AT, thus showing that alterations in these two genes, i.e. disruption of the *ATM* loci and overexpression of *TCL1* or *MTCP1* are necessary for the development of the T-PLL.

Animal models

Evidence for the oncogenic role of the *TCL1* gene is supported also by animal models. Transgenic mice that carried either the *TCL1* gene under the transcriptional control of the $p56^{lck}$ promoter, or the *MTCP1* gene under the control of the CD2 regulatory gene develop a form of chronic leukemia resembling morphologically and phenotypically to the T-PLL. These results indicated that transcriptional activation of the *TCL1* and *MTCP1* oncogene can cause malignant transformation of T lymphocytes. The leukemogenic effect seems to be dependent from the overexpressed copy number of the transgenes. These mice develop mature T-cell leukemias after long latency period, around 15 months. The leukemia in these transgenics is almost invariably of the $CD8^+$ phenotype.

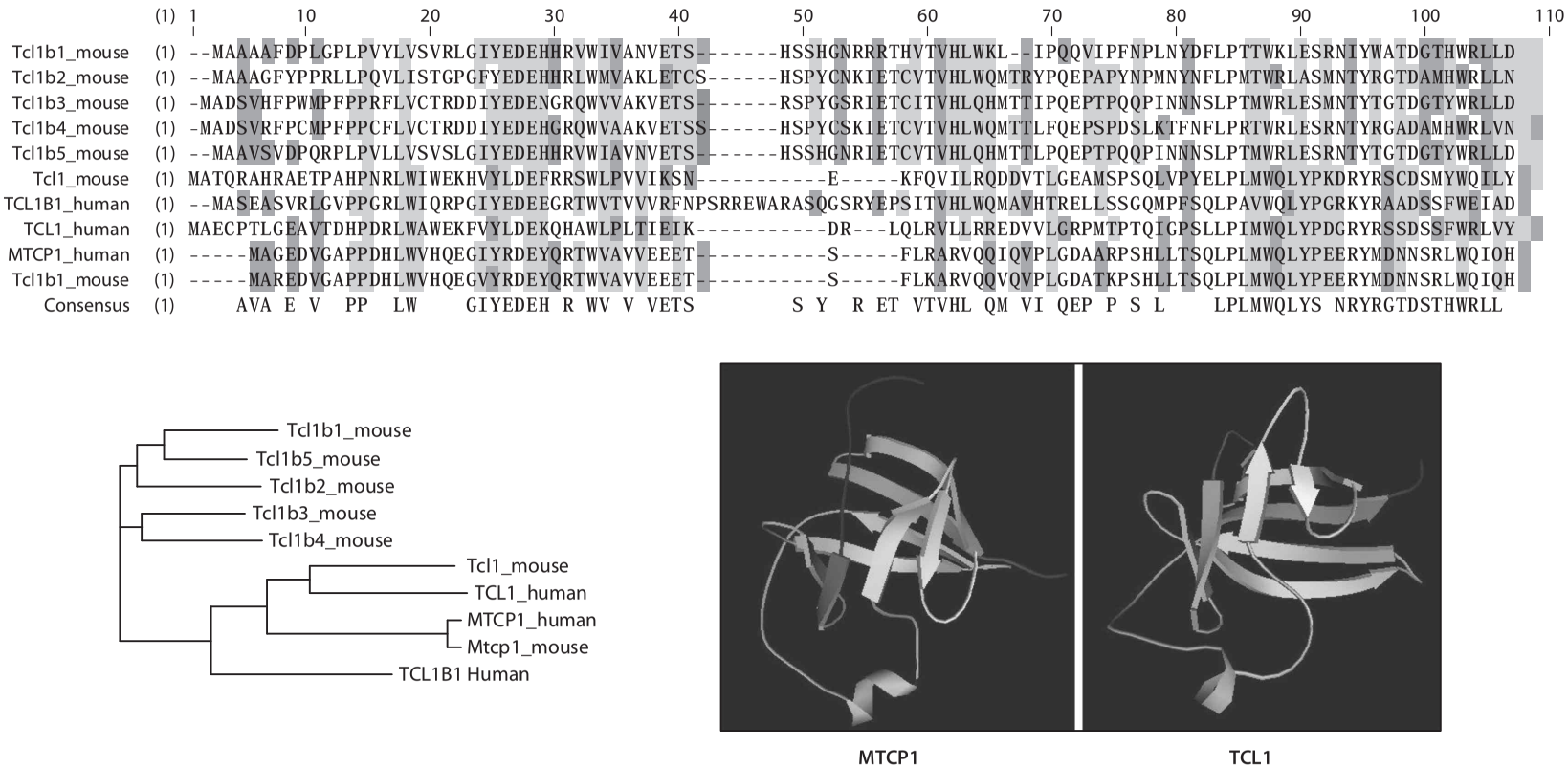
TCL1 protein

TCL1 codes for a transcript of approximately 1.3 kb translated in a protein of 114 aa with a predicted M_r of 14 kD, with homology to no other protein with the exception of the other family members. 10 homologues genes have been identified, 7 in the mouse and 3 in human (Fig. 2). The 3D structure has been solved by NMR and X-Ray crystallography for both the TCL1 and MTCP1 proteins. These proteins



T-cell Leukemia/lymphoma 1 (TCL1). Fig. 1 – top: Schematic representation of rearrangement occurring in T-PLL. **A** Two normal human chromosome 14 with location of the *TCRα* locus and *TCL1* genes. **B** An inverted chromosome 14 is represented on the right showing also that a split occurs in the *TCRα* (usually the break is in the Jα region). **C** A chromosomal translocation involving both the chromosome 14 with a split occurring always in the Jα region of the *TCRα*. In both cases B) and C) TCL1 remains in the same position but in the inversion the *TCRα* is situated centromeric (proximal) to *TCL1* while in the translocation it is situated telomeric (distal).
bottom: Map of the *TCL1* locus on showing the position and orientation of the *TCL1* and *TCL1b/TML1* gene, recently discovered that is also involved in the translocation. *TNG* (*TCL6*) is located between *TCL1b* and the inversion indicated by Lv. The position of two cloned translocation (NL and Pt) and of two inversion (Lv, AT5B1) derived from T-PLL is also indicated.

fold in a compact eight-stranded β-barrel structure with a short helix between the fourth and fifth strand, with a unique topology (Fig. 2). The identical residues of the TCL1 and MTCP1 protein are clustered inside the barrel and on the surface at one side of it. Overall the structure resembles those of other proteins involved in the transporting of small molecules (such as ly-

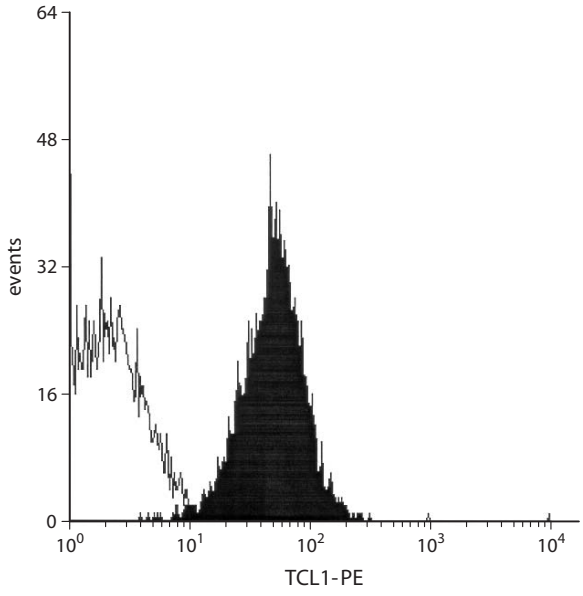


T-cell Leukemia/lymphoma 1 (TCL1). Fig. 2 – top: Amino acid comparison among all the members of the *TCL1* protein family. **Bottom left:** Phylogenetic tree. **Bottom right:** Crystal structure of MTCP-1 and TCL1 showing the β-barrel structure and the 3D homology between the two human proteins.

pocalin and calycine): this might led to predict that TCL1 could bind small ligand and function as transporter. The localization of TCL1 is mainly in the cytoplasm, but a nuclear translocation has also been reported. This last phenomenon has been described recently in association with → AKT1, to whom TCL1 appears to bind.

TCL1 expression in normal and pathological lympoid tissues

Expression of the *TCL1* gene, in normal cells is observed mainly in the lymphocytes, but also in ovary and in very early stages of embriogenesis in the mouse. In human T-cells derived from fetal thymus, the gene is confined to a very early in CD4-CD8 double negative and no other T-cells seems to express it. FACS analysis of peripheral blood lymphocytes, using a monoclonal antibody showed that TCL1 is only detectable in malignant T-cells from T-PLL (Fig. 3) while no other T-cell in the blood express it. The situation is different in B-cells where in the bone marrow TCL1 is absent in the $CD34^+CD19^-$ stem cell rich fraction: weak expression appears in the $CD34^+CD19^+$subpopulation of pro-B cells and the expression peaks in the IgM negative pre B-cells with high CD19 and persist in immature IgM^+ cells. In the lymph-node TCL1 is expressed by a subset of small lymphocytes of the lymphoid mantles and also by the cells of the germinal centres (centrocytes, centroblasts) of the secondary lymphoid follicles and by data obtained analyzing a large series of tumors is downregualted in memory and plasma cells. TCL1 was recently found to be highly expressed in the majority of AIDS immunoblastic lymphoma plasmacytoid.



T-cell Leukemia/lymphoma 1 (TCL1). Fig. 3 – Mo Ab against *TCL1* recognizes specifically $CD3^+$ lymphocytes in a case of T-PLL (black). In this histogram it also shown an isotype control with an irrelevant murine IgG1 (white).

Conclusions

Even if the functional role of the *TCL1* and its related genes must be deciphered, this gene plays an important role in the development of the T-PLL and in the in B-cell differentiation and thus its targeting might have therapeutical consequences.

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T cells

Definition

T cells are lymphocytes of the T (thymic) lineage that may be subdivided into several functional types. They carry TcRs (T-cell receptor) and are involved in the cell-mediated immune response.

TCF16

Definition

Conserved helix-loop-helix ubiquitous kinase [→ BHLH].

TCF21

Definition

Transcription factor 21 (Tcf21), also known as POD1 or Epicardin, is a bHLH protein member of the Twist family. The human TCF21 gene locus maps to 6q23-q24 and the mouse tcf21 gene locus at chromosome 10 (16.00 cM). Tcf21 has been identified in the developing kidney where it may play a role in the regulation of morphogenetic events.

TCL1

Definition

→ T-cell leukemia/lymphoma 1.

TCR

Definition

→ Transcription repair.

Technical Knockout

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Definition

Technical knockout (TKO) is a method for function-based gene isolation in mammalian cells.

Characteristics

Technical knockout application has resulted in the isolation of several novel genes involved in apoptosis and in the genetic dissection of certain processes within this important biological process.

In general, TKO is based on two concepts, both directed at overcoming impediments to the use of genetic screens in cultured cells.

- One concept is the use of nucleic acids as 'virtual mutagens' for trapping genes based on their function. This was achieved by transfections with anti-sense cDNA libraries to randomly inactivate gene expression, followed by a strong phenotypic selection.
- The other concept was to develop efficient strategies for introduction and recovery of complex cDNA libraries, to allow multiple rounds of phenotypic selection and rapid

screens for identification of functionally relevant genes. To this aim, an expression vector that provides an efficient gene transfer system was developed, with the capability of representing a complex library in a single transfection event.

An → EBV-based, self replicating episomal vector, was chosen to express a directional anti-sense cDNA library (Fig.1). The unidirectional strategy increased the probability of acquiring 'recessive mutations' due to loss of expression. The episomal vector had several advantages over vectors that integrate into the genome. It reduced the background of non relevant phenotypic alterations occurring as a result of random integrations into DNA. In addition, the episomes were easily rescued from the transfected cells by a simple DNA extraction procedure with no need for any other genetic manipulations. This yielded a rapid and convenient way to perform multiple rounds of phenotypic selection. It also solved the issue of plasticity of tissue culture cells, since only the individual cDNA fragments that transduced the phenotypic change in subsequent transfections were scored as real positives. The episomal vector accumulated at multiple copies in the stable transfectants, resulting in high expression levels. In addition, the promoter cassette of the vector had been manipulated to confer much stronger expression levels of anti-sense RNAs during the phenotype selection. Since interferon-γ was the trigger that induced apoptosis, an interferon-responsive enhancer element was introduced into the vector to increase expression during the selection by the killing cytokine. In retrospect, the latter manipulation was found to be critical for the success of the functional cloning, since expression of high anti-sense RNA levels during the phenotypic selection was essential to reduce efficiently the protein levels.

Isolation of genes in apoptosis

The application of the TKO approach to apoptosis resulted in the discovery and subsequent characterization of five novel genes that function as positive mediators of this biological process. In addition, an anti-sense cDNA fragment to cathepsin D was also isolated, suggesting that this well known lysosomal protease is actively recruited to the death process. It was found that the five novel apoptotic genes (named DAP genes for: Death Associated Proteins) encode proteins that display a diverse spectrum of biochemical activities and different intracellular localizations (Fig. 2). The list comprises a novel type of calcium/calmodulin-regulated kinase, which carries ankyrin repeats and the death domain, and is localized to the cytoskeleton (DAP-kinase), a nucleotide-binding protein (DAP-3), a small proline-rich, RGD-containing, protein (DAP-1) found in the cytoplasm, and a novel homologue of the eIF4G translation initiation factor (DAP-5). Extensive studies proved that these diverse activities participate in multiple apoptotic scenarios consistent with the initial design of the strategy which was aimed at targeting genes that belong to the basic machinery of apoptosis.

Preliminary results indicate that some of these rescued genes are functionally interconnected; the others may function independently of each other. The DAP genes were highly conserved in evolution. The *C. elegans* orthologue of DAP-3 shares 35% identity to the human protein and induces apoptosis in mammalian cells, indicating that the protein is also conserved at the functional level.

Clinical aspects

The advantage of functional approaches to gene cloning is that they select the relevant rate limiting genes controlling a biological process in a unbiased manner. As a consequence, novel targets and unpredicted mechanisms may emerge, as became evident from the study of DAP proteins. For example, the calcium/calmodulin-dependent DAP-kinase, which was found to be localized to the actin microfilaments, may provide a molecular handle to study the collapse of the microfilament system in apoptosis and/ or may mediate membrane blebbing. Most importantly, DAP-kinase, was found to possess tumor suppressive activities. This has been analyzed initially in mouse model systems where DAP- kinase display strong anti-meta-

static effects and later in other *in vitro* systems that test suppression of oncogenic transformation. In human carcinoma cell lines and B cell lymphomas, DAP-kinase expression was lost at high frequency. Recent clinical studies have revealed that DAP-kinase expression is lost in non-small lung carcinomas, head and neck carcinomas and B cell malignancies. Thus, screening systems for genes that are rate limiting in apoptosis may target tumor suppressor genes. Another breakthrough step relates to the discovery of DAP-5. The structure/function features of this novel translation regulator resemble the proteolytically cleaved eIF4G initiation factor, which appears in cells upon infection with some RNA viruses and directs cap-independent translation. The rescue of DAP-5 proved the existence of a strong link between apoptosis and the control of protein synthesis, which seems to be critical in certain apoptotic systems, and focused some of the mechanistic studies towards this direction. Another example refers to the isolation of cathepsin D by the TKO method suggesting that lysosomal proteases are recruited during apoptosis, in addition to the well known caspase family of proteases.

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TEL

Definition

Translocation-→ ets-leukemia (TEL), also known as ETV6. The TEL/ETV6 gene is a ETS family transcription factor disrupted by multiple translocations in acute myeloid and lymphoid leukemia. That is, the DNA binding domain shows homology to other ETS family members, which are defined by a conserved DNA binding domain.

Telomerase

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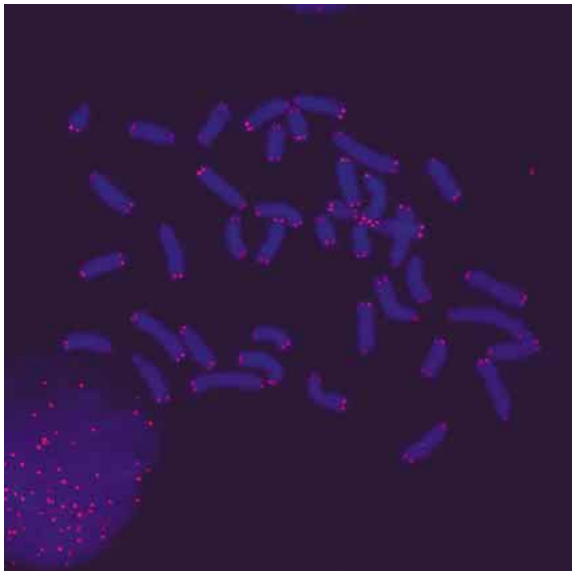
Definition

Telomerase (TE-LÓM-ER-ACE) is a ribonucleoprotein enzyme complex (a cellular reverse transcriptase) that maintains chromosome ends and has been referred to as a cellular immortalizing enzyme. Telomerase is composed of both RNA and proteins and uses its internal RNA component (complementary to the telomeric single stranded overhang) as a template in order to synthesize telomeric DNA (TTAGGG)n directly onto the ends of chromosomes. Telomerase is present in most fetal tissues, normal adult male germ cells, inflammatory cells, in proliferative cells of renewal tissues and in most tumor cells. After adding six bases, the enzyme is thought to pause while it repositions (translocates) the template RNA for the synthesis of the next six base pair repeat. This extension of the 3′ DNA template end in turn permits additional replication of the 5′ end of the lagging strand, thus compensating for the → end-replication problem.

Characteristics

→ Telomeres are the repetitive DNA sequences at the end of all linear chromosomes. In humans there are 46 chromosomes and thus 92 telomere ends that consist of thousands of repeats of the six nucleotide sequence, TTAGGG. The telomere-telomerase hypothesis of aging and cancer is based on the findings that the cells of most human tumors have telomerase activity, while normal human somatic cells do not.



Telomerase. Fig. 1 – Telomeres in human chromosomes. Metaphase human chromosomes that have been both stained with DAPI (blue color which stains DNA/chromosomes) and also in situ hybridized with a PNA (peptide nucleic acid) fluorescently labelled telomere probe (red ends of each chromosome).

Telomere length is maintained by a balance beween processes that lengthen telomeres (telomerase) and processes that shorten telomeres (the end-replication problem). Telomerase is a cellular reverse transcriptase that stabilizes telomere length by adding hexameric (TTAGGG) repeats onto the telomeric ends of the chromosomes, thus compensating for the continued erosion of telomeres that occurs in its absence. The core catalytic subunit of telomerase, → hTERT, is expressed in embryonic cells and in adult male germline cells, but is undetectable in normal somatic cells except for proliferative cells of renewal tissues (e.g. hematopoietic stem cells, activated lymphocytes, basal cells of the epidermis, proliferative endometrium and intestinal crypt cells). The *hTERT* gene maps to chromosome band 5p15.33.

In normal somatic cells, progressive telomere shortening is observed, eventually leading to greatly shortened telomeres and to a limited ability to continue to divide. It has been proposed that telomere shortening may be a molecular clock mechanism that counts the number of times a cell has divided, and when telomeres are short, cellular → senescence (growth arrest) occurs. It has been proposed, but not proven, that shortened telomeres in mitotic (dividing) cells may be responsible for some of the changes we associate with normal aging.

What are telomeres and what do they do?

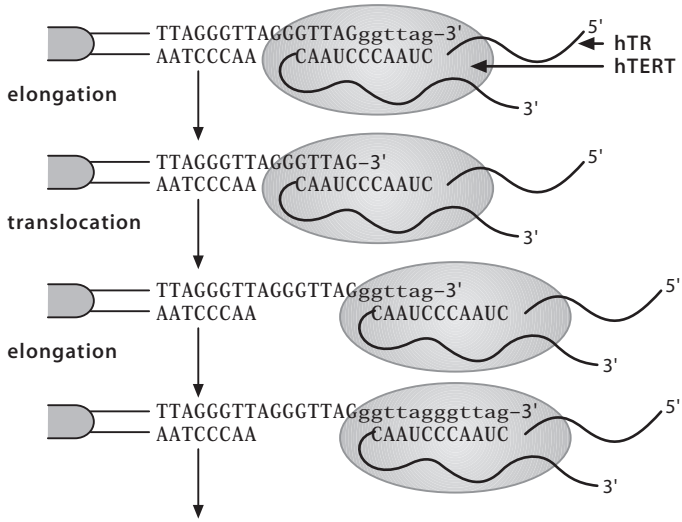
→ Telomeres are repeated DNA sequences that protect the ends of chromosomes from being treated like a broken piece of DNA needing repair. Without telomeres, the ends of the chromosomes would be 'repaired', leading to chromosome fusion and massive genomic instability. Telomeres are also thought to be the 'clock' that regulates how many times an individual cell can divide. Telomeric sequences shorten each time the DNA replicates. When at least some of the telomeres reach a critically short length, the cell stops dividing and ages (senesces) which may cause or contribute to some age-related diseases. In cancer, a special cellular reverse transcriptase, telomerase, is reactivated and maintains the length of telomeres, allowing tumor cells to continue to proliferate.

Why do telomeres shorten?

The mechanisms of DNA replication in linear chromosomes is different for each of the two strands (called leading and lagging strands). The lagging strand is made as series of discrete fragments, each requiring a new RNA primer to initiate synthesis. The DNA between the last RNA priming event and the end of the chromosome cannot be replicated because there is no DNA beyond the end to which the next RNA primer can anneal, thus this gap cannot be filled in (this is referred to as the '→ end replication problem'). Since one strand cannot copy its end, telomere shortening occurs during progressive cell divisions. The shortened telomeres are inherited by daughter cells and the process repeats itself in subsequent divisions.

What is cellular senescence?

In contrast to tumor cells, which can divide forever (are 'immortal'), normal human cells have

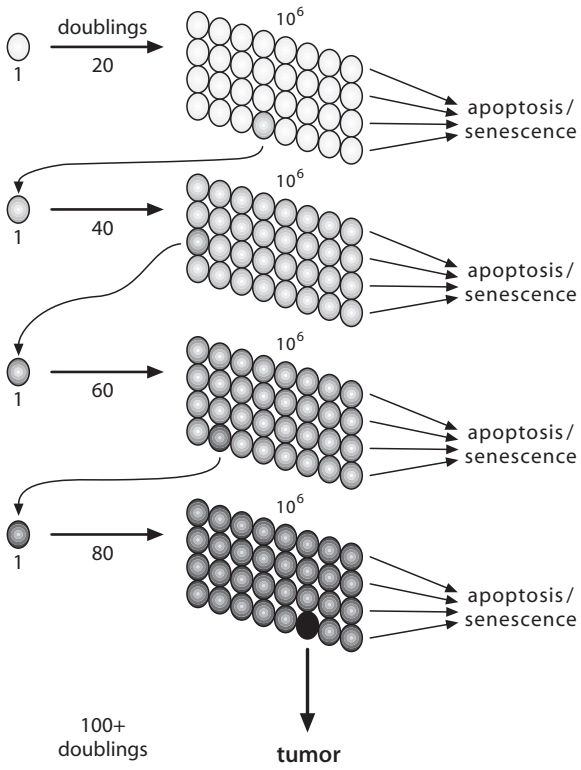


Telomerase. Fig. 2 – Telomeric sequences are synthesized by telomerase, a ribonucleoprotein enzyme (composed of both RNA and protein). Telomerase contains RNA-dependent DNA polymerase activity which uses its RNA component (complementary to the telomeric single stranded overhang) as a template in order to synthesize TTAGGG repeats (elongate) directly onto telomeric ends. After adding six bases, the enzyme is thought to pause while it repositions (translocates) the template RNA for the synthesis of the next six bp repeat. This extension of the 3′ DNA template end in turn permits additional replication of the 5′ end of the lagging strand, thus compensating for the telomere shortening that occur in its absence.

a limited capacity to proliferate (are 'mortal'). In general, cells cultured from a fetus divide more times in culture than those from a child, which in turn divide more times than those from an adult. The length of the telomeres decreases both as a function of donor age and with the number of times a cell has divided in culture. There appear to be two mechanisms responsible for the proliferative failure of normal cells. The first, M1 (Mortality stage 1), occurs when there are still at least several thousand base pairs of telomeric sequences left at the end of most of the chromosomes. M1 may be induced by a DNA damage signal produced by one or a few of the 92 telomeres that have particularly short telomeres. The M1 mechanism causes growth arrest mediated by the tumor suppressor genes *p16*, *RB1* and *p53*. If the actions of *p53* and *p16/pRB* are blocked, either by mutation or by binding to viral oncoproteins, then cells can continue to divide and telomeres continue to shorten until the M2 (Mortality stage 2) mechanism is induced. M2 represents the physiological result of critically short telomeres when cells are no longer able to protect the ends of the chromosomes, so that end-degradation and end-to-end fusion occurs and causes genomic instability and cell death. In cultured cells, a focus of immortal cells occasionally arises. In most cases, these cells have reactivated the expression of telomerase, which is able to repair and maintain the telomeres.

If you can stop the shortening of telomeres will this prevent cellular aging?

While there have been many studies indicating that there is a correlation between telomere shortening and proliferative failure of human cells, the evidence that it is causal has only recently been demonstrated. Introduction of the telomerase catalytic protein component into normal human cells without detectable telomerase results in restoration of telomerase activity (2). Normal human cells stably expressing transfected telomerase demonstrate extension of lifespan, providing more direct evidence that telomere shortening controls cellular aging. The cells with introduced telomerase maintain a normal chromosome complement and continue to grow in a normal manner. In-



Telomerase. Fig. 3 – It has been argued that it may not be necessary to exhaust the replicative potential of normal cells in order to form a massive tumor (e.g. after 50 doublings a single cell could generate a tumor of a size greater than 1000 kilograms). However, this theoretical argument assumes that all cells survive, which is highly unlikely to be correct. If the frequency of spontaneous mutations is approximately 10^{-6}, at least a million cells are needed for a second mutation to occur with reasonable probability. Since these mutations must accumulate in the same cell, a series of clonal expansions must occur as is illustrated in this figure. Since it requires 20 cell doublings to generate approximately one million cells, 20 divisions would accompany each mutation. For example if we assume five mutations are necessary for cancer to arise from a normal cell, more than 100 divisions (doublings) would be required to render a cell malignant. Losses of cells due to → apoptosis or inhibition of cell proliferation due to senescence would limit the number of cells in a such a tumor to $10^6 - 10^8$ cells which is less than a 1 gram biomass. Thus, with the possible exception of certain stem cells from the bone marrow, skin, and perhaps the intestine, most normal human cells only divide 50 – 70 times before they growth arrest. Thus cellular senescence could act as a very effective 'brake' on the proliferation of cells that had accumulated a few mutations but not all those prerequisite for malignancy.

itial concerns that the introduction of telomerase into normal cells may substantially increase the risk of cancer have not proven true. One way to think about this is that special reproductive tissues maintain high levels of telomerase throughout life, and there is no increased incidence of cancers in these special cells compared with other types of cancer. Thus, the major role of telomerase is to maintain telomere stability and keep the cells dividing. These observations provide the first direct evidence for the hypothesis that telomere length determines the proliferative capacity of human cells.

Can telomerase be used as a product to extend cell lifespan?

The ability to immortalize human cells and retain normal behavior holds promise in several areas of biopharmaceutical research, including drug development, screening and toxicology testing. The development of better cellular models of human disease and production of human products are among the immediate applications of this new advance. This technology has the potential to produce unlimited quantites of normal human cells of virtually any tissue type and may have most immediate translational applications in the area of transplantation medicine. In the future it may be possible to take a persons own cells, manipulate and rejuvenate them without using up their lifespan and then give them back to the patient. In addition, genetic engineering of telomerase-immortalized cells could lead to the development of cell-based therapies for certain genetic disorders such as muscular dystrophy.

Cell and molecular regulation

Proteins have been identified that directly interact with telomerase, such as p23/hsp90 (molecular chaperones) and TEP1 (telomerase associated protein 1 with unknown function). In addition, there are likely to be other proteins that help regulate telomerase function that have yet to be identified. The transcriptional regulation of the catalytic subunit of telomerase (→ hTERT) is clearly complex, but there is recent evidence that the *c-myc* gene may be important

in some aspects of the transcriptional activation of hTERT. In addition, there is evidence that a gene on chromosome 3 may be involved in the transciptional repression of hTERT. Since telomerase interacts with the telomeres, there has been a number of proteins identified that directly or indirectly bind to telomeres (TRF1, TRF2, tankyrase, TIN2, hRap1) that are also important in the regulation of telomerase.

There is also regulation of the level of telomerase activity in specific cell types. Telomerase activity is low in the most primitive stem cells of renewal tissues (e.g. crypts of the intestine, bone marrow cells, resting lymphocytes, basal layer of the epidermis), while telomerase activity is increased in the proliferative decendents of these cells. Thus, there are telomerase competent cells that have low activity when quiescent (not dividing) and increased activity when proliferating (dividing). However, these telomerase competent stem cells do not fully maintain telomere length since such cells obtained from older individuals have shorter telomeres than those derived from younger individuals. Thus, in germline (reproductive) cells and tumor cells, telomerase fully maintains telomere length in contrast to stem cells (with regulated telomerase acitivty) and most somatic cells (with no detectable telomerase activity) in which telomeres progressively shorten with increased age.

Cellular senescence may have evolved, in part, to protect long lived organisms, such as humans, against the early development of cancer. Thus, it has been proposed that upregulation or reexpression of telomerase may be a critical event responsible for continuous tumor cell growth (1). In contrast to normal cells, tumor cells show no net loss of average telomere length with cell division, suggesting that telomere stability may be required for cells to escape from replicative senescence and proliferate indefinitely. Most, but not necessarily all, malignant tumors may need telomerase to sustain their growth (3). → Immortalization of cells may occur through a mutation of a gene in the telomerase repression pathway. Thus, upregulation or reactivation of telomerase activity may be a rate-limiting step required for the continuing proliferation of advanced cancers. There is experimental evidence from hundreds of independent laboratories that telomerase activity is present in approximately 90 percent of all human tumors, but not in tissues adjacent to the tumors. Thus, clinical telomerase research is currently focused on the development of methods for the accurate diagnosis of cancer and on novel anti-telomerase cancer therapeutics.

Clinical Relevance

There is mounting evidence that cellular senescence acts as a 'cancer brake' as it takes many divisions to accumulate all the changes needed to become a cancer cell. In addition to the accumulation of several mutations in oncogenes and tumor suppressor genes, almost all cancer cells are immortal and, thus, have overcome the normal cellular signals that prevent continued division. Young normal cells can divide many times, but these cells are not cancer cells since they have not accumulated all the other changes needed to make a cell malignant. In most instances a cell becomes senescent before it can become a cancer cell. Therefore, aging and cancer are two ends of the same spectrum. The key issue is to find out how to make our cancer cells mortal and our healthy cells immortal, or at least longer lasting. Inhibition of telomerase in cancer cells may be a viable target for anti-cancer therapeutics, while expression of telomerase in normal cells may have important biopharmaceutical and medical applications (4). In summary, telomerase is both an important target for cancer and for the treatment of age-related disease.

Could telomerase be the 'Achilles heel' of cancer?

We believe that progressive telomere shortening is halted in cancer cells by the presence of the enzyme telomerase which maintains and stabilizes the telomeres, allowing cells to divide indefinitely. Telomerase activity is detected in almost all human tumors. It is hoped that a therapy can be developed that inhibits telomerase activity and interferes with the growth of many types of cancer.

Will inhibiting telomerase restore the senescence program in cancer cells and if so will this therapy cure cancer?

One research strategy is to inhibit the activity of telomerase, forcing immortal cells into a normal pattern of permanent growth arrest (senescence) or death (apoptosis). Following conventional treatments (surgery, radiotherapy, chemotherapy), anti-telomerase agents would be given to limit the proliferative capacity of the rare surviving tumor cells in the hope that this would prevent cancer recurrence. We believe this treatment would be very selective, in that only cells with an activated telomerase would be affected. As far as we know, that includes only 'immortal' tumor cells and germline (reproductive cells) and at lower levels stem cells in renewal tissues.

Will telomerase activity be useful in cancer diagnostics?

Telomerase activity is detected in premalignant specimens (*in situ* lung and breast cancers), while colon and pancreatic cancer have detectable telomerase activity at later (carcinoma) stages. The ability to use almost any clinical specimen and to demonstrate telomerase may allow the detection of cancers at an earlier stage. For example, telomerase activity is detected in lung cells in cancer patients obtained by bronchial alveolar lavage. In addition, fine needle aspirations (breast, liver and prostate cancer), washes (bladder and colon), and sedimented cells from urine (bladder and prostate) provide minimally invasive sources of cells to detect telomerase activity and are likely to have immediate diagnostic utility. Telomerase may also be important in the monitoring of minimal residual disease. In an effort to improve the diagnostic value of telomerase determinations, *in situ* hybridization methods for the demonstration of telomerase on archival paraffin embedded clinical specimens appears to distinguish cancer from normal cells, correlates well with telomerase activity, and thus may provide added value to telomerase activity assays. In addition, the presence or absence of telomerase may have prognostic value and help risk-stratify patients into those with favorable outcomes (to avoid unnecessary treatments for patients with low or no detectable telomerase) and those with high telomerase activity and with unfavourable outcomes (to help oncologists manage patient treatments more effectively).

Have any telomerase therapeutic agents been identified and what are the potential complications of such strategies?

Since telomerase is expressed in most advanced cancers, methods for telomerase inhibition using small molecules such as modified oligonucleotides may have utility. There are potential risks in the use of such therapy that must be considered, for example the affects of inhibitors on telomerase-expressing stem cells. However, it is likely that this approach will be less toxic than conventional chemotherapy which affects all proliferating cells, including stem cells. The rate of division of the most primitive stem cells is so much slower than that of most cancer cells that the amount of telomere shortening in the stem cells should be relatively small. Some of the side effects of standard chemotherapy, such as thrombocytopenia, leukopenia, nausea and hair loss due to the death of the cells in rapidly proliferating tissues, may be reduced by the use of telomerase inhibitors, which are predicted to induce cellular senescence or cell death only after a period of growth. This raises what many consider the most important concern with this proposed treatment regimen, the prolonged time potentially required for a telomerase inhibitor to be effective. Since the mode of action of telomerase inhibitors may require telomeric shortening before inhibition of cell growth or induction of apoptosis, there may be a significant delay in efficacy. Thus methods may have to be devised to increase the rate of telomere shortening when telomerase inhibitors are used therapeutically. Telomerase inhibitors will likely be used together with or following conventional therapies, so that once the bulk of the tumor mass is eliminated, anti-telomerase therapy might prevent the large number of cell divisions required for the regrowth of rare resistant cancer cells.

They may also be used in early stage cancer to prevent overgrowth of metastatic cells, as well as in high-risk patients with inherited susceptibility to cancer syndromes to prevent the emergence of telomerase-expressing cells (chemoprevention).

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Telomeres

Definition

Telomeres are repeated DNA sequences [$(TTAGG)_n$ in humans] found at the ends of linear chromosomes that protect the ends of the chromsome from degradation (→ telomerase).

TEP1

Definition

→ PTEN.

Teratogenic

Definition

Teratogenic substances cause developmental malformations during gestation.

Terminal Protein

Definition

The terminal protein of → adenovirus binds to the ends of the viral genome where it functions as a protein primer for DNA synthesis, a requirement of the adenoviral polymerase.

TERT

Definition

Telomerase reverse transcriptase (Tert), also known as TRT, TP2, TCS1 or hEST2, is the telomerase catalytic subunit (ec 2.7.7.-) of 1132 amino acids and 126 kD. The human TERT, TRT, EST2 or TCS1 gene locus maps at 5p15.33 and the mouse tert gene locus at chromosome 13 (43.00 cM). Tert, a part of the telomerase ribonucleoprotein enzyme, is a reverse transcriptase that adds simple sequence repeats to chromosome ends by copying a template sequence within the RNA component of the enzyme. Telomerase is essential for the replication of chromosome termini in most eukaryotes, by elongating telomeres.

Tetraploid

Definition

A tetraploid cell contains four times the haploid genome. This exists in a cell that has completed DNA replication but has not yet divided, or a cell that has been generated from two diploid cells by cell fusion.

TFE Family

Definition

The Tfe family is a group of → bHLH proteins comprising Tfe3, Tfeb, Tfec, Mitf and Mi. They bind to the immunoglobulin μ heavy chain gene enhancer and are involved in placenta vascularization and also in development of melanocytes, osteoclasts and masts cells.

TFIIH

Definition

TFIIH is a protein complex that plays a key role in both transcription and DNA repair.

TGF-β

Definition

→ Transforming growth factor β (TGF-β) is a multifunctional dimeric 25 kD polypeptide growth factor, whose main functions are growth inhibition, immunosuppression and regulation of extracellular matrix formation and turnover. There are three different mammalian gene products. Activation renders the biologically latent TGF-β into its active form, which can bind to the cell surface receptors and initiate signaling. The TGFβ superfamily contains at least forty cytokines currently divided into two classes: the bone morphogenetic proteins (BMP) and the TGFβ/activin. These cytokines regulate cell fate (proliferation, differentiation and → apoptosis) and extracellular matrix deposition.

TGF-α

Definition

Transforming growth factor α (TGF-α) is an epidermal growth factor (EGF)-like growth factor that binds to the epidermal growth factor receptor. TGF-α acts synergistically with TGF-β to promote anchorage-independent cell proliferation. The protein is 160 aa long and has a molecular weight of 17 kD; the gene maps to 2p13.

Thalidomide

Definition

Thalidomide is a therapeutic drug that was administered to pregnant women in the late 1950s for the prevention of sickness. However, major teratogenic effects occurred, especially phocomelia, related to the anti-angiogenic properties of thalidomide. Recently, thalidomide has been reintroduced in therapy for the treatment of many conditions such as chronic → graft versus host disease, leprosa or Behcets disease. More recently, exciting results have been reported in the treatment of refractory myeloma, but also in other hematopoietic malignancies such as myelodysplastic syndromes or myelofibrosis. Anti-angiogenic effects are probably not the sole therapeutic properties of this drug.

T Helper

Definition

CD4+ T helper (Th) subsets are characterized by their distinct cytokine production profiles. Th1 cells secrete interleukin-2 (IL-2), IFNγ and TNFβ, which promote cellular immune responses against intracellular pathogens and viruses. Th2 cells produce → IL-4, IL-5, → IL-6 IL-10 and IL-13, which promote humoral immunity by aiding in B cell growth and differentiation.

Therapy, genetic

Definition

→ Gene therapy.

THRA

Definition

→ Thyroid hormone receptor α.

THRB

Definition

→ THRB.

Thyroid C Cells

Definition

Thyroid C cells, also known as parafollicular cells, lie in the substance of the thyroid gland, adjacent to the thyroid follicles which produce thyroid hormone, but are of different origin and function from thyroid follicular cells. C cells are derived from the neural ectoderm and they synthesise and secrete the hormone calcitonin; → MEN 2.

Thyroid Carcinogenesis

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Definition

Malignant transformation of thyroid follicle-lining epithelial cells and progression to a clinically manifest thyroid carcinoma. Thyrocytes, embryologically derived from the primitive pharynx, produce thyroid hormones and are thus involved in the regulation of metabolic pathways. Malignant transformation of these cells gives rise to various types of differentiated and undifferentiated thyroid carcinomas. In contrast, medullary carcinomas (5–15% of all thyroid carcinomas) originate from calcitonin-producing neural crest-derived C cells and form a separate entity.

Characteristics

The main groups of non-medullary epithelial thyroid carcinomas include

- Follicular carcinoma (< 20% of all thyroid carcinomas), composed of closely packed follicles lined by cuboidal or columnar cells with dark-staining round nuclei and eosinophilic cytoplasm. Capsular and/or vascular invasion, missing in follicular adenoma, is crucial for diagnosis of follicular carcinoma. The tumor spreads via the blood stream, with metastasis preferentially to bone and lung, occasionally to brain and liver.
- Papillary carcinoma (> 60% of all thyroid carcinomas), cytologically characterized by typical changes of nuclei with ground glass appearance, overlapping pattern, irregular shape with clefts, grooves and pseudo-inclusions. The main histological variants include
  - typical papillary variant, classical pattern, showing arborized papillae with fibrovascular core, often containing calcified psammoma bodies
  - follicular variant with irregularly shaped colloid-containing follicles, composed of cells typical for papillary carcinoma
  - solid variant exhibiting closely packed cell clusters with the characteristic cytology of papillary carcinoma, separate by a thin fibrovascular stroma
  - diffuse sclerozing variant containing large areas of fibrosis with small foci of cells typical of papillary carcinoma.

  Multicentricity is common to papillary carcinoma. Lymphatic spread and metastasis formation in regional lymph nodes is frequent in all variants of papillary carcinoma.

- Undifferentiated anaplastic carcinoma (5 – 10%), composed of pleomorphic polygonal, round or spindle cells with mitotic figures, shows rapid growth and invasion and early metastasis to regional lymph nodes and to lungs. This type is more common in older individuals.

General features

Thyroid carcinomas are the most common endocrine malignant tumors with a variable annual incidence in different parts of the world ranging from 5 to 100 per million, with females two to four times more often affected than males. The incidence of spontaneous tumors increases with age. Tumors are detected and diagnosed by palpation, ultrasound, thyroid scans and fine needle aspiration biopsy.

- Therapy involves surgery (total or near-total thyroidectomy, often combined with lymph node dissection) and, when distant metastasis of iodine-concentrating carcinomas has to be assumed, radioiodine ablation of disseminated metastatic cells. Metastases that do not take up radioiodine may be treated by surgery, external radiation or chemotherapy.
- Prognosis: Young patients have a more favourable prognosis than older persons, irrespective of the tumor type. Widely invasive follicular or undifferentiated anaplastic carcinomas have an adverse prognosis, particularly in males at older age. Anaplastic carcinomas are most aggressive with a mortality of more than 90%. In contrast, the mortality of patients suffering from papillary carcinomas is low; more than 90% have a 10-year survival. Papillary microcarcinomas may remain clinically undetected and are often found only at autopsy.
- Cases of familial thyroid carcinomas (about 3% of all thyroid carcinomas) indicate that hereditary genetic factors might play a role in thyroid carcinogenesis. However, linkage analyses did not reveal typical susceptibility genes. Instead, genetic heterogeneity in familial nonmedullary thyroid carcinomas is most probable.
- Environmental factors contribute to thyroid carcinogenesis. Iodine deficiency is a risk factor: In iodine deficient regions, particularly in mountain areas, an excess of follicular carcinomas is observed in goiterous thyroid glands. High TSH production, because of the negative feedback regulation by a decreased thyroid hormone level due to iodine deprivation, may act as a continuous mitotic stimulus for thyrocytes and thus as a cofactor for thyroid carcinogenesis. Iodine supplementation in such areas reduces the risk of follicular carcinoma. However, high thyroid tumor incidence has been observed also in areas of high iodine (Hawaii, Iceland) where nutritional factors might interfere with iodine uptake and/or metabolism. It has been hypothesized that papillary carcinomas may be more frequent in iodine-rich areas. Upward trends of this tumor type, however, are observed in most affluent countries. The reasons are unknown.

Role of ionizing radiation

Ionizing radiation is an accepted risk factor for thyroid carcinogenesis. The thyroid gland is one of the most radiation sensitive organs of the body. External therapeutic radiation that had been administered in former years to head, neck or mediastinal regions of children suffering from tinea capitis, enlarged tonsils, adenoids or thymus, hemangioma or Hodgkin disease resulted, after a 10 to 30 years latency period, in an increased incidence of thyroid carcinomas. It was concluded from large epidemiological studies that the risk for thyroid carcinoma is significantly increased in children before an age of 15 years even after a thyroid dose as low as 0.1 Gy, with a linear dose/risk relation except for very high doses at which the cytocidal effect may be more pronounced than the transforming action. A mean excess relative risk of 7.7 per Gy thyroid dose has been calculated for children. A comparable risk has not been observed for adults after external radiation or after ^{131}I treatment because of benign thyroid diseases or for diagnostic purpose.

Knowledge about correlations between radiation exposure and thyroid carcinogenesis

has greatly been increased by studies on survivors of the atomic bomb explosions in Japan, or after the testing of nuclear weapons at Bikini with contamination of the Marshall Island, and, above all, by studies after the Chernobyl reactor accident. After the Chernobyl nuclear power plant explosion in April 1986, about 2×10^{18} Bq of radioiodine isotopes, besides other radioisotopes, were released to the environment. Children who lived in the most severely contaminated regions of Belarus, the Oblast Gomel in particular, received thyroid doses in the range of 0.15 to 4.7 Gy, in exceptional cases even 10 Gy and more. Thyroid doses in adults were lower by a factor of 3 to 5. After a latency period of about 4 years, thyroid cancer incidence increased steeply in exposed children, up to a factor of nearly 200 in the regions of largest radioactive fallout. Congruence of areas of radioiodine contamination and of elevated thyroid cancer incidence argues for radioiodine as an etiological factor.

Genetic aberrations

Molecular studies on structural genetic aberrations did not disclose a characteristic tumor-specific pattern in sporadic thyroid carcinomas: Mutations of codons 12, 13 or 61 of *H-*, *K-* or *N-RAS* are found in 20 to 30% of follicular adenomas and carcinomas. Obviously, a constitutive activation of the ras/raf signal transduction cascade as a consequence of these *RAS* mutations is an important molecular change at an early stage of follicular carcinogenesis. Connections to iodine deficiency or radiation exposure are not apparent. The prevalence of *RAS* mutations is lower in papillary carcinomas.

Mutations at codon 201 or 227 of the Gsa subunit of the → *GSP* gene or TSH receptor mutations have been observed in hyperfunctioning thyroid adenomas. These mutations are almost completely missing in follicular or papillary carcinomas. Obviously, the TSH receptor-adenylate cyclase-protein kinase A pathway is not primarily affected in epithelial thyroid carcinomas. The prevalence of mutations of the tumor suppressor gene *p53* (exons 5 to 8) is high in anaplastic thyroid carcinomas, but rarely found in other thyroid tumors. *p53* mutational inactivation appears closely related to progression from a differentiated to an undifferentiated anaplastic, highly aggressive thyroid carcinoma.

→ Loss of heterozygosity on a variety of chromosomes has been reported to be a common feature of follicular neoplasms, particularly carcinomas, suggesting loss of tumor suppressor genes during thyroid carcinogenesis. It is less frequently found in papillary carcinomas.

RET gene changes

Rearrangements of the → receptor tyrosine kinase gene → *RET* have been detected in about 17% of sporadic papillary thyroid carcinomas of adults, with very high geographic variation. In addition, another receptor tyrosine kinase gene, *NTRK1*, is rearranged in rare cases of this tumor type. In contrast to sporadic thyroid carcinomas, radiation-induced tumors of the thyroid show a more homogeneous pattern of molecular aberrations, as evident from analyses of post-Chernobyl papillary thyroid carcinomas: In more than 60%, rearrangements of the proto-oncogene *RET* were found. Oncogenic *RET* rearrangements are characterized by loss of the 5′ part of *RET* including exon 11 and replacement by part of a *RET*-fused gene. In the rearranged fusion gene, the *RET* tyrosine kinase domain remains intact at the 3′ end, while the *RET* transmembrane and extracellular domains, located upstream of the tyrosine kinase domain and essential for the stringent normal regulation of *RET* activity by ligand-receptor complex interactions, are missing. They are replaced by 5′ regulatory subunits of the *RET*-fused genes. This leads to an unphysiological activation of the *RET* tyrosine kinase in thyrocytes which, under normal conditions, are devoid of this activity.

The preferential *RET* fusion partners in radiation-induced thyroid carcinomas are

- *H4*, a gene of yet unknown function, giving rise to *H4/RET* fusions (PTC1),
- *ELE1*, a transcriptional activator of the androgen receptor (*ELE1/RET* fusions, PTC3).

Both types of rearrangement are formed by a balanced reciprocal intrachromosomal para-

centric inversion of *RET* at 10q11.2, *ELE1* also at 10q11.2, or *H4* at 10q21. Less frequently, *RET* rearrangements involve interchromosomal translocations, as found with the

- *RIα* gene, coding for the catalytic domain of cAMP-dependent protein kinase A, at 17q23, leading to *RIα/RET* fusions (PTC2),

or in the newly detected types of *RET* rearrangement with the fused genes located on chromosomes other than 10:

- *GOLGA5*, coding for golgin-84 located in the membrane of Golgi vesicles (*GOLGA5/RET* fusions, PTC5),
- *HTIF1*, a transcriptional activator of several nuclear receptors (*HTIF1/RET* fusions, PTC6),
- *RFG7*, a homologue of *HTIF1* (*RFG7/RET* fusions, PTC7),
- *RFG8* of a yet unknown function (*RFG8/RET* fusions, PTC8) on 18q21-22,
- *ELKS* with unknown function, on 12p13.3 (*ELKS/RET* fusion).

It is common to all *RET*-fused genes detected so far that they are ubiquitously expressed and contain coiled-coil structures. Thus a unique type of fusion protein is formed in thyrocytes combining two characteristic peculiarities: the potential for dimerization and the tyrosine kinase domain. Dimerization of this fusion protein may lead to constitutive, RET-ligand-(GDNF)-independent activation of the RET tyrosine kinase, autophosphorylation and activation of signal transduction pathways not normally active in thyrocytes. The processes involved in the aberrant signal transduction which will finally end up in clonal expansion of the transformed cell(s) are not yet understood. However, the dominant thyroid tumor-inducing effect of the fusion genes has convincingly been demonstrated in *H4/RET* or *ELE1/RET* transgenic mice.

The balanced reciprocal translocation does not only cause dysregulation of RET tyrosine kinase by the *RET*-fused gene, but leads also to a reciprocal transcript with RET at the 5′ end and the *RET*-fused gene at the 3′ part of the fusion protein. The ensuing disturbance of the physiological function of the RET-fused genes may influence the mode of tumor development. For example, *ELE1/RET* rearrangements induce preferentially solid variants of papillary thyroid carcinomas, in contrast to *H4/RET* rearrangements that are related to classical papillary or follicular variants.

The relevance of *RET* rearrangement as a characteristic radiation effect in human thyroid tissue has recently been experimentally corroborated; radiation leads to the formation of *H4/RET* rearrangements in normal human thyroid tissue transplanted to → SCID mice. Epidemiological studies revealed that patients who had undergone radiotherapy earlier in life disclosed more often *RET* rearrangements in papillary thyroid carcinomas than patients without radiation exposure. On the other side, epidemiological data are yet insufficient to conclude that the presence of *RET* rearrangement proves radiation as a causative factor in an individual papillary thyroid carcinoma.

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Thyroid Hormone Receptor α

Definition

Thyroid hormone receptor α is also known as THRA1, THRA2, ERBA1, avian erythroblastic leukemia viral (v-erb-A1) oncogene homolog. It is a protein of 410 amino acids and 46 kD that is a high affinity receptor for triiodothyronine. Two forms, α-1 and α-2, are produced by alternative splicing, THRA1 and THRA2. Thyroid hormone receptor α belongs to the nuclear hormone receptor family and the gene maps to locus 17q11.

Thyroid Hormone Receptor β

Definition

Thyroid hormone receptor β, also known as avian erythroblastic leukemia viral (v-erb-A) oncogene homolog 2, is a protein of 461 amino acids and 52 kD that is high affinity nuclear receptor for triiodothyronine. It belongs to the nuclear hormone receptor family, subfamily NR1, and the gene maps to locus 3p24.

Tight Junctions

Definition

Tight junctions are an intercellular junctional structure, typically found in epithelia and endothelia. In the tight junction, the two membranes of neighboring cells are brought into close proximity through binding of specific transmembrane proteins. This results in a selectivity barrier that seals the apical lumen from the basolateral intercellular space and also establishes cellular polarity by preventing membrane linked molecules from freely diffusing between the apical and the basolateral cell surface (→ cell adhesion molecules).

Tissue Microarray

Definition

Tissue microarrays are microscope slides containing hundreds to thousands of tissue specimen sections arrayed at high density. The tissue sections are approximately 0.6 mm in diameter and 4 to 5 μm thick. They facilitate the simultaneous high-throughput parallel analysis of protein levels (IHC), genomic DNA copy number (FISH) and transcript levels (mRNA *in situ*) of hundreds of clinical tissue sections.

Tissue-specifically Expressed Genes

Definition

Tissue-specifically expressed genes are genes that are expressed in only a subset of tissues or developmental stages and express proteins with functions specific for certain cell types.

TKO

Definition

→ Technical knockout (TKO) is a method to isolate genes by a functional approach.

TNF

Definition

→ Tumor necrosis factor α.

TNF Receptor-associated Factor 2

Definition

→ Tumor necrosis factor receptor-associated factor 2.

TNF-α in HIV Infection

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Characteristics

Tumor necrosis factor-α: molecular characteristics, and receptors

In the 1950s-1960s, it was reported that several patients with malignant tumors had a spontaneous regression of their tumors after bacterial infections. In the 1970s, a bacterially-induced circulating host factor was associated with this anti-tumor activity and was designated 'tumor necrosis factor' (TNF). Subsequently, TNF was isolated, cloned and found to be the leader of the 'TNF superfamily' and a pleiotropic cytokine representing a major mediator of the inflammatory and immune responses. The human *TNF* gene is located on chromosome 6p23-6q12 between class I HLA region for *HLA-B* gene and the gene encoding the complement factor c; it contains four exons in a total length of 3.6 kb. Human TNF-α is a non-glycosylated protein of 157 amino acids with a molecular weight of 17 kD. A 233 amino acid precursor (26 kD) is synthetized, expressed at cell surface and cleaved by the 85 kD TNF-α converting enzyme (TACE). This TNF precursor is biologically active and considered as the 'membrane TNF' form. The 17 kD peptides interact together and form circulating homotrimers. The level of circulating TNF ranges between 10 and 80 pg/mL in non-pathological conditions. TNF is secreted by various cell populations such as cells of the macrophage lineage (monocytes, macrophages, microglial cells), CD4+ and CD8+ T cells, dendritic cells, neutrophils, adipocytes, keratinocytes, astrocytes, neurons and pancreatic cells.

Two distinct membrane receptors for TNF have been identified and cloned:

- a 55 kD receptor (p55) or TNF-R1, newly designated as CD120a and referred as TNF receptor superfamily member 1A (TNFRSF1A);
- a 75 kD receptor (p75) or TNF-R2, newly designated as CD120b and referred as TNF receptor superfamily member 1B (TNFRSF1B).

TNF-R1 and -R2 are glycoproteins of 455 and 461 amino acids, respectively. Soluble forms of TNF-R1 (at least two molecular weights 32 and 48 kD) and -R2 (42 kD) are generated by proteolytic cleavage. Cells such as monocytes/macrophages, endothelial cells express both TNF-R1 and -R2. It was first suggested that TNF-α was bound by TNF-R2 and transferred to TNF-R1, which then is activated; in fact, TNF-R1 may be the main receptor for soluble TNF-α, whereas membrane TNF preferentially interacts with TNF-R2.

Physiopathological role of TNF-α in HIV infection

Human immunodeficiency virus (HIV) is a retrovirus that infects preferentially T CD4+ lymphocytes and macrophages, a major source of TNF-α. As a consequence, close relationships exist between HIV and TNF-α; an implication of TNF-α has been reported in HIV replication, neuroAIDS, → cachexia and in the development of opportunistic infections and tumors.

Relationships between HIV replication and TNF-α

TNF-α is a pro-inflammatory cytokine and thus has been considered as a cytokine that could increase HIV replication. In fact, the effects of TNF-α on HIV replication is dual and opposite:

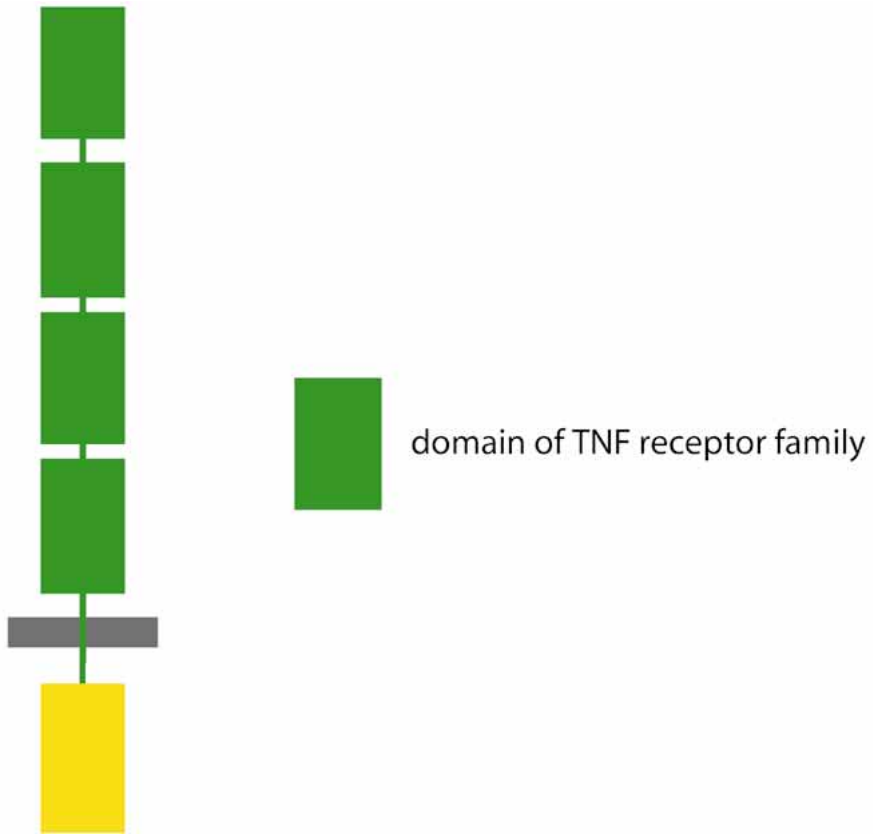
- inhibition of HIV entry, and
- increase of expression of proviral genome and production of HIV particles.

The inhibitory effects on HIV entry is due to the capacity of TNF-α to favor the synthesis of β-chemokines e.g. regulated upon activation, normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1α and MIP-1β, the natural ligands that compete with HIV particles to bind their receptor, which is also the co-receptor of HIV. The transcription factor NFκB participates, in part, to the deleterious effects of TNF-α on HIV production; this transcription factor is activated by TNF-α, and interacts with domains regulating the expression of HIV (long terminal repeat or LTR), in which two binding sites has been identified (Fig. 1).

TNF-α is a cytokine that is secreted by cells of the immune system in the area of microbial infections e.g. viral infections. In HIV infection, immune cells are HIV targets, and dysregulations of TNF-α synthesis induced by the HIV infection were explored in macrophages. Several authors demonstrated that the infection of macrophages by HIV is not sufficient to induce the synthesis of TNF-α in the absence of other environmental factors. In contrast, TNF-α synthesis may be increased in the presence of soluble factors or cells; only few contaminating lymphocytes favor, for example, the macrophagic TNF-α synthesis in response of HIV infection.

Role of TNF-α in neuroAIDS

TNF-α is also a neurotoxic factor that is implicated in the neuronal death in HIV disease. TNF-α can be synthetized in central nervous system (CNS), and the passage of blood brain barrier is not required for its presence *in*



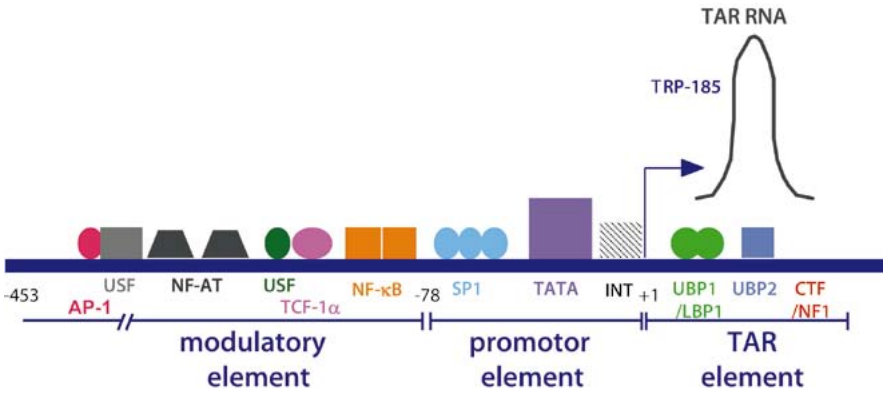
TNF-α in HIV Infection. Fig. 1 – Structure of TNF receptor superfamily members. TNF-R1 (p55, CD120a, TNF-RSF1A), TNF-R2 (p75, CD120b, TNF-RSF1B).

situ. The mechanisms of this neurotoxicity involve the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor and the N-methyl-D-aspartate (NMDA) receptor. TNF-α inhibits the glutamate uptake by astrocytes and favors the synthesis or release of the phospholipid mediator platelet-activating factor (PAF) which enhances excitatory transmission. Moreover, TNF-α also participates in the pathogenesis of neuroAIDS by regulating NFκB activation and HIV replication levels in perivascular macrophages and microglial cells. Indeed, viral factors such as the envelope glycoprotein gp120 and the transactivator protein → Tat are known to be neurotoxic factors, and Tat can induce TNF-α synthesis. Both TNF receptors are present in the CNS, and on neurons in particular. The role of TNF-R2 in the CNS remains largely unknown. TNF-R1 can promote apoptosis of neurons via the release of silencer of death domains (SODD) permitting the association with intracellular proteins such as TNF receptor-associated death domain (TRADD), FAS-associated death domain (FADD) and caspase-8 (FLICE/CASP8). The activation of acid sphingomyelinase by caspase-8 contributes to the cleavage of sphingomyelin to phosphocholine and ceramide, which activates various enzymes e.g. phosphatases, protein kinases involved in apoptosis (Fig. 2). A new neurodegeneration process has recently emerged; the silencing of survival signal (SOSS). TNF-α, at picogram concentrations, inhibits the survival signaling mediated by insulin-like growth factor 1 (IGF 1) in neurons; TNF-α inhibits tyrosine phosphorylation of insulin receptor substrate 2 (IRS2) and the activation of the survival enzyme, phosphatidylinositol 3′ kinase (PI3 kinase) and the formation of phosphatidylinositol(3,4,5)P3, known to play a pivotal role in the regulation of cell proliferation and survival.

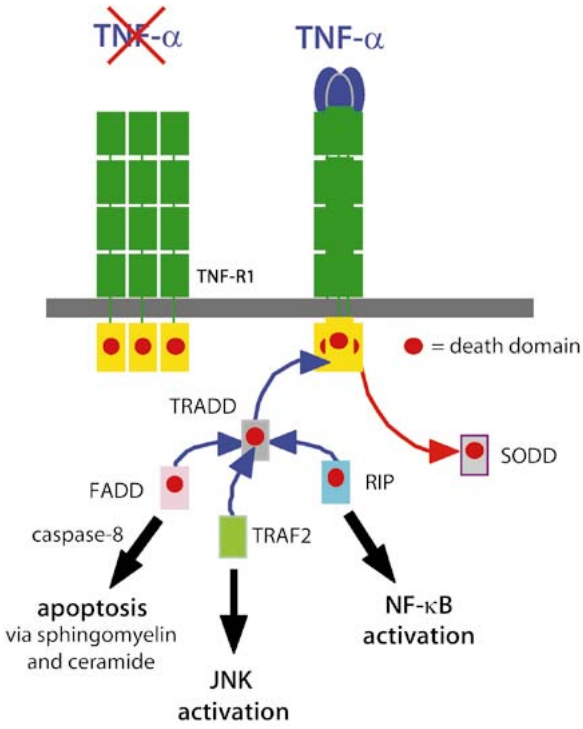
However, as the effects of TNF-α on HIV replication, the effects of TNF-α in apoptosis may be opposite. TNF-α may be also a neuroprotective factor, for example, in excitotoxic conditions. Members of the family of TNF receptor-associated factors (TRAF), particularly TRAF2 (Fig. 3), may play a major role in this process; it increases the TNF-α-induced JNK and NFκB activation known to favor cell proliferation, and a dominant-negative mutant enhances apoptosis.

Role of TNF-α in cachexia, opportunistic infections and tumors

TNF-α plays a major role in cachexia in HIV-infected patients but also in the development of opportunistic infections e.g. *Mycobacterium avium*, and tumors e.g. malignant lymphoma and → Kaposi sarcoma. The immune activation and TNF-α synthesis induced by the *Mycobacterium* infection amplifies the infection of proximal cells by HIV and their level of viral replication. This viral replication accentuates the TNF-α secretion, and harmful connections between the replication of HIV or opportunistic



TNF-α in HIV Infection. Fig. 2 – Different regulatory elements in the HIV LTR domain (LTR: long terminal repeat).



TNF-α in HIV Infection. Fig. 3 – Different signaling pathways mediating biological effects of TNF-α. Tumor necrosis factor-a triggers the release of SODD and the DD domain of TNF-R1, that recruits TRADD via an interaction with the DD domain of this cell factor. Then, TRADD interacts with FADD, TNF-R-associated factor 2 (TRAF2) or kinase receptor-interacting protein (RIP). FADD is involved in the apoptotic signal cascade via caspase-8, TRAF2 in JNK activation, and RIP in NFκB activation.

pathogens, and immune activation or inflammation, are created. In Kaposi sarcoma, TNF-α favors the acquisition of the phenotype and functional features of AIDS-related KS spindle cells by endothelial cells. Moreover, TNF-α could be also responsible for deleterious effects of compounds delivered as treatment of opportunistic infections e.g. amphotericin B and cryptococcal meningitis.

Clinical relevance of molecules inhibiting TNF-α synthesis

In order to reduce the TNF-α -dependent HIV replication and the chronic inflammation that is associated with HIV infection, several inhibitors of TNF synthesis and TNF-receptor antagonists were evaluated *in vitro* or *in vivo* as therapeutical agent (e.g. RP-55778, pentoxifylline, chimeric humanized monoclonal antibody cA2). These molecules decreased *in vitro* TNF-α synthesis and HIV-1 replication. *In vivo*, pentoxifylline and cA2 were tested but no beneficial effects were observed on HIV viral load; only TNF-α synthesis was decreased. These data confirm that an inhibitor of TNF synthesis could not be used as pharmacological agent in monotherapy of HIV disease.

Systemic inflammation is decreased by highly active antiretroviral therapy (HAART); therefore, TNF-α and TNF-R2 may constitute reliable predictive markers of efficiency of HAART, or its failure. In tissues, particularly in CNS, HAART is less efficient to decrease HIV replication and chronic inflammation. As a consequence, anti-TNF-α molecules could be theoretically a good pharmacological strategy as adjuvant therapy. Nevertheless, caution is also required because it is important to preserve antimicrobial effects promoted by TNF-α against a variety of infectious agents, and particularly HIV.

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TNFR-1

Definition

→ Tumor necrosis factor receptor-1.

TNFRSF6

Definition

Tumor necrosis factor receptor superfamily, member 6; → Fas.

TNFRSF12

Definition

Tumor necrosis factor receptor superfamily, member 12 (TNFRSF12) is primarily expressed on thymocytes and lymophocytes. It induces → NFκB activation and → apoptosis when overexpressed. There is frequent allelic loss in human → neuroblastoma and the gene maps to locus 1p36.3.

TNM

Definition

TNM stands for tumor, nodes, metastases. It is a staging system used to evaluate the extent and burden of the tumor that considers its site, size and spread within the prostate gland and the degree to which it has metastasized to the lymph nodes or to distant sites.

Tobacco Carcinogenesis

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Definition

Tobacco carcinogenesis is the study of cancer induction by tobacco products and their constituents in laboratory animals and humans.

Characteristics

Tobacco and human cancer

Worldwide tobacco use is staggering. According to estimates by the World Health Organization, there are about one billion smokers in the world, representing approximately one third of the global population aged 15 years or higher. 30% of all cancer death in developed countries is caused by tobacco products. Lung cancer is the dominant malignancy caused by smoking, with 514,000 lung cancer deaths attributed to smoking in developed countries in 1995. Cigarette smoking is also an important cause of oral cancer, oropharyngeal cancer, hypopharyngeal cancer, laryngeal cancer, esophageal cancer, pancreatic cancer, cancer of the renal pelvis and bladder cancer. Other cancers that may be caused by smoking include renal adenocarcinoma, cancer of the cervix, myeloid leukemia, colon cancer and stomach cancer. Environmental tobacco smoke can cause lung cancer in non-smokers, although the risk is far less than in smokers. Smokeless tobacco products, such as moist snuff and betel quid-containing tobacco, are accepted causes of oral cavity cancer.

Tumor induction in laboratory animals

Experimental studies evaluating the ability of cigarette smoke and its condensate to cause cancer in laboratory animals have collectively demonstrated that inhalation of tobacco smoke as well as topical application of tobacco smoke condensate cause cancer in experimental animals. Inhalation studies have achieved the most success using the Syrian golden hamster. Studies in other rodents and dogs have been less frequent. Cigarette smoke condensate has been extensively tested on mouse skin, where it consistently induces benign and malignant skin tumors. Extracts of unburned tobacco have shown carcinogenic activity in some rodent tests, but the results are inconsistent.

Chemistry of tobacco smoke

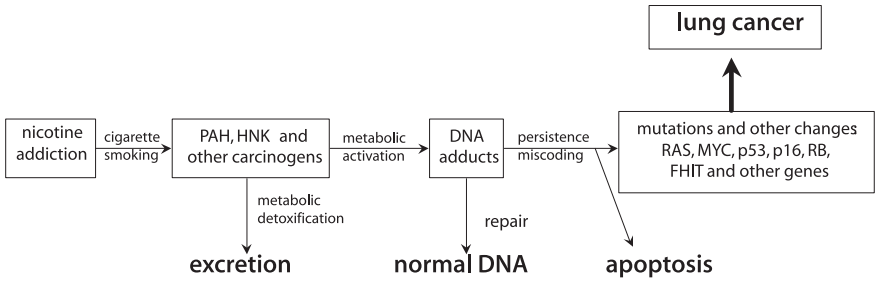
When cigarette tobacco is burned, mainstream and sidestream smoke are generated. Mainstream smoke is the material drawn from the mouth end of a cigarette during puffing. Sidestream smoke is the material released into the air from the burning tip of the cigarette plus the material which diffuses through the paper. Environmental tobacco smoke is a composite of sidestream smoke and exhaled smoke. Mainstream smoke is an aerosol containing about 1×10^{10} particles per ml. About 95% of the smoke is made of gases, chiefly nitrogen, oxygen and carbon dioxide. The particulate phase of mainstream smoke contains more than 3500 compounds and most of the carcinogens. Many components are present in higher concentrations in sidestream than in mainstream smoke, but a person's exposure to sidestream smoke is far less than to mainstream smoke because of dilution with room air.

There are 55 carcinogens in cigarette smoke that have been evaluated by the International Agency for Research on Cancer, and for which there is sufficient evidence for carcinogenicity in laboratory animals or humans. Carcinogens in cigarette smoke come from various classes of compounds including polycyclic aromatic hydrocarbons (PAH), aza-arenes, *N*-nitrosamines, aromatic amines, heterocyclic aromatic amines, aldehydes, low molecular weight organic compounds and inorganic compounds. In addition, cigarette smoke contains tumor promoters, co-carcinogens and toxic agents such as acrolein and nitrogen oxides. The most important compounds with respect to human lung cancer appear to be the PAH, typified by benzo[*a*]pyrene (BaP) and the tobacco-specific *N*-nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Whereas PAH occur in all products of incomplete combustion, tobacco-specific *N*-nitrosamines are found only in tobacco products because they are derived from nicotine and related compounds. Tobacco-specific *N*-nitrosamines are the most prevalent strong carcinogens in unburned tobacco and are believed to play a significant role in the induction of oral cavity cancer by these products.

Mechanisms of tumor induction

The mechanisms by which tobacco causes cancer can best be illustrated by considering the relationship between cigarette smoking and lung cancer, because it is here that the most information is available. The overall framework for discussing this information is illustrated (Fig.). This general scheme is also applicable to other types of cancer caused by tobacco products. Carcinogens form the link between nicotine addiction and cancer. Nicotine addiction is the reason that people continue to smoke. While nicotine itself is not considered to be carcinogenic, each cigarette contains a mixture of carcinogens, including a small dose of PAH and NNK among other carcinogens, tumor promoters and co-carcinogens. Carcinogens such as NNK and PAH require metabolic activation, that is, they must be enzymatically transformed by the host into reactive intermediates in order to exert their carcinogenic effects. There are competing → detoxification pathways that result in harmless excretion of the carcinogen. The balance between metabolic activation and detoxification differs among individuals and will affect cancer risk.

The metabolic activation process leads to the formation of → adducts to DNA, which are carcinogen metabolites bound covalently to DNA, usually at guanine or adenine. There have been major advances in our understanding of DNA adduct structure and its consequences in the past two decades, and we now have a large amount of mechanistic information. If DNA adducts escape cellular repair mechanisms and persist they may lead to miscoding, resulting in a permanent mutation. This occurs when DNA polymerase enzymes read an adducted DNA base incorrectly, resulting in the insertion of the wrong base or other errors. As a result of clever strategies that combine DNA adduct chemistry with the tools of molecular biology, we know a great deal about the ways in which carcinogen DNA adducts cause mutations. Cells with damaged DNA may be removed by → apoptosis, or programmed cell death. If a permanent mutation occurs in a critical region of an → oncogene or a → tumor suppressor gene, it can lead to activation of the on-



Tobacco Carcinogenesis. Fig. – Linking nicotine addiction and lung cancer via tobacco smoke carcinogens and their induction of multiple mutations in critical genes. Persistence refers to DNA adducts that remain unrepaired through one or more cycles of replication; miscoding refers to the incorrect insertion of a base opposite a DNA adduct, resulting in a permanent mutation. [PAH, polycyclic aromatic hydrocarbons; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone]

cogene or deactivation of the tumor suppressor gene. Oncogenes and tumor suppressor genes play critical roles in the normal regulation of cellular growth. Changes in multiple tumor suppressor genes or oncogenes lead to aberrant cells, with loss of normal growth control and ultimately to cancer. While the sequence of events has not been well defined, there can be little doubt that these molecular changes are important. There is now a large amount of data on mutations in the human K-*ras* oncogene and *p53* tumor suppressor gene in lung tumors from smokers.

The → p53 gene plays a central role in the delicate balance of cellular proliferation and death. It is mutated in about half of all cancer types, including over 50% of lung cancers, leading to loss of its activity for regulation of cellular growth. Point mutations at guanine (G) are common. In a sample of 550 *p53* mutations in lung tumors, 33% were G→T transversions, while 26% were G→A transitions (a purine→pyrimidine or pyrimidine→purine mutation is referred to as a transversion, while a purine→purine or pyrimidine→pyrimidine mutation is called a transition). A positive relationship between lifetime cigarette consumption and the frequency of *p53* mutations and of G→T transversions on the nontranscribed DNA strand has also been noted. These observations are generally consistent with the fact that most activated carcinogens react predominantly at G, and that repair of the resulting adducts would be slower on the nontranscribed strand, and thus support the scheme outlined in the Figure.

Mutations in codon 12 of the K-*ras* oncogene are found in 24% to 50% of human primary adenocarcinomas, but are rarely seen in other lung tumor types. When K-*ras* is mutated, a complex series of cellular growth signals is initiated. Mutations in K-*ras* are more common in smokers and ex-smokers than in non-smokers, which suggests that they may be induced by direct reaction with the gene of an activated tobacco smoke carcinogen. The most commonly observed mutation is GGT→TGT, which typically accounts for about 60% of the codon 12 mutations, followed by GGT→GAT (20%) and GGT→GTT (15%).

Using a weight-of-the-evidence approach, specific PAH and the tobacco-specific nitrosamine NNK can be identified as probable causes of lung cancer in smokers, but the contribution of other agents cannot be excluded. The chronic exposure of smokers to the DNA-damaging intermediates formed from these carcinogens is consistent with our present understanding of cancer induction as a process that requires multiple genetic changes. Thus, it is completely plausible that the continual barrage of DNA damage produced by tobacco carcinogens causes the multiple genetic changes that are associated with cancer. While each dose of carcinogen from a cigarette is extremely small, the cumulative damage produced in years of smoking is substantial.

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Tolerance

Definition

Tolerance is a specific non-responsiveness of a recipients immune system to graft antigens that would normally elicit a rejection reaction.

Topoisomerase III

Definition

Topoisomerase III is an enzyme that changes the degree of supercoiling of DNA by cutting one strand of DNA and passing an intact strand through the break.

Topoisomerase Enzymes as Drug Targets

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Definition

Topoisomerases are nuclear enzymes that change the conformation of DNA. These conformational changes facilitate distribution of cellular DNA to daughter cells during cellular replication and division. Topoisomerase activity is required for separation of the chromosomes and, therefore, is essential for cell division. In human cells, the two major types of topoisomerases that are targets for anticancer drugs are:

- topoisomerase I
- topoisomerase II

Several different forms of each of these classes of topoisomerases have been described, but the main difference between the two classes is that the Type I enzymes change DNA structure by cleaving single strands of DNA, while the Type II enzymes cleave double strands of DNA. Each enzyme then passes another strand of DNA through the cleaved site and rejoins the cleaved DNA. The nucleotide sequence is unchanged, but the DNA is more or less twisted (coiled) than it was prior to the enzymatic reaction. Type III topoisomerases have also been reported, but their precise molecular function is still being investigated.

Characteristics

Cellular functions

All nucleated human cells and tumor cells contain topoisomerases and the activity of these enzymes is essential for cell division. Because topoisomerase activity is needed for cell division and because tumor cells by definition divide in an uncontrolled manner, inhibitors of

topoisomerases have been investigated extensively for their potential in anticancer therapy.

Additionally, recent evidence suggests that topoisomerases also have functions that are unrelated to their ability to change the conformation of DNA. These functions include:

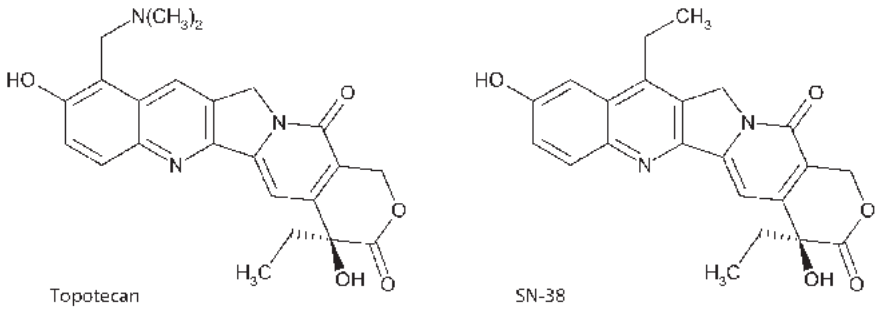
- binding to transcription factors or splicing factors prior to RNA and protein production,
- binding to stress response proteins such as hsp70 or to proteins that 'shuttle' back and forth between the nucleus and cytoplasm of cells,
- binding to proteins such as p53 which are thought to inhibit tumor formation,
- interaction with helicases to contribute to genomic stability,
- phosphorylation of other proteins to modify their activity.

Inhibitors of topoisomerases

Inhibitors of topoisomerases may inhibit either the Type I or Type II enzymes, or both. Effects of chemotherapeutic agents on Type III topoisomerases have not yet been investigated. Drugs that inhibit both Types I and II enzymes are not necessarily the most effective agents for all tumor types. Interestingly, some topoisomerase inhibitors are chemically very similar, but differ markedly in the types of tumors against which they are effective. For example, the → camptothecin derivatives topotecan and CPT-11 inhibit topoisomerase I. Topotecan and the active form of CPT-11 (SN-38) differ only in the position of a short side chain at position 7 (SN-38) or 9 (topotecan)(Fig.). CPT-11 is efficacious in the treatment of colon adenocarcinoma, while topotecan is not. The molecular basis for this difference is not understood. The dose limiting side effects of chemically similar compounds can also differ markedly. The limiting side effect of topotecan is bone marrow suppression, while the limiting toxicity of CPT-11 is diarrhea.

Mechanism by which inhibitors of topoisomerases kill tumor cells

Historically, the way topoisomerase inhibitors were thought to kill tumor cells could be explained simply: The inhibitor decreased the ability of the topoisomerase enzyme to rejoin the strand of DNA that it had cleaved. The broken DNA strand was nonfunctional and interpreted by the cell as DNA damage. DNA damage induced → apoptosis (cell death). However, this simple explanation no longer suffices, as new classes of inhibitors are being developed that do not inhibit religation and therefore do not cause direct DNA damage, but do cause cell



Topoisomerase Enzymes as Drug Targets. Fig. – The camptothecin derivatives topotecan (left) and SN-38 (right) have very similar chemical structures but have different antitumor activities. Both of the compounds belong to the same chemical class of drugs and both inhibit topoisomerase I; however, they are effective in treating different types of tumors. Factors that have been postulated to affect the antitumor efficacy of these two drugs include such things as how each drug is distributed throughout the body, the ability or inability of a certain tissue or tumor to convert CPT-11 to its active form SN-38 and whether a tumor cell may express proteins (multi drug-resistance protein, MRP or P-glycoprotein, Pgp) that transport specific drugs out of a tumor cell.

death. Further, the extent to which topoisomerase inhibitors that are used in chemotherapy alter relatively newly described functions, such as interactions with p53 or helicases, is unknown.

Numerous theories have been proposed as to why topoisomerase inhibitors are selectively toxic to tumor cells compared to normal cells. While it is true that some tumors overexpress topoisomerases thereby increasing the number of targets (topoisomerase molecules bound to DNA) and that tumors have a greater propensity to divide than normal cells, there is no single cellular characteristic that predicts whether a tumor cell will or will not respond to topoisomerase inhibitors. Several cellular factors have been identified that do contribute to the overall efficacy of these drugs. These factors include relatively high levels of topoisomerases in the nucleus of the cell, actively dividing cells, and the absence of cell membrane transport proteins (Pgp, MRP, BCRP, cMOAT) that export chemotherapeutic agents from cells.

Topoisomerase inhibitors used clinically

The following table contains topoisomerase inhibitors used frequently in the clinic. All of the topoisomerase I inhibitors listed belong to the chemical class of camptothecins; these compounds are either extracted from the Chinese tree *Camptotheca acuminata* or are semisynthetic derivatives of the parent camptothecin compound. The topoisomerase II inhibitors are of several chemical classes including the epipodophyllotoxins (VM-26 and VP-16), anthracyclines (doxorubicin and mitoxantrone) and aminoacridines (m-AMSA). Actinomycin D (and perhaps doxorubicin and m-AMSA) is likely a dual topoisomerase I/II inhibitor. Like the camptothecins, these topoisomerase II inhibitors are isolated or derived from botanical or bacterial sources.

Antitumor efficacy of topoisomerase inhibitors, scheduling and side effects

Topoisomerase II inhibitors, such as doxorubicin and actinomycin D, have antitumor effects against many types of cancers. VP-16 is used extensively in treating pediatric acute lymphocytic leukemia (ALL). The camptothecin class of topoisomerase I inhibitors is used for the treatment of various solid tumors.

Of all the combinations and schedules of administration that have been investigated using these agents, two observations deserve specific mention. Inhibitors of topoisomerases I and II are mutagenic in experimental systems; further, VP-16 is associated with secondary malignancies in patients. However, these secondary malignancies are remarkably schedule-dependent in that, for example, the incidence of secondary acute myelocytic leukemia (AML) in pediatric lymphocytic leukemia patients increased only in patients given relatively low doses of VP-16 for extended periods of time. The second observation concerning unique characteristics of these agents is the recent evidence suggesting that topotecan and CPT-11 are much more effective when given in lower doses over more prolonged times, compared to fewer, relatively high doses.

The side effects of the topoisomerase inhibitors may or may not be related to their ability to inhibit topoisomerases themselves. The muta-

Topoisomerase Enzymes as Drug Targets. Table – Anticancer agents that inhibit topoisomerases.

DNA Topoisomerase I	DNA Topoisomerase II
9-aminocamptothecin (9-AC)	VM-26 and VP-16 (teniposide and etoposide)
9-nitrocamptothecin (9-NC)	mitoxantrone
topotecan	m-AMSA (amsacrine)
irinotecan (CPT-11)	doxorubicin (adriamycin)
	actinomycin D (act D)

genicity of VP-16 is probably related to the DNA damage that results from prolonged inhibition of topoisomerase II. In contrast, the cardiotoxicity of doxorubicin and the gastrointestinal toxicity of CPT-11 is probably not topoisomerase dependent, suggesting that analogues of these compounds might be designed that retain their antitumor properties, but have fewer dose limiting side effects than analogues currently used in the clinic.

In summary, inhibitors of topoisomerases are extremely important as chemotherapeutic agents for treating both hematopoietic and solid malignancies. Antitumor efficacy may be related to inhibition of not only DNA conformational changes, but to inhibition of the other multiple functions that are now being identified for these enzymes.

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TOR

Definition

The phosphatidylinositol (PI) kinase homologue TOR ('target of → rapamycin') is the cellular target of the complex of FK506 binding protein (FKBP) with the immunosuppressant rapamycin. TOR is part of the general mitotic signalling pathway involving a tyrosine kinase, a phosphatidylinositol 3-kinase (PI3K), Akt/PKB kinase, p70S6 kinase (p70S6k) and 4E-BP1 (PHAS-I). TOR is conserved from yeast to mammals and is also known as FRAP, RAFT, RAPT or mTOR.

TP53

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Synonyms

- TP53 (p53) is a p53 tumor suppressor gene

Definition

TP53 is located on chromosome 17p13.1 and encodes an ubiquitous phosphoprotein of molecular mass 51-53,000, essentially expressed in the nucleus. This gene is frequently inactivated by somatic mutation or by loss of alleles in many common human cancers. More than 14,000 such mutations have been described so far. Inherited, heterozygous mutations have been identified in about 150 families with → Li-Fraumeni Syndrome (LFS), a rare familial pathology characterised by the early occurrence of cancers at multiple organ sites. *TP53* belongs to a gene family which also includes *TP73* (1p36) and *P63* (3p28). In contrast with *TP53*, these two genes have a restricted, tissue specific and developmental expression pattern and are not frequently mutated in cancer (1).

The p53 protein is a latent transcription factor, which is activated in response to multiple forms of physical and chemical stress to exert diverse, complementary effects in the regulation of cell proliferation, genetic integrity and survival. These effects include:

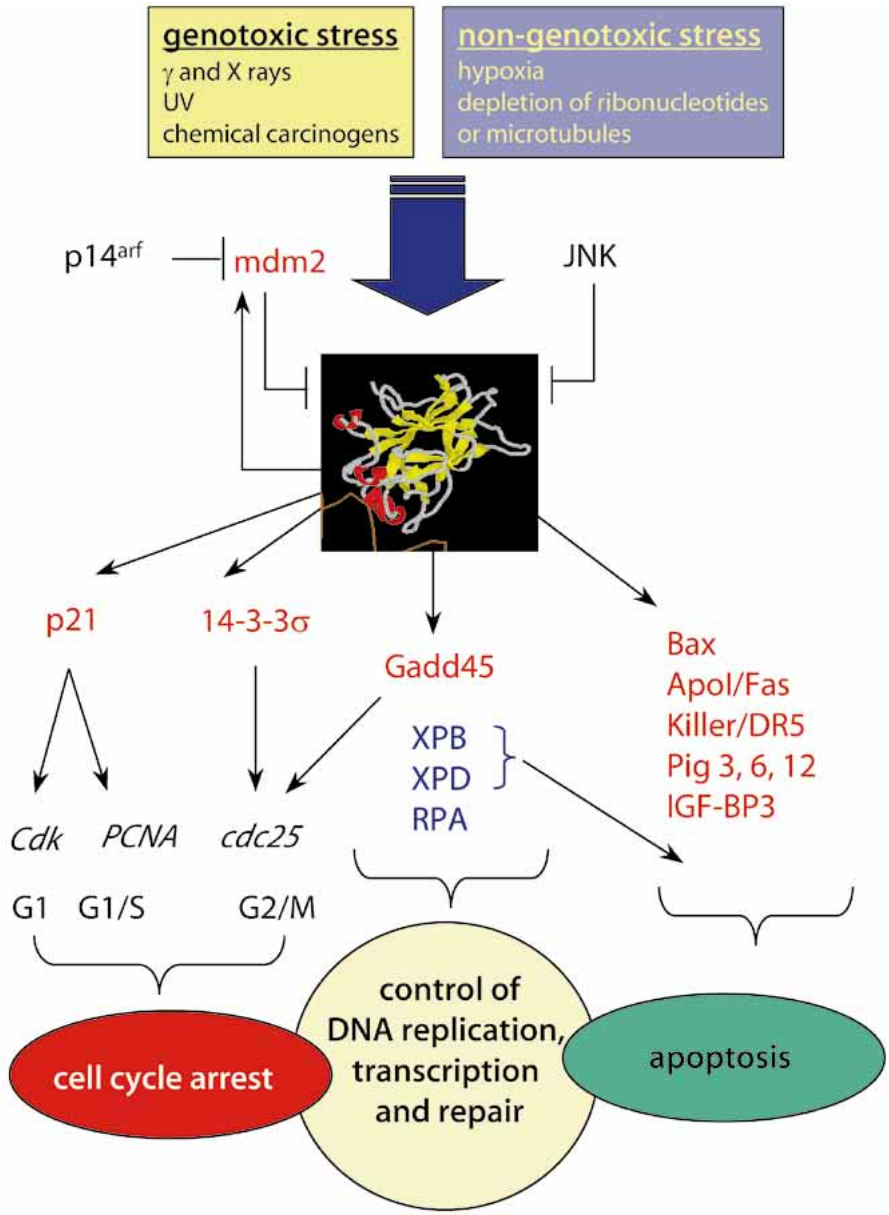
- induction of apoptosis
- control of cell division through regulation of cell-cycle progression in G1 and G2, of centrosome duplication and of mitosis
- modulation of DNA replication and repair.

The main function of the p53 protein is to act as an 'emergency brake' to prevent the proliferation of cells with damaged genetic material, as a

result of exposure to genotoxic agents (Fig. 1) (2). In a broader context, the protein acts as an integrator of multiple exogenous and intracellular signals to regulate cell proliferation during replicative senescence, differentiation and development. Inactivation of *TP53* by homologous recombination in mice results in accelerated development of multiple tumors, while a fraction of p53-deficient embryos display a lethal defect in neural tubule closure resulting in exencephaly (3).

Characteristics

The *TP53* gene spans 20 kb and contains 11 exons, the first one being non-coding. The cod-



TP53. Fig. 1 – The p53 pathway. The p53 protein is induced in response to various forms of stress and mediates a set of coordianted, anti-proliferative responses including cell-cycle arrest, control of replication, transcription and repair and apoptosis. Blue: factors that bind to p53 and that are regulated by protein interactions. Red: factors that are regulated by p53 at the transcriptional level.

ing sequence contains five regions showing a high degree of conservation in vertebrates, located in 2, 5, 6, 7 and 8. An orthologue has recently been described in *Drosophila*. Several polymorphisms are identified in the human population, with allele frequencies that vary with ethnic origin. However, there is only limited evidence that these polymorphisms play a role in tumor susceptibility.

The *TP53* gene gene does not contain a conventional TATA box, but is under the control of several ubiquitous transcription factors including NFkB, Sp1 and c-Jun. It is generally expressed in the form of one major transcript of 2.8 kb, although several shorter forms corresponding to alternatively spliced variants have been described.

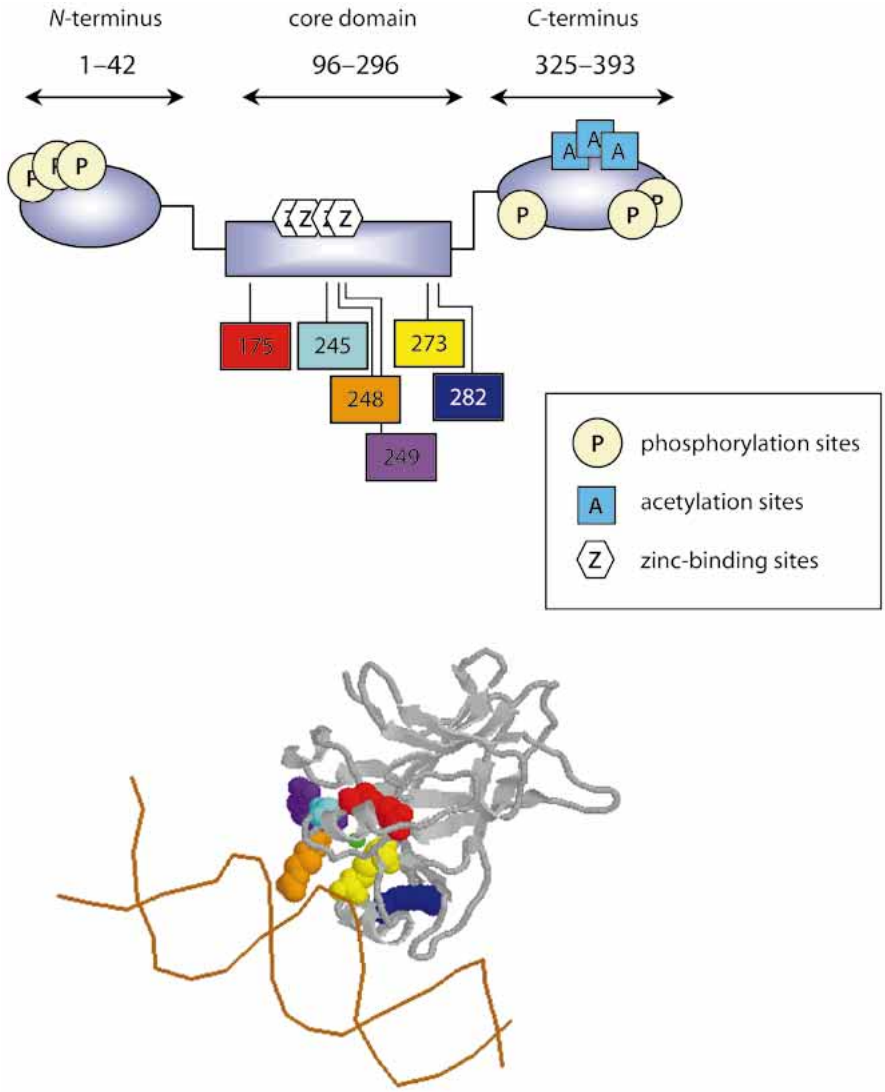
The protein contains 393 residues and is organized in a hydrophobic, central core (residues 110-296, encoded by exons 5 to 8), flanked by an acidic N-terminus and a basic C-terminus (Fig. 2 top). The N-terminus contains two complementary transcriptional activation domains, with a major one at residues 1-42 and a minor one at residues 55-75, specifically involved in the regulation of several pro-apoptotic genes. The central core is made of a scaffold of two β-sheets supporting a set of flexible loops and helices stabilized by the binding of an atom of zinc. These loops and helices make direct contact with DNA sequences containing inverted repeats of the motif RRRC(A/T) (4). The C-terminus contains the main nuclear localisation signals and oligomerisation domains (residues 325-366). The active form of the protein is a tetramer (in fact, a pair of dimers). The extreme C-terminus has multiple regulatory functions and exerts a negative control on sequence-specific DNA binding activities. Both N- and C-terminal regions contain multiple phosphorylation sites, while none has been identified so far in the central core (see Table 1).

Upstream of p53: signalling of DNA damage

The p53 protein is constitutively expressed in most cells and tissues as a latent factor. Due to its rapid turnover (5-20 minutes), the protein does not accumulate unless it is stabilized in response to a variety of intracellular and extracellular stimuli. Signals that activate p53 include diverse types of DNA damage (strand breaks, bulky adducts, oxidation of bases), blockage of RNA elongation, hypoxia, depletion of microtubules, ribonucleotides or growth factors, modulation of cell adhesion and alteration of polyamine metabolism. Most of the current knowledge on p53 protein activation is derived from studies using DNA strand breaks as inducing signals.

The main regulator of p53 protein activity is mdm-2, a protein which binds p53 in the N-terminus (residues 17-29); it conceals its transcription activation domain, redirects p53 from nucleus to the cytoplasm and acts as an ubiquitin-ligase to target p53 for degradation by the proteasome. The MDM-2 gene is a transcriptional target of p53, thus defining a regulatory feedback loop in which p53 controls its own stability. The p53/mdm-2 complex is regulated by Arf (Alternative Reading Frame), a 14 kD protein encoded by the *p16/ CDKN2A* gene (5).

The kinetics, extent and consequences of p53 activation vary according to the nature and intensity of the inducing signals (2). In response to ionizing radiation, activation of the p53 protein in response to stress is thought to proceed through several consecutive steps, with first phosphorylation of p53 in the N-terminus by kinases involved in the sensing of DNA damage such as Atm (the product of the → ataxia telangiectasia mutated gene) and Chk-2 (a cell-cycle regulatory kinase). These phosphorylations contribute to the dissociation of the p53/mdm-2 complex and stabilize the protein. Second, p53 binds co-activators with acetyltransferase activity such as p300/CBP and pCAF. These factors acetylate p53 in the C-terminus. This process, as well as other coordinated post-translational modifications of the C-terminus, induce conformational changes that turn the protein into an active form with a high affinity for specific DNA sites. The third step involves redox-regulation of sensitive cysteines within the DNA-binding domain of the protein. This three-step mechanism may account for p53 induction in response to most forms of DNA damage (1, 2). However, how p53 becomes activated by non-DNA damaging signals is still largely unknown.



TP53. Fig. 2 – Diagram of the p53 protein structure. top: linear structure, showing the three main structural domains. Codon numbers of the main mutation hotspots are shown as coloured boxes. Sites of post-translational modifications are shown as 'P' (phosphorylations), 'A' (acetylations) and 'Z' (zinc binding sites). bottom: 3-D structure of the central core of p53 in complex with target DNA. Hotspot residues are shown in the same color code as above.

Downstream of p53: cell-cycle control, apoptosis and DNA repair

Once activated, p53 exerts its effects through two major mechanisms: transcriptional control (activation or repression of specific genes) and complex formation with other proteins. Important downstream effectors of p53 (Table 2) include regulators of cell-cycle checkpoints (in G1/S, G2 and during mitosis), factors involved in the signalling of apoptosis, and components of the transcription, replication and repair machineries. At the cellular level, activation of p53 most frequently results in either cell cycle arrest (mostly in G1 and/or G2/M) or apoptosis. How a given cell 'chooses' between cell cycle arrest and apoptosis in response to specific stimuli may depend upon many factors, such as the nature and intensity of the stress, as well as the cell type. In many tissues, p53 plays a role in drug-induced apoptosis and is

TP53. Table 1 – Factors involved in the activation and post-translational modification of p53.

factor	biochemical function / activated by	interaction with p53
PARP	ADP-ribose polymerase / DNA strand breaks, nucleotide depletion	ADP-ribose polymers bind to p53
HMG-1	high mobility group 1 / ?	binding to N-terminus or to DNA-binding domain
E6AP	E6 accessory protein / ubiquitin-mediated degradation	binding to p53
Hif-1	hypoxia-inducible factor / Hypoxia	binding to p53
14-3-3 s	cell-cycle regulator / ionizing radiations	binding, C-terminus (Ser-376)
p300/CBP	histone acetyl-transferases / co-activators of transcription	binding, N-terminus acetylation, C-terminus
c-abl	tyrosine kinase / irradiation, DNA-strand breaks	binding, proline-rich region
mdm-2	oncogene / negative control of p53	binding, residues 13-29
NO	nitric oxide / oxidative stress, inflammation, irradiation	oxidation of cysteines in DNA-binding domain
Cdc2/Cdk2-Cyclin A/B	cell-cycle dependent kinases	phosphorylation of Ser-315; forms complexes with p53
cdk7-cyclin H	component of TFIIH	phosphorylation of Ser-33
CKII	kinase / UV	phosphorylation of Ser-389; forms complexes with p53
MAPK	mitogen-activated protein kinase / UV?	phosphorylation, Thr-73 and 83 (mouse p53)
ATM	kinase / ionizing radiations	phosphorylation, Ser-15
DNA-PK	kinase / UV	phosphorylation, Ser-15 and Ser-37
Chk-2	cell-cycle-dependent kinase	phosphorylation, Ser-20
JNK/p38	stress-activated kinases / UV	phosphorylation, Ser-34, mouse p53
PKC	protein kinase C	phosphorylation, Ser-378
CKI	kinase / ?	phosphorylation, several N-terminal serines (including Ser-6 and Ser-9)
p19arf	cell-cycle inhibitor, alternative product of CDKN2A	prevents p53-mdm2 interactions
Ref-1	redox-repair enzyme / oxidative stress, hypoxia	reduction of cysteine in DNA-binding region binding to C-terminus

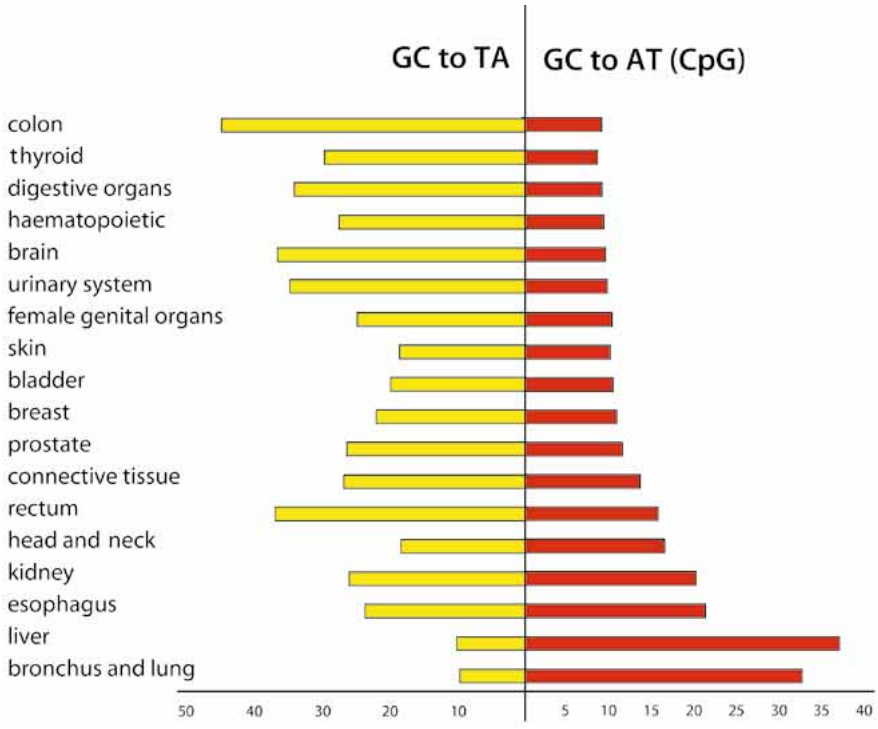
thus an important effector in the response of cancer cells to chemo- or radio- therapy (6). In addition, loss of p53 function results in deficient cell cycle arrest, inefficient mitotic spindle checkpoint, aberrant centrosome duplication, premature re-entry into S phase, genomic instability and aneuploidy.

Clinical Relevance

The *TP53* gene is often inactivated by missense mutations, in contrast with many other tumor suppressors such as *APC*, *RB1*, *BRCA1* or *p16/CDKN2A*. The mutations described to date mostly occur in the region of the gene encoding the DNA binding domain. Most of these mutations impair DNA binding by disrupting the structure of the domain or crucial contact points between the protein and target DNA. About 30% of missense mutations affect 6 'hotspot' codons (175, 245, 248, 249, 273 and 282) (Fig. 2 bottom). The other mutations are scattered over 200 different codons. Mutations are very common in the invasive stages of many epithelial tumors. A database of all published mutations is available at the International Agency for Research on Cancer (http://www.iarc.fr/p53/homepage.htm).

In many cancers, the pattern of mutations show variations revealing clues about the mechanisms responsible for the formation of the mutations (1). Specific carcinogen-induced mutations have been identified in hepatocellular carcinoma (mutations induced by aflatoxins in sub-Saharan Africa and in south-east Asia), in skin tumors (double transitions at adjacent cytosines, a typical signature of mutagenesis by UV in squamous and in basal cell carcinomas) and in lung cancers (G to T transversions associated with exposure to tobacco smoke; → tobacco carcinogenesis; - Fig. 3).

The usefulness of *TP53* mutation detection in molecular pathology is still a matter of debate. As mutation often results in the accumu-



TP53. Fig. 3 – Prevalence of two common mutation types: G to T transversions and C to T transitions at dipyrimidine (CpG) repeats in tumors of various organs. Tumors with high prevalence of G to T transversions often have a low prevalence of transitions and vice-versa. G to T transitions are a common molecular signature of many environmental carcinogens, such as tobacco smoke components (lung and esophageal cancers) or dietary mycotoxins (liver cancer).

TP53. Table 2 – Some important downstream effectors of p53 functions.

factor	activity	mode of regulation	function
Apo-1/Fas/CD95	death signalling receptor	transcriptional activation?	apoptosis
Bax-1	dominant-negative inhibitor of bcl2	transcriptional activation	apoptosis
Bcl-2	repressor of apoptosis	transcriptional repression	apoptosis
IGF-BP3	inhibitor of IGF-I	transcriptional activation	apoptosis
Killer/DR5	death signalling receptor	transcriptional activation	apoptosis
P85	regulatory subunit of PI3 kinase	transcriptional activation	apoptosis
Pig-12	glutathione transferase homologue	transcriptional activation	apoptosis
Pig-3	quinone oxidase homologue	transcriptional activation	apoptosis
Pig-6	proline oxidase homologue	transcriptional activation	apoptosis
IGF-I	growth factor	transcriptional repression	apoptosis?
IL-6	survival factor	transcriptional repression	apoptosis?
thrombo-spondin-1	inhibitor of angiogenesis	transcriptional activation	apoptosis?
Gadd45	binding to PCNA	transcriptional activation	cell-cycle arrest ?
BTG2	inhibitor of proliferation	transcriptional activation	cell-cycle arrest, G1
p21waf-1	inhibitor of CDK2-4 and 6	transcriptional activation	cell-cycle arrest, G1 and G2/M
cyclin A	cell-cycle regulation, S phase	transcriptional repression	cell-cycle arrest, G1/S
cyclin G	cell-cycle regulation	transcriptional activation	cell-cycle arrest?
GPx	glutathione peroxidase	transcriptional repression	control of oxidative stress
NOS2/iNOS	inducible Nitric Oxide synthase	transcriptional repression	control of oxidative stress
COX2	inducible cyclooxygenase	transcriptional repression	control of oxidative stress?
Pig-1	galectin-7	transcriptional activation	differentiation?
PCNA	auxiliary subunit of poly-merase δ	transcriptional activation	DNA repair/replication
RPA	replication protein A	inhibition by protein binding	DNA repair/replication
ERCC2/ERCC3	helicases, TFIIH complex	activation by protein binding	DNA repair/transcription
P53RR2	ribonucleotide reductase homolog	transcriptional activation	DNA repair?
TBP	TATA box-binding protein	inhibition by protein binding	inhibition of transcription
Mdm-2	oncogene	transcriptional activation	repression of p53
MDR-1	multi-drug resistance	transcriptional repression	resistance to chemotherapy

lation of the protein, immunohistochemistry (IHC) has often been used as a criterion to detect *TP53* abnormalities. However, positive IHC does not always correlate with mutation as several common missense mutants, as well as most frameshift and nonsense mutants, do not result in protein accumulation. Several, well established methods have been described for the detection of mutations in *TP53*, including SSCP (Single Stranded Conformation Polymorphism analysis), TTGE (Temporal Temperature Gradient Electrophoresis), yeast-based functional assays and, recently, micro-array hybridization assays.

TP53 mutations are good markers for the clonality of tumor lesions. In many tissues, mutation correlates with bad prognosis and poor response to therapy, but only in rare cases has *TP53* mutation been shown to behave as an independent marker of prognosis. Recent evidence suggest that the nature and position of the mutation may help to predict poor response to treatment. Detection of circulating anti-p53 antibodies as well as of free plasmatic DNA containing mutant *TP53* may have an interest for the early detection of cancer lesions.

TP53 is the target of several experimental therapeutic approaches. Gene transfer of wild-type *TP53* into cancer cells has been tested in several human tumors. However, the effects reported to date are limited and, at best, transient. Another approach is based on the use of cytolytic viruses selectively replicating in *TP53*-deficient cells (ONYX vectors)(7). Recently, several pre-clinical studies have investigated the use of small lipophilic coumpounds or peptides to activate *TP53* function or to restore the activity of mutant proteins (8).

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TP73

Definition

→ Tumor protein 73.

TPA

Definition

12-O-tetradecanoyl-phorbol-13-acetate, a tumor promoting agent; → tumor promoters.

T-PLL

Definition

T-Prolymphocytic leukemia; → T-cell leukemia/lymphoma 1 (TCL1).

TRAF1

Definition

Tumor necrosis factor receptor-associated factor 1 (TRAF1) is one of a number of adaptor molecules that are involved with tumor necrosis factor receptor superfamily signaling.

TRAF2

Definition

→ Tumor necrosis factor receptor-associated factor 2.

TRAIL

Definition

TNF-related → apoptosis-inducing ligand (TRAIL).

Transactivation

Definition

Transactivation is the stimulation of gene expression by diffusable mediators, for example proteins that are usually encoded on distant regions of the genome.

Transcription

Definition

Transcription is the synthesis of RNA from a DNA template (DNA synthesis from RNA template: → reverse transcription). It is a process that results in the production of mRNA, complementary to DNA sequences encoded by genes; → RNA polymerase.

Transcription Factor

Definition

A transcription factor is a protein that binds to DNA and regulates the transcription of genes. The term is generally used to indicate a protein that binds to specific DNA sequences to selectively increase or decrease the transcription of a specific gene or set of genes.

Transcription Repair

Definition

Transcription repair (TCR) is a subpathway of nucleotide excision repair that removes lesions from actively transcribed DNA.

Transcription Unit

Definition

Transcription unit is the distance between the sites of RNA polymerase initiation and termination, which may include more than one gene.

Transcription-coupled Repair

Definition

Transcription-coupled repair is the rapid repair of DNA damage in the transcribed strand of expressing genes.

Transcriptome

Definition

A transcriptome is the entire set of transcribed sequences (RNAs) of a cell.

Transduction of Oncogenes

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Synonyms

- oncogene transduction
- retroviral transduction

Definition

Transduction generally refers to the transfer of genetic information from one organism to another. In the case of retroviruses, the transduction of oncogenes refers to the ability of the retroviruses to acquire cellular proto-oncogenes as integral parts of their genomes and then express those transduced genes as viral oncogenes in another cell within the context of viral replication.

Characteristics

Many viruses have the potential to acquire cellular genetic material. In fact, the many cellular homologs found among the large DNA viruses likely represent examples of how a virus can use a cellular gene to its own advantage. Perhaps it is not surprising that viruses can extract such important commodities from cells given the intimate relationship between viruses and their hosts. However, the mechanisms at work to place these cellular genes in the viral genome and the selective pressures that allow their appearance in the natural biological setting are areas of continuing interest.

The first retrovirus was identified prior to the time when we appreciated what a retrovirus is. Thus, an infectious agent that passed through a filter that retained bacteria (the working definition of a virus at the end of the nineteenth century) and caused anemia in horses represents the first reported retrovirus, now known as equine infectious anemia virus (EIAV). The first cancer causing retrovirus, leukemia in chickens, was reported a few years later, but at that time leukemias were not considered to be a cancer. It was only slowly over the course of the twentieth century that these viruses were appreciated as being part of the retrovirus family, and their role in cancer was revealed. We now know that all retroviruses have an RNA genome in the virus particle but go through the unusual step of copying the RNA genome into DNA, which is then integrated into the host cell genome and is referred to as a provirus. In the integrated state, the DNA is copied into new strands of viral RNA that are either translated into protein or are packaged into new virus particles made from the viral proteins. It is this reverse flow of genetic information, i.e. the copying of the RNA into DNA, that gives retroviruses their name.

Retroviruses and cancer

The conceptual link between viruses and cancer was made in 1911 by Peyton Rous (6). He observed that a filtered extract of a passaged chicken tumor could itself pass the tumor, again defining the agent as smaller than bacteria. This virus was named Rous sarcoma virus (RSV). It took 60 years to understand that a gene in the virus genome was responsible for eliciting tumorigenesis, and that the gene that is responsible for tumorigenesis was derived from the host, i.e. that the retrovirus had stolen, or transduced, a copy of a chicken gene and turned it into a tumor gene. In the case of Rous sarcoma virus this gene is called *v-src*, a viral gene that causes sarcomas. In general genes that can induce or contribute to cancer are called oncogenes. In their non-oncogenic state we refer to these cellular genes as proto-oncogenes (*c-onc*). The ability of retroviruses to transduce cellular proto-oncogenes and mutate these genes into oncogenes has provided one of our most important tools in our search for the molecular basis of cancer (5). Retroviruses actually cause cancer in at least three ways: One way is through the transduction of cellular proto-oncogenes. However, there are at least two additional mechanisms through which retroviruses cause cancer. In a second mechanism, integrated viral DNA, which appears to integrate randomly through out the host cell genome, increases the expression of a nearby gene. When the nearby gene is a proto-oncogene, the overexpression of that gene might be enough to contribute to the evolution of that cell into a tumor cell. Thus the molecular genes that are involved in cancer through insertional activation can be the same cellular genes that have been found as transduced oncogenes in retroviruses. The other additional mechanism is poorly understood and is associated with leukemias caused by the human T-cell leukemia virus type I. This

virus can cause inappropriate cell growth but tumors only rarely form and then only after a delay of several decades. It may be that early inappropriate cellular proliferation puts the cell at risk for accumulating other genetic changes that increase the risk of cancer later in life.

Retroviruses that induce a tumor by insertional activation and retroviruses that induce tumors because of the presence of transduced oncogene have a very distinctive biological difference. Insertional activation occurs after a long period of infection, while a retrovirus that carries its own transduced oncogene causes a tumor rapidly. Since integration is almost random, many rounds of replication and integration must occur before the chance integration near a cellular proto-oncogene. By contrast, each cell infected with a retrovirus that is already carrying an oncogene has the potential to turn into a cancer cell, thus the appearance of the tumor is very rapid. This difference in timing of tumor appearance was an important observation in understanding the mechanisms involved.

Rous sarcoma virus, at age 60, took center stage in unraveling the mechanisms of acute tumor formation by a retrovirus. Viruses very similar to RSV had been identified, collectively called avian leukosis virus (ALV), but they caused a different type of cancer in chickens and only after a long period of time. An analysis of the genomes of these two types of viruses showed that RSV was about 20% bigger, and this led to the hypothesis that RSV had an extra cancer-causing gene compared to the smaller ASV relatives. The key experiment came when this extra region of the RSV genome was isolated and shown to be derived from the chicken genome (7), demonstrating that this cancer-causing gene was derived from the normal gene present in the host cell genome. This observation pointed to the potential role mutated versions of normal genes might play in tumor formation in the complete absence of the involvement of a virus. It also pointed to a biological phenomenon where retroviruses were capable of extracting normal genes from the host cell and placing them in their own genomes.

This insight led to the characterization of a number of retroviruses that had been isolated from tumors in a number of animal systems. Many of these could be shown to carry a cell DNA-derived insert that was responsible for the oncogenic properties of the virus. In one way, RSV has remained unique among all of these oncogene-carrying retroviruses; it is the only such virus that retains all of the normal replicative genes (*gag*, *pro*, *pol* and *env*). All other known retroviruses that have transduced a cellular oncogene are replication defective due to the deletion of one or more of the viral replicative genes. Put another way, RSV is the only replication competent member of this group, all the rest require a replication competent helper virus to grow.

A wide range of cellular genes have now been found associated with various retroviral genomes. These include genes encoding growth factors, growth factor receptors, intracellular signaling proteins and nuclear transcription factors. Since expression of these cellular genes in their normal context does not lead to cancer (they are normal cellular genes) there is something different about the way these genes are expressed by the virus. One type of change is the incorporation of mutations that can subtly change the activity of the protein. Examples of such mutations are ones that make the protein more active or cause it to localize or concentrate in an inappropriate compartment of the cell. Another form of mutation is the truncation of the cellular gene concomitant with its fusion in frame with a viral gene. The cellular gene may also be expressed at a higher level than the normal gene, when expression occurs within the context of the viral genome. An analogous effect is the expression of the viral oncogene in a cell where the normal counterpart of that gene is never expressed. These different mechanisms of conferring oncogenic potential on the cell-derived proto-oncogene are not mutually exclusive.

Incorporation of cellular sequences into retrovirus genomes

Why do these cell-virus genomic chimeras appear? There is no obvious selective pressure for them, since as cancer-causing agents they are detrimental to the host. In addition, the inser-

tion of the cellular oncogene into the viral genome typically results in a replication defective virus, unable to propagate without a helper virus, making transmission a difficult proposition. It is likely that they arise by accident and are rapidly lost in nature unless they themselves are captured by a laboratory investigator. In this view, chance recombinants between the viral and cellular genomes arise as random acts. Most recombinants would go unnoticed and be rapidly lost. Those recombinants that are capable of altering cell growth would be amplified as part of a growing tumor cell population. The linkage to a tumor brings the recombinant to the attention of a scientist, but normally leads to the death of the host and the recombinant virus with it. Even in an experimental setting, these recombinant viruses are genetically unstable, frequently giving rise to mutants with deletions of the cell-derived insert.

Given that the recombination events that give rise to acutely oncogenic retroviruses are rare, we are not able to observe the actual generation of these viruses. We are left to puzzle over the mechanisms by looking at the structures of the recombinant genomes, evaluating the potential of retroviruses to engage in different types of recombination events and testing hypotheses of recombination mechanisms in model systems. However, our inability to view the actual creation of the recombinant retrovirus in nature has precluded a definitive selection of one specific mechanism among the several that have been proposed, and in fact there may be more than one mechanism giving rise to these viruses. At present, there are three models for how cell-virus chimeric genomes arise.

- **Model 1:** *Recombination during viral DNA synthesis.* Viral DNA synthesis includes synthesis through several template discontinuities, or jumps, as part of the intricate process of synthesizing a double-stranded DNA product from the virion RNA template. In addition to the programmed jumps between ends of the templates, the replication complex of reverse transcriptase and the growing DNA chain can jump between the two strands of the dimeric viral RNA present in the virus particle, making the DNA product a patchwork of the two RNAs via a copy-choice mechanism. If the two strands of RNA are at all different (making a heterodimer), then the resulting single copy of DNA can represent a recombinant of the sequences in the two copies of viral RNA. A similar mechanism has been proposed for the capture of cellular sequences. In this model, the viral replication complex jumps to a cellular RNA in the cytoplasm, uses the cellular RNA as a template, then jumps back to the viral DNA to finish synthesis. The net effect is to incorporate a segment of cellular sequence into an interior region of the viral genome. The cellular RNA for such an event could be co-packaged during virion assembly and brought into the next cell where the subsequent round of DNA synthesis would occur, or be drawn from the milieu of cellular RNAs in the cytoplasm of the newly infected cell. The evidence that this is a potential pathway for recombination comes from the analysis of newly synthesized viral DNA. In one such experiment, a recombinant viral genome was isolated prior to integration and this genome had a cellular insert (2). Further examination revealed that the insert was derived from the cell that was infected, not the cell that produced the virus particle, and that the insert sequence was present in the cell as an expressed RNA. This led to the proposal that recombination had occurred during viral DNA synthesis in a jumping mechanism between the viral RNA template and a nearby cellular RNA. Evidence for the co-packaging of cellular RNA during virion assembly comes most dramatically from a mutant of RSV that largely fails to package its own RNA but packages cellular RNAs instead (1). These RNAs can serve as templates for reverse transcription (3).
- **Model 2:** *Transcriptional readthrough and RNA packaging.* Proviral DNA, integrated into the host genome, carries signals for the initiation of transcription and the termination of transcription within the bounding long terminal repeats (LTR) of the viral DNA. The former is active predominantly

in the upstream LTR, the latter in the downstream LTR. Termination actually consists of a transcript that is read through the LTR into the flanking cellular sequence, then cleaved with poly A added at the cleavage site, which is encoded within the LTR sequence. However, this cleavage event is not 100% efficient, leaving a subset of viral readthrough transcripts with cellular sequences at the 3′ end. This process can have important biological consequences as this is one mechanism of expressing a flanking cellular oncogene, a mechanism that has been observed in the activation of the *c-erb* by ALV (4). Chimeric readthrough transcripts can be packaged, limited only by the packaging size limit of the virus particle, which can accommodate transcripts at least several kilobases larger than the wildtype viral genome. Thus it is possible that at some frequency, readthrough transcripts are being packaged into virus particles along with a normal sized RNA to make a heterodimer. These RNAs could be rearranged during subsequent rounds of DNA synthesis to effect a recombination event that would result in the 3′ cellular sequence ending up at an interior position of a completed copy of viral DNA. The potential for such a mechanism was tested using a dominant selectable marker placed downstream of a copy of viral DNA. Recombinants were found in a subsequent round of infection that carried the hallmarks of a transduced cellular gene (8).

- **Model 3:** *Rearrangement of integrated viral DNA*. The mechanism for the insertional activation of cellular oncogenes is through integration of viral DNA near the cellular gene. If viral DNA were integrated upstream of such a gene and there was an intervening deletion event, this would result in the fusion of the left half of the viral genome with the right half of the cellular gene. The viral promoter in the upstream LTR, and the adjacent viral RNA packaging signal, would still be intact, and a chimeric transcript would be synthesized. If a cell harboring such a chimeric provirus were to express also another viral genome, a wildtype transcript could be co-packaged with the chimeric transcript. The heterodimer could then undergo rearrangement during viral DNA synthesis in the subsequent round of infection, resulting in a recombinant. Evidence for this mechanism comes from the structure of the RSV genome. The upstream recombination site between the viral sequences and the cellular *c-src* sequences are within a *c-src* intron (9). In the RSV genome, *v-src* is expressed via a splicing event involving the upstream viral splice donor site with the *c-src*-derived splice acceptor site just downstream of the recombination site. The presence of cell-derived intron sequences would not likely be derived from a cellular RNA, since it would have its introns spliced out. This leads to a model where a rearrangement between viral and cellular sequences takes place at the DNA level followed by a second joining of viral sequences downstream of the cell-derived sequence during a subsequent round of viral DNA synthesis.

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Transfection

Definition

Transfection is an experimental method used to introduce purified nucleic acids or proteins into cells, e.g. by electroporation, lipofection or calcium phosphate precipitation. It is the introduction of genetic material into a cell, where the DNA is usually complexed with cell surface active agents.

Transformation

Definition

Malignant transformation is the collection of events that leads to loss of normal cellular function and acquisition of tumorigenic properties. Transformation of eukaryotic cells describes the failure to observe the normal constraints of growth. It refers to their conversion to a state of unrestrained growth in culture, resembling or identical with the tumorigenic condition. Transformed cells become independent of growth factors usually needed for cell growth. *In vitro* cell transformation tests are used as a model for predicting *in vivo* carcinogenesis.

Transforming Gene

Definition

→ Oncogene.

Transforming Growth Factor α

Definition

→ TGF-α.

Transforming Growth Factor β

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Synonyms

- TGF-β (Transforming growth factor β)

Definition

Transforming growth factors were originally identified on the basis of their ability to induce soft agar growth and morphological changes in nonmalignant cells. The original observation was of an activity, which was named sarcoma growth factor. Subsequently, the term transforming growth factor (TGF) was adopted. Sarcoma growth factor was subsequently found to be composed of an epidermal growth factor-like protein, which was called transforming growth factor-α (TGF-α) and of TGF-β. TGF-α is a member of the → epidermal growth factor family, and is unrelated to TGF-β.

TGF-βs (three different mammalian gene products, TGF-βs 1-3) are multifunctional polypeptide growth factors involved in the regulation of cellular growth and differentiation and of immune functions (1). TGF-βs are in many senses unique among growth factors in their potent and widespread actions. Almost all types of cells in the body make some forms of TGF-β, and nearly all cells have receptors for it. One of their major effects is inhibition of cell proliferation. TGF-βs have also an important role in the control of the proteolytic balance

of cells and in the regulation of the production and structure of the components of the connective tissue and extracellular matrix. TGF-β stimulates the synthesis of various components of the extracellular matrix like collagens, fibronectin, vitronectin, tenascin and proteoglycans. TGF-βs are chemotactic for many cell types like fibroblasts, eosinophils and various inflammatory cells at low concentrations. They also suppress matrix degradation by decreasing the expression of proteinases, such as plasminogen activators, numerous metalloproteinases, and by inducing proteinase inhibitors, such as plasminogen activator inhibitor-1 and tissue inhibitors of metalloproteinases (TIMPs). In addition, TGF-βs regulate the expression of matrix receptors, the integrins. It is thus evident that the activities of TGF-βs must be tightly regulated.

Characteristics

TGF-β receptors and signalling mechanisms

Members of the TGF-β superfamily have diverse functions in cell-cell signalling (2). TGF-βs play different roles in tissue homeostasis and at various stages of development. The mechanisms of regulation of TGF-β activity are multifaceted and complex. Three different TGF-β isoforms and the types, affinity and signalling functions of its receptors also add complexity to the regulation of their effects. The effects of TGF-βs and the other family members are mediated from the cell membrane to nucleus through distinct combinations of type I and type II serine/threonine kinase receptors, Tβ-RI and Tβ-RII and downstream effectors, the → Smad proteins. The receptor-regulated Smads become phosphorylated by activated type I receptors, and they form heteromeric complexes with a common partner, Smad4, which gets translocated into the nucleus for gene transcription control. In addition to the signal transducing Smads, also inhibitory Smads have been identified. They downregulate the activation of receptor-regulated Smads. In contrast to the growing TGF-β growth factor superfamily, relatively few type I and type II receptors or Smads have been identified. The signaling specificities between different TGF-β superfamily members vary, and a certain family member can elicit a broad spectrum of biological responses.

Latency of TGF-β

TGF-βs are produced by the majority of cells in latent complexes unable to associate with TGF-β signaling receptors. Only a few primary cells and established cell lines secrete active TGF-β. TGF-βs are secreted from cells as latent dimeric complexes containing the mature C-terminal TGF-β and its N-terminal pro-domain, LAP, the TGF-β latency associated protein (LAP). The two polypeptide chains of pro-TGF-β associate to form a disulfide bonded dimer. TGF-β is cleaved from its propeptide by furin-like endoproteinase during secretion, at RRXR sequence. The LAP propeptide dimer remains associated with the TGF-β dimer by non-covalent interactions. This complex is referred to as small latent TGF-β. TGF-βs are secreted in most studied cultured cell lines as large latent TGF-β complexes, consisting of small latent TGF-β covalently bound to one of the three latent TGF-β binding proteins (LTBPs -1, -3 or -4) covalently linked to LAP (Fig.). LTBPs have a central role in the processing and secretion of TGF-βs, but they do also have structural roles like fibrillins. The expression of LTBPs and TGF-βs is, in general, coordinately regulated.

Matrix association and release of TGF-β

LTBPs have a central role in the targeting of TGF-β to extracellular matrix structures (3). LTBPs are produced in excess to TGF-β, and since TGF-β secretion is very inefficient in the absence of LTBP, it is probable that most secreted cellular TGF-β is in the large latent complexes. Release of active TGF-β from matrix-associated latent complexes may require two steps, the release of the complex from extracellular matrix (ECM) by proteolysis and subsequent activation, which can be achieved by different mechanisms. Since TGF-β regulates the cellular production of ECM components as well as the proteolytic balance, the ma-

trix association and activation of TGF-β complexes form a finely tuned control network for the maintenance of the organization of extracellular structures. Cancer cells have often been found to produce aberrant amounts of TGF-β. They also fail to deposit TGF-β complexes to the extracellular matrix, probably due to their decreased deposition of fibronectin-collagen matrix, as well as decreased LTBP production.

Latent complexes of TGF-β in the ECM may provide tissues with a readily available storage form of this growth factor. The release and activation of stored growth factors can generate rapid and highly localized signals, like in wound healing or during radiotherapy. Rapid activation of extracellular signaling mechanisms could be important in the healing of tissues after damage, in the control of cells of the immune system during acute infections and in the initial stages of angiogenesis. It is unclear how soluble growth factors could form gradients in highly cellular tissues. Matrix-bound growth factors may generate this kind of an immobilized activity gradient.

LTBPs: expression and functions

Very few functions have been identified for LTBPs thus far. Structurally they resemble fibrillins, which are components of the extracellular microfibrils. They have a typical structure consisting of four eight-cysteine (8-Cys) repeats and several EGF-like repeats. The association of latent TGF-β1 with the matrix is mediated by LTBPs. The N-terminal parts and, putatively, also parts of the C-terminus of LTBP are important in this association. The N-terminus contains transglutaminase substrate motifs, and transglutaminase is required for the covalent ECM association. Also TGF-β2 and -β3 become associated with LTBPs. It is thus likely that LTBPs mediate the binding of all three TGF-β isoforms to various extracellular matrices. By transfection and overexpression systems it has been found that LTBPs facilitate the secretion of small latent TGF-β complexes from the cells.

The TGF-β1 binding region in LTBPs is located close to their C-terminus in the second 8-Cys repeat from C-terminus. The association between LTBP-1 and the propeptide part, LAP, is mediated by disulfide bonding. The respective 8-Cys repeats of LTBPs-3 and -4 bind also small latent TGF-βs. Of the numerous known 8-Cys repeats of the LTBPs and fibrillins, only three have been found to have the capacity to associate with the small latent TGF-β.

Activation of soluble forms of latent TGF-β

TGF-β can be activated *in vitro* by multiple mechanisms, including proteolysis, enzymatic deglycosylation and extremes of pH (Table). Activation of latent TGF-β involves proteolytic disruption of the non-covalent interaction between the propeptide LAP and TGF-β, which releases biologically active TGF-β capable of binding to its signaling receptors. LAP may

Transforming Growth Factor β. Table – Activation methods of latent forms of TGF-β. [Reviewed in Saharinen et al. (1999)]

physicochemical
acidic cellular microenvironment
extremes of pH
γ-irradiation
reactive oxygen species
enzymatic and non-specified protein interactions
proteases • plasmin, cathepsin G • calpain • Kato III cells (unidentified protease) • various MMPs
cell co-cultivation (u-PA)
glycosidases
thrombospondin-mediated
integrin $\alpha_v\beta_6$- and $\alpha_v\beta_1$-mediated
drug-induced
antiestrogens
retinoids
vitamin D_3 derivatives
glucocorticoids

also undergo conformational changes, in such a manner that LAP is not degraded but releases or exposes mature TGF-β to its receptors. The existence of different TGF-β isoforms and latent complexes, as well as the number of different LTBPs suggests that multiple pathways exist for the activation of TGF-βs.

The electrostatic interaction between LAP and TGF-β can be dissociated *in vitro* by extremes of pH, chaotropic agents and heat treatment. From the physiological point of view, the acidic environment in the bone (osteoclasts) or during wound healing could induce the activation of TGF-β.

In vivo experiments using tumor-bearing mice have indicated that irradiation causes rapid activation of TGF-β in the tumors. This effect appears to result from the activation of existing, most probably of matrix-bound latent TGF-β. Irradiation produces reactive oxygen species leading to redox-mediated activation of latent TGF-β complexes. Redox-mediated TGF-β activation may be involved in chronic tissue processes where oxidative stress is implicated, such as carcinogenesis.

TGF-β can be activated by deglycosylation of LAP. Mechanisms involving proteolysis are, however, more diverse and more likely to operate *in vivo*. Various proteases can degrade the propeptide LAP and release active TGF-β. Protease inhibitors can prevent the activation of TGF-β in cell culture. Plasmin-mediated proteolysis appears to be a major mechanism by which TGF-β activation is achieved. Cell-cell contacts, targeting of TGF-β, as well as transglutaminase activity appear to be important in the generation of active TGF-β in cell co-cultivation models.

LTBPs most likely have a distinctive role in the proteolysis-mediated TGF-β activation, since excess amounts of free LTBP or specific LTBP antibodies inhibit the activation process. One explanation is that activation is carried out at or near the cell surface by another proteolytic cleavage, and that LTBP in the solubilized, truncated large latent complex would be required for this association with the cell surface. Plasmin-mediated TGF-β activation is neutralized via feedback inhibition, since TGF-β induces the production of the plasminogen activator inhibitor PAI-1, which decreases the formation of active plasmin. Certain other serine proteases also have the ability to activate TGF-β.

Thrombospondin (TSP), a platelet α-granule and extracellular matrix protein, plays a role in the activation of latent TGF-β complexes via a mechanism that does not involve cell surfaces or proteases. Using purified plasma TSP or the recombinant protein it was found that it is able to activate both small and large latent TGF-β complexes. The activation mechanism is not fully understood, but seems to involve the N-terminal end of LAP and the type I repeats of TSP, possibly by inducing a change in the conformation of LAP and thus releasing the active TGF-β. TSP interacts with LAP as a part of a biologically active complex, and this may prevent the re-association of the inactive complex of LAP and TGF-β. The expression of TSP is induced during wound healing. TGF-β activation may thus get focally increased at sites of injury by enhanced TSP synthesis. Accordingly, TSP deficient mice display many phenotypic features, similar to those detected in TGF-β1 deficient mice. The abnormalities in some tissues of the TSP null animals were even reverted by TSP-derived TGF-β activating peptides, further emphasizing the role for TSP in TGF-β activation.

Hormonal effectors can also affect TGF-β activation. Originally it was found that antiestrogens could induce the production and secretion of active TGF-β in cultured breast cancer cells. Activation of TGF-β has subsequently been found in a number of cell culture models using a number of estrogens and antiestrogens. Steroid hormone superfamily members are efficient regulators of the expression of TGF-β isoforms, and TGF-βs are likely to act as local mediators of the diverse actions of steroids. Estrogens and antiestrogens regulate TGF-β1 formation in different cells and tissues like in mammary carcinoma cells and in fetal fibroblasts. TGF-β functions, for instance, as an autocrine negative growth regulator in breast carcinoma cells.

Perspective

Growth factors of the TGF-β family are important → autocrine and → paracrine regulators of

cell proliferation and differentiation (4). The regulation levels of their activities include the expression of TGF-β receptors, availability of TGF-βs, their activities and modulation of the cellular response. Most cells secrete TGF-β in a large latent complex that associates with the extracellular matrix and is unable to bind to the TGF-β signaling receptors. LTBPs have a central role in TGF-β secretion, extracellular matrix deposition and activation. In addition, LTBPs have structural and other functions not directly related to TGF-β signaling. Structural diversity in LTBP proteins is tremendous, and the possible functions of the different forms include among others the modulation of cell adhesion and the functions of integrins.

Focal activation of latent TGF-β in the matrix by physicochemical means offers a rapid way to induce TGF-β signaling. In addition to plasmin mediated TGF-β activation, novel mechanisms have been found including other proteases, reactive oxygen species, thrombospondin and integrin mediated activation.

The modulation of pericellular proteolytic activity by TGF-β supports a general model where proteinases and latent matrix-bound growth factors are components of an extracellular signal transduction machinery that directs tissue construction and remodeling, and probably also regulates the activity of infiltrating immune cells. Disturbances in these control systems could participate in the pathogenesis of a variety of disease states like atherosclerosis, cancer, various fibrotic diseases and chronic inflammation.

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Transgenic

Definition

Transgenic animals or plants are created by introducing new DNA sequences into the germ line via addition to the egg. The use of transgenic mice carrying an alien gene in every cell is a widely used experimental system in biomedical science. They are generated by microinjection of DNA (transgene) into fertilized eggs, which are then implanted into pseudopregnant foster mothers. The offspring often carries the transgene in all cells, including germ cells. Thus, the transgene will be transmitted according to Mendelian genetics. Transgenic mice represent a tool to study gene effects in the context of the whole organism.

Transition

Definition

Transition or transversion in DNA is a purine to purine or pyrimidine to pyrimidine mutation.

Translation

Definition

Translation is the second step in gene expression, whereby the genetic code contained in the nucleotide sequences of messenger RNA molecules (transcribed sequences) is translated into amino acid sequences, the building blocks of proteins. A variety of enzymes is required for protein synthesis.

Translin

Definition

Translin (TSN), also known as testis brain-RNA binding protein (TB-RBP), is an RNA- and single-stranded DNA-binding protein. Although widely expressed, the RNA-binding interactions of TSN have been associated specifically with regulation of translation during spermiogenesis and the transport of particular mRNAs in the nervous system. DNA-binding interactions of TSN have been associated with chromosome translocation breakpoint sites in malignancy, and a role in DNA repair or meiotic recombination mechanisms has been suggested.

Translocase

Definition

Translocase is an enzyme that translocates lipids between the two leaflets of the lipid bilayer.

Translocation

Definition

- Translocation of chromosomes is the illegitimate recombination between non-homologous chromosomes, a rearrangement in which part of a chromosome is detached by breakage and then becomes attached to another chromosome. The translocation may or may not be reciprocal; in reciprocal balanced translocations, genetic material is exchanged without loss between non-homologous chromosomes; in unbalanced translocations chromosomal material, often the translocated material from one of the non-homologous chromosomes is deleted.
- Translocation of proteins is the regulated movement of proteins or other molecules from one cellular compartment or organelle to another.

Transmembrane Signalling

Definition

The plasma membrane of mammalian cells provides a protective barrier and many extracellular agonists are unable to enter the cell. Instead, they recognize and activate specific receptors at the cell surface which in turn stimulate activity of proteins (e.g. lipid-hydrolyzing enzymes) present inside the cell and in this way transmit the signal across the membrane i.e. from extracellular to intracellular environment.

Transrectal Ultrasound

Definition

Transrectal ultrasound is the examination of the prostate gland *via* an ultrasound transducer built into a cylindrical probe which is inserted into the rectum.

Transurethral Resection

Definition

Transurethral resection is the endoscopic excision of bladder or prostate tissue through the urethra.

Transversion

Definition

Transversion or transition in DNA is a purine to purine or pyrimidine to pyrimidine mutation.

TRAP

Definition

1. → Telomeric repeat amplification protocol;
2. → Tumor necrosis factor receptor-associated factor 2

Telomeric Repeat Amplification Protocol

Definition

Telomeric repeat amplification protocol (TRAP) is a highly sensitive polymerase chain reaction [→ PCR]-based assay for measuring → telomerase activity.

TRE

Definition

TPA-responsive element (TRE) is an → AP-1 binding site, first defined in the human collagenase gene to mediate induction by the phorbol ester TPA.

TRF (Terminal Restriction Fragments)

Definition

An estimate of telomere [→ telomerase] length is measured by digesting cellular DNA with restriction enzymes having 4-base recognition sites, so that most of the DNA is reduced to short fragments. Telomeric repeats are non-palindromic and lack restriction sites. Therefore, they remain as relatively long Terminal Restriction Fragments (TRFs) that can be identified by probing with labeled telomeric oligonucleotides (→ telomerase).

Trinucleotide Repeat

Definition

A trinucleotide is a series of three nucleotides in a DNA sequence and represents the basic genetic coding unit (codon) for individual amino acids. Repeats of the same trinucleotide are found in a number of human genes in both coding and non-coding regions, and in some cases can become unstable and mutate, leading to large expansions of the repeat regions. Such expansions are associated with at least 12 human genetic diseaes including the → fragile X syndrome, Huntington disease, spinocerebellar ataxias and others. In cases where the trinucleotide is a (CCG), expansion can lead to the appearance of a chromosomal → fragile site.

TRITC

Definition

Tetramethyl-rhodamin; is a fluorescence dye, used to label biomolecules (proteins, nucleic acids). TRITC can be used to determine the subcellular localization of proteins by fluorescence microscopy. TRITC excitation is at 560 nm, the excited TRITC molecule emits light of 570 nm.

TRK

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Definition

High-affinity tyrosine kinase receptors for neurotrophins:

- TRK-A (NTRK1) is a receptor for nerve growth factor (NGF);

- TRK-B (NTRK2) is a receptor for brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5);
- TRK-C (NTRK3) is a receptor for neurotrophin-3 (NT-3).

TRK can also form a heterodimeric receptor complex with the low-affinity neurotrophin receptor, $p75^{NTR}$.

Characteristics

The neurotrophin signaling through activation of the TRK tyrosine kinase receptor is important in regulating differentiation and survival of neurons. The rearrangement of the *TRK* gene makes it oncogenic.

- Ligand-dependent activation of the prototype TRK tyrosine kinase receptor sends intracellular signals into nucleus and induces growth arrest, differentiation and promotion of survival in neuronal cells.
- In → neuroblastoma and → medulloblastoma, expression levels of the prototype TRK receptors modulate tumor cell biology (Fig. 1).
- The oncogene *TRK*, caused by rearrangement of the *TRK-A* gene is often observed in certain kinds of human cancers (e.g. thyroid papillary carcinoma). The constitutively activated fusion receptor acquires transforming activity.
- Gene targeting: TRK-A signaling supports the survival of sympathetic neurons and sensory neurons, responsive to temperature and pain. TRK-B signaling supports the survival of sensory neurons responsive to tactile stimuli, and TRK-C signaling supports the survival of sensory neurons responsive to limb movement and position.

Cellular and Molecular Regulation

In general principle, neurotrophins are supplied from the target cells to neurons by retrograde transport. In tumor tissue, the neurotrophin-mediated cross talk between stromal cells and tumor cells expressing TRK receptor may regulate growth, differentiation and cell death. In neuroblastoma with amplified → MYCN, the autocrine/paracrine system of BDNF or NT-4/5 and TRK-B is functional and enhances invasion and metastasis. On the other hand, the product of the *TRK* oncogene (e.g., a fusion gene of tropomyosin and *TRK-A*) is constitutively activated and stimulates growth in some tumors.

Clinical Relevance

Expression of TRK is associated with the biology of cancer. In neuroblastoma, TRK-A is highly expressed in favorable tumors, while it is down-regulated in aggressive ones (Fig.2). In medulloblastoma, however, high expression of TRK-C is associated with favorable prognosis. The TRK oncogene was originally cloned from a colon cancer. The oncogene *TRK*, with replacement of ligand-binding domain by heterologous sequences including tropomyosin, is found in about 25% of thyroid papillary carcinoma. Mutation of the *TRK* genes are rare in human cancers.

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TRK-A

Definition

TRK-A is a receptor for nerve growth factor (NGF); → TRK.

TRK-B

Definition

TRK-B is a receptor for brain-derived neurotrophic factor (BDNF); → TRK.

TRK-C

Definition

TRK-C is a receptor for neurotrophin-3; → TRK.

TSN

Definition

→ Translin.

Tumor Associated Macrophages

Definition

Tumor associated macrophages are macrophages in the vicinity of a tumor. Macrophages are normally part of the immune defense against tumors, but can be re-programmed by the tumor cells to facilitate tumorigenesis.

Tumor Initiators

Definition

Tumor initiators are DNA damaging agents. Exposition to low doses of initiators can result in cancer when the organism is subsequently exposed to → tumor promoters.

Tumor Necrosis Factor α

Definition

Tumor necrosis factor α is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation. It is responsible for a diverse range of signalling events within cells, leading to necrosis or → apoptosis, and binds to cell membrane receptor → TNFR-1.

Tumor Necrosis Factor Receptor-1

Definition

Tumor necrosis factor receptor-1 is a member of the tumor necrosis factor receptor superfamily. It is a 55 kD cell membrane receptor which has an intracellular functional domain that is called death domain (DD), responsible for the receptor signaling activities, and an extracellular domain, which binds tumor necrosis factor α (TNF α).

Tumor Necrosis Factor Receptor-associated Factor 2

Definition

Tumor necrosis factor receptor-associated factor 2 is also known as TRAF2, TNF receptor-associated factor 2 or TRAP. It has a broad expression spectrum in different tissues and is involved in both interleukin 1B and TNF-α signaling cascades (→ stress response).

Tumor Progression

Definition

Tumor progression is a process characterized by progressive accumulation of genetic defects in tumor cells and the outgrowth of tumor cell populations possessing more invasive properties, increased ability to spread to other sites and reduced response to therapeutic intervention. It represents genetic and epigenetic changes of tumor cells resulting in higher malignancy of the tumor; → progression of tumors; → multistep development.

Tumor Promoters

Definition

Tumor promoters are chemicals that are not carcinogenic on their own but enhance carcinogenesis when applied to a tissue after the application of a low dose of a DNA damaging (mutagenic) agent. The latter agents are called → tumor initiators.

Tumor Protein 73

Definition

Tumor protein 73, also known as p73 or TP73, is a protein that activates transcription of p53 responsive genes and inhibits cell growth by apoptosis. The gene maps to locus 1p36, and is a member of → p53 gene family.

Tumor Suppression

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Definition

Tumor suppression is the consequence of the functional presence and activity of → tumor suppressor genes (TSGs). TSGs are recessive genes whose protein products appear to directly or indirectly negatively regulate cell proliferation, promote apoptosis and maintain *in vivo* homeostatic growth and differentiation potential.

Characteristics

Three major classes of genes are involved in cancer causation and progression:

- dominantly-acting → oncogenes, whose proteins serve to stimulate cell growth and survival;
- recessive genes involved in → repair of DNA;
- recessive TSGs.

The recessive TSGs and DNA repair genes are often included under the rubric of tumor suppressor genes.

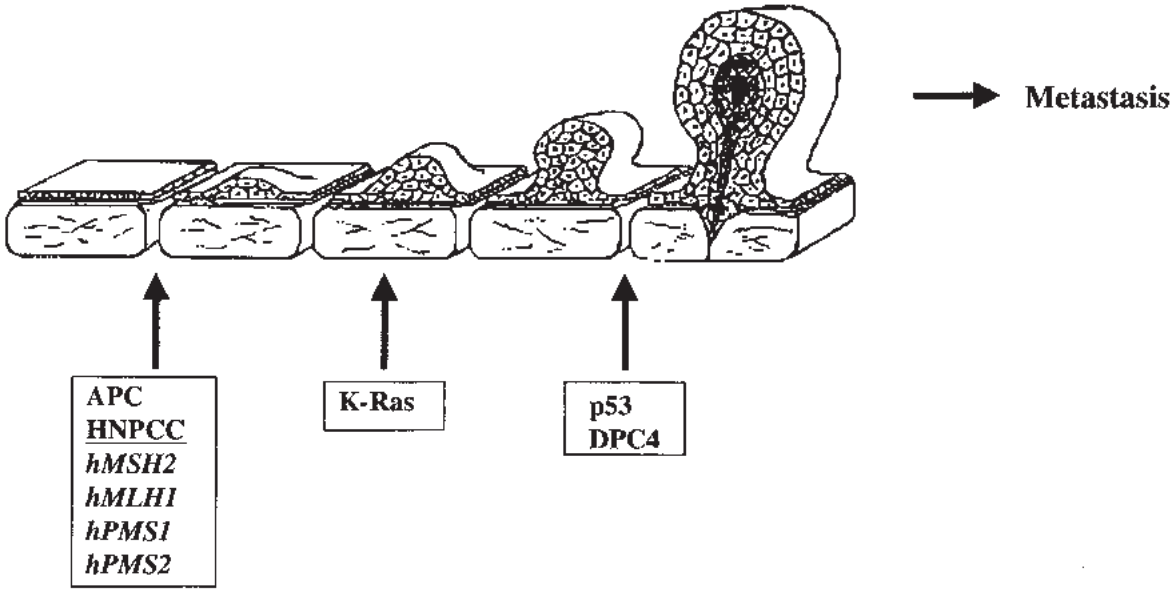
The importance of TSGs in the genesis of cancer became apparent when individuals predisposed to early onset cancer were found to contain a mutated allele of a certain TSG in their germline. This condition predisposes the individual to earlier onset cancer at a significantly higher probability than individuals who possess a sporadic cancer of the same histologic type. This is best illustrated by the prototypic TSG, → *RB1*, that predisposes to childhood → retinoblastoma. The incidence of sporadic retinoblastoma is approximately 1:40,000, and is often unilateral, whereas the incidence of heritable retinoblastoma within an affected family is approximately 40%, and often involves both eyes (bilateral retinoblastoma). The affected proband inherits the mutant allele from one of the parents. Thus, the classic presentation

of a heritable recessive TSG is one of dominant autosomal inheritance. In the classic → two-hit model, the affected individual inherits one mutant *RB1* allele and the second allele is eliminated somatically in the retinoblastoma tumor. In the case of sporadic retinoblastoma both alleles are eliminated somatically.

Since the identification of the *RB1* TSG, a large number of cancer-predisposing germline mutations in TSGs have been found (Table), and they include DNA repair genes. Many, but not all (e.g. → *BRCA1* and → *BRCA2*), are commonly found to be mutated in sporadic cancers of the same histologic type as those seen in the relevant familial cancer cases.

Progression to the cancerous condition is a multistep phenomenon. This is best illustrated by the colorectal cancer model (→ multistep development; Fig. 1). Tumorigenesis proceeds through a series of cellular alterations, including hyperplasia of the colonic epithelium, benign polyps of increasing size and disordered growth, and → carcinoma in situ with localized invasion. Distant metastases may occur. Accompanying these cellular alterations are genetic alterations, including activating mutation of the *K-ras* proto-oncogene and loss-of-function mutations in multiple TSGs. In the case of TSGs, mutations in both alleles must occur for complete loss of function. Loss-of-function of multiple TSGs is a hallmark feature of most, if not all cancers.

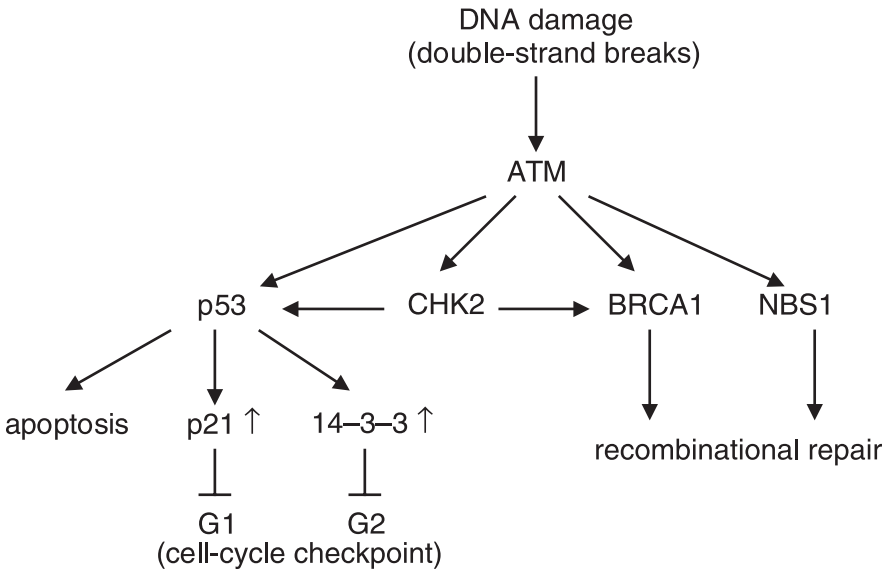
TSGs span a broad range of functions (Table). These include kinases, phosphatases, cyclin-dependent kinase inhibitors, transcription factors, cell adhesion molecules, proteins involved in specific protein degradation pathways and a variety of DNA repair processes. There is increasing evidence that tumor suppressor proteins and DNA repair proteins interact in functional networks (an example is given in Fig. 2). The ATM kinase phosphorylates and activates the tumor suppressor p53 and CDSI/CHK2 proteins, and the DNA repair proteins, BRCA1 and NBS1. These activations lead to cell-cycle checkpoint arrest and repair of DNA damage. Loss-of-function of any one of these factors compromises DNA repair, culminating in genomic instability and increased probability of progression to the cancer phenotype.



Tumor Suppression. Fig. 1 – Multistep progression model for colorectal cancer. Tumorigenesis proceeds through a series of cellular alterations, including hyperplasia, benign polyps of increasing size and disordered growth, and carcinoma *in situ* with localized invasion. Genetic alterations associated with this progression include inactivation of *TSGs*, e.g. *APC* , *p53* and *DPC4*, mutational activation of the *K-Ras*, oncogene and inactivation of one of the DNA mismatch repair genes.

Tumor Suppression. Table – Predisposing Germ Line Mutations in Tumor Suppressor Genes.

associated cancer syndrome	tumor suppressor gene	human chromosomal location	gene function	cancer type
familial retinoblastoma	*RB1*	13q14	transcriptional regulator of cell cycle	retinoblastoma, osteosarcoma
Wilms tumor	*Wt1*	11p13	transcriptional regulator	nephroblastoma
Li-Fraumeni	*p53*	17q11	transcriptional regulator/growth arrest/ apoptosis	sarcomas, breast/brain tumors
von Recklinghausen	*NF1*	17q11	Ras-GAP activity	neurofibromas, sarcomas, gliomas
neurofibromatosis type 2	*NF2*	22q12	ERM protein/cytoskeletal regulator	schwannomas, meningiomas
von-Hippel Lindau	*VHL*	3p25	regulates proteolysis	hemangiomas, renal, pheochromocytoma
familial adenomatous polyposis	*APC*	5q21	binds/regulates β-catenin activity	colorectal cancer
familial melanoma	*INK4a*	9p21	$p16^{Ink4a}$ cdki for cyclinD/cdk4/6; $p19^{ARF}$ binds mdm2, stabilises p53	melanoma, pancreatic
Gorlin syndrome	*PTC*	9q22.3	receptor for sonic hedgehog	basal cell carcinoma, medulloblastoma
juvenile polyposis	*DPC4*	18q21.1	transduces TGF-β signals	pancreatic, colon, hamartomas
Cowden syndrome BZS, LDD	*PTEN*	10q23	dual specificity phosphatase	glioblastoma, prostate, breast
tuberous sclerosis	*TSC2*	16	cell-cycle regulator	renal, brain tumors
familial prostate carcinoma	*NKX3.1*	8p21	homeobox protein	prostate
Peutz-Jeghers	*LKB1*	19p13	serine/threonine kinase	hamartomas, colorectal, breast
familial gastric cancer	*E-Cadherin*	16q22.1	cell adhesion regulator	breast, colon, skin, lung carcinoma
ataxia telengiectasia	*ATM*	11q23	P13K-like kinase	leukemias, lymphomas
HNPCC	*MSH2*	2p22	Mut S homologue, mismatch repair	colorectal cancer
HNPCC	*MLH1*	3p21	Mut L homologue, mismatch repair	colorectal cancer
HNPCC	*PMS1*	2q31	mismatch repair	colorectal cancer
HNPCC	*PMS2*	7p22	mismatch repair	colorectal cancer
HNPCC	*MSH6*	2p16	mismatch repair	colorectal cancer
Bloom Syndrome	*BLM*	15q26.1	DNA helicase	multiple
Fanconi anemia				
complementation Gr A	*FAA*	16q24.3	involved in DNA cross-link repair	leukemia
complementation Gr C	*FAC*	9q22.3	involved in DNA cross-link repair	leukemia
Xeroderma pigmentosum (seven complementation groups	*XPA* *XPB* *XPC* *XPD* *XPE* *XPF* *XPG*	9q34.1 2q21 3p 19q12.3 ?11 16p13 13q23-33	binds damaged DNA helicase; part of TFIIH ? helicase; part of TFIIH binds damaged DNA structure-specific endonuclease structure-specific endonuclease	skin
Nijmegen breakage syndrome	*NBS1*	8q21	involved in DNA doublestrand break repair	lymphomas
familial breast cancer	*BRCA1*	17q21	transcriptional regulator/DNA repair	breast/ovarian tumors
familial breast cancer	*BRCA2*	13q12	transcriptional regulator/DNA repair	breast/ovarian tumors



Tumor Suppression. Fig. 2 – Networking of tumor suppressor and DNA repair proteins. In response to DNA damage, ATM phosphorylates and activates p53 and the cell cycle kinase CHK2. This kinase also enhances activation of p53 via phosphorylation. Activated p53 upregulates expression of the cyclin dependent kinase inhibitor, p21, and 14-3-3 proteins, resulting in cell-cycle arrest at the G1 and G2 checkpoints, respectively. The activated p53 protein may also induce apoptosis. Transient cell-cycle arrest allows for repair of damaged DNA by preventing the duplication and propagation of damaged DNA. ATM also plays an important role in DNA repair by phosphorylating and activating BRCA1 and NBS1 proteins. See Table for identification of the genes. → = activation; ⊣ = inhibition.

Clinical aspects

Identification of specific germline mutations in TSGs in affected families allows the recognition of those members who are carrying the mutant allele and, therefore, are at a significantly higher risk of getting cancer. Detection of mutant tumor suppressor proteins, e.g. p53, or loss of expression of such proteins may aid in cancer diagnosis and prognosis. Experimental investigations have established that restoration of TSG function in cancer cells that are defective for that function result in suppression of tumor growth or death of the cancer cells. Clinical trials are in progress using → gene therapy and pharmacologic approaches that aim to apply these procedures to the treatment of human cancers.

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Tumor Suppressor Genes

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Synonyms

- recessive oncogenes

Definition

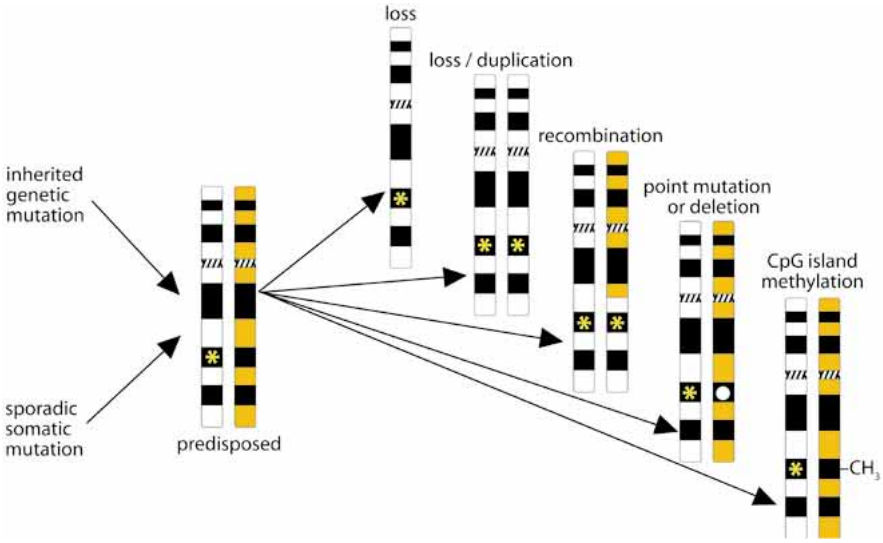
Tumor suppressor genes are genes whose products normally negatively regulate cell growth or cell behavior.

Characteristics

The hallmark of a tumor suppressor gene is that its function is lost during tumor initiation or progression. This typically occurs by one of a set of chromosomal processes called → loss of heterozygosity but, in some cases, can occur by forming dominant negative forms of the tumor suppressor gene product. Their presence is usually inferred through the cytogenetic or molecular detection of subchromosomal loss. Upon molecular isolation, the genetic inference can be confirmed and dissected by demonstrating a restoration of growth regulation upon ectopic expression of the gene and/or by the formation of tumors or growth abnormalities in animals lacking the functional gene, either naturally occurring mutant strains or those constructed by *in vivo* homologous recombination 'gene knockout' techniques.

What was the evidence for tumor suppressors?

The primary lines of evidence are genetic: One is that specific kinds of cancer can cluster in families. In most cases, the inheritance pattern is autosomal dominant which means that it is not sex-linked, may be transmitted from either parent and involves the transmission of a gene whose presence is sufficient to cause disease. In addition to familial clustering of the common cancers, two additional clinical observa-



Tumor Suppressor Genes. Fig. – Chromosomal mechanisms for tumor suppressor gene inactivation. Left side, the first mutation (*) can occur in a single somatic cell and result in sporadic disease. Alternatively, it can occur in a germ cell (*de novo* mutation) or be inherited from an affected parent and result in heritable disease. Right side, the first mutation can become completely inactivated by (from top to bottom) physical deletion or recombination of the wild type chromosome, by a targetted second mutation or deletion of the remaining wild type gene or by methylation of the promoter of the wild type gene leading to loss of expression.

tions provide strong epidemiological support for the contention that cancer has a genetic etiology.

- First, some individuals and their families have an autosomal dominant transmission of cancer predisposition, not to a single tumor as described above, but to multiple tumors occurring independently at different body sites.
- Second, individuals with a variety of multiorgan developmental defects often also develop specific rare tumors. A statistical argument can thus be made that the combined occurrence of multiple independent tumors or the routine association of developmental defects with tumors which are very rare in the general population is so unlikely as to suggest an etiologic relationship.

The apparent dominant transmission of cancer traits is paradoxical in light of three observations. First, hybrid cells formed from the experimental fusion of highly malignant tumor cells with normal cells are not usually tumorigenic, suggesting that the normal phenotype is dominant in the presence of tumorigenic mutations. Furthermore, the occasional hybrid cell that regains tumorigenicity in these experiments has lost specific chromosomes originally contributed by the normal cell, implying that it is not gain of a dominant cancer trait but specific chromosomal loss that is responsible for the tumor phenotype. Second, if a single mutation was sufficient in itself to elicit a tumor, then families segregating for autosomal dominant forms of cancer would be expected to have no normal tissue in the diseased organ. This expectation is in direct contrast to the clinical description of these tumors as focal lesions surrounded by normal, functioning tissue of the same organ. Finally, epidemiological analyses of sporadic and familial forms of several human cancers have indicated that the conversion of a normal cell to a tumor cell requires multiple events.

Retinoblastoma - the first suppressor

→ Retinoblastoma is a relatively rare tumor (1 in 20,000 births) of young children and occurs in both a sporadic and autosomal dominant inherited form. Based entirely on statistical data from epidemiology and clinical observations, several remarkable conclusions were made regarding the nature of events leading to retinoblastoma tumor formation. First, the inherited mutation alone was not sufficient to cause the disease, since there are at least 10^7 retinoblast cells which are potential targets for retinoblastomas, each carrying the inherited mutation, yet on average, only 3 independent tumors form per affected individual. This also suggested that at a genetic level, mutations leading to retinoblastoma may be recessive, rather than dominant as suggested by the inheritance pattern. The hereditary tumors were proposed to arise through an initial germline mutation followed by a second mutation in a somatic cell. The rate at which somatic mutations occurred was similar in hereditary and sporadic cases, although sporadic tumors required two somatic mutations, each in the same retinoblast for tumor formation. Entirely consistent with this was the observation that hereditary cases usually occurred at an earlier age, were often bilateral and had multiple tumors, whereas the sporadic cases were invariably unilateral and single tumors. Because of the small possibility that a second somatic mutation may never occur in hereditary cases, approximately five percent of carriers do not develop any tumor. The nature of the two mutational targets in the genome was unknown at the time of these clinical observations, but cytogenetics and molecular genetics eventually led to the answer as well as to a general approach to other human cancers.

Analysis of the chromosome banding patterns from hereditary and sporadic retinoblastoma patients revealed a deletion of chromosome 13q14 (chromosome 13, q or long arm, band one-four), suggesting that the gene for retinoblastoma (Rb) resided somewhere within this region. DNA from hereditary tumors was then analyzed with cloned DNA probes (termed 'DNA markers') that could distinguish the two copies, or alleles, of chromosome 13 within each cell. It was found that, in tumors of affected individuals, the region containing the suspected Rb gene on chromosome 13 was pre-

sent in a mutant only state. This conversion from a heterozygous state to homozygosity for the mutation was termed loss of heterozygosity (LOH), and constituted the second hit required for tumor formation in hereditary cases. Furthermore, LOH on chromosome 13q14 also occurred in sporadic retinoblastoma. These data lent strong support to the idea that retinoblastoma tumor formation occurs by the unmasking of a recessive genetic defect. The discovery that LOH occurs in other hereditary and most sporadic cancers in humans marked the simultaneous emergence of somatic cell cancer genetics and its coupling to the genetics of hereditary cancer. By identifying the region of chromosome 13q14 with the most consistent LOH in tumor DNA, the gene responsible for retinoblastoma was eventually isolated and

Tumor Suppressor Genes. Table

gene	chromosomal location	function	cancer sites
RB1	13q14.2	cell-cycle regulator	retina, bone, bladder, breast, pancreas
p53	17p13.1	genome-stability regulator	brain, breast, leukemia, soft tissue
p16	9p21	cyclin dependant kinase inhibitor	brain, melanocyte
p15	9p21	cyclin dependant kinase inhibitor	leukemia
p18	1p32	cyclin dependant kinase inhibitor	esophagus, lung, bladder, pancreas
p21	6p21	cyclin dependant kinase inhibitor	prostate, lung
E2F1	20q11	transcription factor	erythroleukemia
BRCA1	17q21	transcription factor (?)	breast, ovary
BRCA2	13q12-13	transcription factor (?)	breast, ovary
WT1	11p13	transcription factor	kidney
VHL	3p25-26	modulator of RNA polymerase	kidney, central nervous system
PTC1	9q22.3	transcription repressor	skin
TGFβR1	9q33-34	TGF-β receptor	colon, retina, liver, stomach
TGFβR2	3p21.3	TGF-β receptor	colon, retina, liver, stomach
DPC4	18q21.1	TGF-β pathway growth inhibitor	pancreas, colon, bladder, liver
CDH1	16q22.1	intercellular adhesion	breast, ovary, liver, skin, endometrium
APC	5q21	cell signalling	colon
MCC	5q21	(?)	colon
NF1	17q11.2	cell signalling	peripheral nervous system, skin
NF2	22q12	cell signalling	central nervous system
MSH2	2p22	mismatch repair protein	colon
MLH1	3p21.3	mismatch repair protein	colon
DCC	18q21	differentiation factor	colon
PTEN	10q23.3	protein/lipid phosphatase	brain, melanocytes, prostate, thyroid, breast

its functionality assessed. Most importantly, the gene was shown to be mutationally inactivated in retinoblastoma tumors. When a normal copy of the gene was transferred to tumor cells, their growth and tumorigenic behavior was reduced. Thus, the conjoint application of epidemiology, cytogenetics, molecular genetics and molecular biology allows the identification of a gene with tumor suppressing function.

Are there other tumor suppressors and what cellular role do they normally play?

Since the first suppressor was isolated, more than 23 others have been molecularly identified. As might be expected, these represent genes whose products are involved in many different aspects of cell growth and behavior. These include regulators of the cell cycle, growth and transcriptional regulators, DNA repair enzymes, differentiation factors, elements of cell motility and regulators of cellular signalling. Thus, elucidation of the function and nature of tumor suppressors is not only of importance for understanding cancer etiology but also useful for dissecting normal cellular function.

Clinical Relevance

The intimate involvement of tumor suppressor genes in the etiology of most human cancers places them at the center of cancer research. Such knowledge has been exploited for the first prenatal and premorbid predictions of cancer occurrence, for molecular pathology approaches to tumor subtyping, for → gene therapy approaches toward gene replacement and as targets for agonist/antagonist development in rational drug design. Just as in research, the continued exploitation of tumor suppressor genes for clinical benefit to cancer patients is likely to assume a central role in modern therapies.

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Tumor Surveillance

Definition

Tumor surveillance, also known as immune surveillance, is a collective term for mechanisms that are mediated by the immune system and serve to hinder tumor formation or to eliminate tumor cells.

TUNEL

Definition

TdT (terminal deoxynucleotidyl transferase)-mediated d**U**TP **n**ick **e**nd **l**abeling (TUNEL), also known as ISEL (***in situ*** **e**nd **l**abeling), is a DNA-tailing reaction using TdT. DNA degradation is considered the key biochemical event in → apoptosis, resulting in cleavage of nuclear DNA into nucleosome-sized fragments (approximately 180 base pair unit size). TUNEL can be used to determine apoptosis in individual cells, preferentially labeling apoptotic rather than necrotic cells. The principle is that DNA ends generated during apoptosis are extended by TdT using biotin- or digoxigenin-labeled BrdUrd (bromodeoxyuridine). The incorporated material is detected by fluorescent reagents.

Turcot Syndrome

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Definition

Turcot syndrome (TS) is a rare inherited neoplastic disease characterized by the association of primary malignant neuroepithelial tumors of the central nervous system and colon cancers and/or multiple colorectal adenomas.

Characteristics

Clinical criteria

The 130 or so Turcot syndrome (TS) cases described to date include various histopathologic types of → brain tumors, e.g. glioma, → medulloblastoma and astrocytoma, associated with a broad spectrum of colorectal findings, from a single adenoma to typical adenomatous polyposis. Usually, polyps are fewer in number than in familial adenomatous polyposis (FAP [→ APC gene in Familial Adenomatous Polyposis]) but are larger in size, and multiple adenomas or colorectal cancers occur at an early age and undergo an earlier malignant transformation than in FAP or in hereditary non-polyposis colorectal cancer (→ HNPCC).

The clinical definition and the mode of inheritance of Turcot syndrome is controversial; some authors propose that TS is an allelic variant of FAP and support an autosomal dominant inheritance, while others postulate that TS is a disease independent of FAP with an autosomal recessive pattern of inheritance.

Genetics

Dominantly inherited cases have been associated with germline mutations in either the tumor suppressor adenomatous polyposis coli gene (APC), usually mutated in FAP, or in the DNA → mismatch repair (MMR) genes, which are usually mutated in HNPCC.

Few recessive cases have been reported and only in two cases were the causative mutations found to be within the *PMS2* gene, a minor MMR gene that is only rarely involved in HNPCC. In the first recessive case described, a germline nonsense mutation (*PMS2*-134) that was inherited from the healthy mother was found in one allele. The second recessive case was found to be a compound heterozygote for two frameshift germline mutations within the *PMS2* gene; a G deletion (1221delG) in exon 11 and a four-base pair deletion (2361delCTTC) in exon 14, both of which were inherited from the patient's unaffected parents. This was the first evidence of recessive dominance of TS because two germline mutations in *PMS2* are not individually pathogenic, but become so when occurring together in a compound heterozygote. Since all carriers of only one *PMS2* gene mutation are clinically healthy, a lower penetrance of *PMS2* gene mutations may be inferred for colorectal tumor development compared with mutations in other MMR genes, e.g. *MLH1* and *MSH2*. Accordingly, there are no reports of HNPCC patients compound heterozygous for *MLH1* or *MSH2* mutations, which suggests compound heterozygosity is lethal.

PMS2 gene

The *PMS2* gene, localized to chromosome band 7p22, encompasses 16 kb and consists of 15 exons. It encodes a protein involved in the mismatch repair system that physically interacts with the MLH1 protein through the carboxyl terminus. Both *PMS2* mutations (1221delG and 2361delCTTC) found in the compound heterozygous TS patient, caused the loss of the MLH1 interaction domain with PMS2, thus indicating that a disruption of the MLH1-PMS2 heterodimer might block the process of the MMR system.

Microsatellite instability

→ Microsatellite instability, an indicator of the defective DNA mismatch repair system, is characteristic of HNPCC colorectal tumors, of a small fraction of sporadic colon and brain cancers,

and of colon carcinomas and adenomas in TS patients. It results from germline mutations in each of the MMR genes, i.e. *MSH2*, *MLH1*, *PMS1* and *PMS2*, with somatic mutation inactivating the second allele in the tumor tissues or loss of protein expression in the tumor tissues. Brain and colon tumors in TS patients exhibit severe microsatellite instability at several DNA marker loci, at the repeated regions of the TGFβRII and of *MSH3* and *MSH6* genes as well as somatic mutations within the *APC* and *p53* genes. High frequencies of the microsatellite instability have also been found in normal colon mucosa from TS patients bearing germline *PMS2* mutations, in contrast to HNPCC patients in which microsatellite instability is very rare in normal tissues. Therefore, the high DNA hypermutability in normal tissues of TS patients is associated with a severe biochemical defect in the MMR system and it might trigger the early development of multiple cancers in this syndrome.

Clinical aspects

Patients with hereditary forms of colon cancer and neurologic symptoms require immediate and thorough investigation because of their significantly increased risk of developing central nervous system (CNS) tumors. Patients diagnosed with a CNS tumor with a family history of colon tumors should undergo screening and surveillance colonoscopy because the CNS lesion may precede colonic symptoms. The elucidation of the gene defects responsible for TS has therefore important implications in the genetic testing of relatives at risk and in the management of young patients with brain tumors and asymptomatic subjects.

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TWIST

Definition

Twist, also known as ACS3 or h-twist, is a → bHLH protein member of the Twist family of 202 amino acids and 20 kD. The human TWIST gene locus maps to 7p21. Twist seems to be involved in the negative regulation of cellular determination and in the differentiation of several lineages including myogenesis, osteogenesis and neurogenesis. It inhibits myogenesis by sequestrating bHLH proteins, inhibiting trans-activation by Mef2 and inhibiting DNA-binding by MyoD through physical interaction.

TWIST Family

Definition

The Twist family is a → bHLH protein family comprising Twist, Paraxis, Scleraxis, Dermo-1, Tcf21. The members are involved in regulation of cellular determination and in differentiation, including myogenesis, osteogenesis and neurogenesis.

Two Component Signaling Pathway

Definition

Two component signaling pathway is a signal transduction pathway found in prokaryotes and eukaryotes (including yeast and plants). An environmental signal, such as a change in the concentration of certain ions or chemotactic factors, activates a receptor, resulting in the autophosphorylation of an associated histidine kinase. In the following, the aspartate residue of a receiver protein becomes phosporylated.

In prokaryotes, the receiver protein functions as a transcription factor, while in eukaryotes it ties into the → MAP kinase pathway.

Two-hit Model

Definition

Based on epidemiological and clinical data, it has been inferred that as few as two mutations are sufficient to initiate the development of → retinoblastoma (Rb). Specifically, it was hypothesized that

- in hereditary Rb one mutation is transmitted *via* the germline and the second mutation occurs in a somatic cell;
- in non-hereditary Rb both mutations arise in somatic cells.

In principle this applies to the inactivation of all tumor suppressor genes.

TX

Definition

→ Caspase 4.

Tyrosine

Definition

Tyrosine is one of twenty amino acids used to synthesize proteins based on sequence information encoded within RNA molecules.

Tyrosine Kinase

Definition

Tyrosine kinase is an enzyme whose activity catalyses the covalent attachment of a phosphate group to tyrosine residues.

Tyrosine Kinase Receptor

Definition

Tyrosine kinase receptor is a class of transmembrane receptors for growth factors and cytokines. They contain an extracellular ligand binding domain and an intracellular catalytic domain, which mediates ATP-dependent auto-phosphorylation and phosphorylation of other proteins on tyrosine residues. The intrinsic tyrosine kinase activity of the receptor is required for signal transduction (→ receptor tyrosine kinases).

U

Ubiquitin

Definition

Ubiquitin is a small 76 amino acid protein that can be conjugated to other proteins via its C-terminus. The primary function of → ubiquitination is to target acceptor proteins for degradation by the → proteasome.

Ubiquitin Pathway Enzymes

Definition

→ Ubiquitin pathway enzymes belong to the ubiquitin-mediated degradation mechanism of proteins in eukaryotic cells (from yeast to mammalian cells); → ubiquitination. Ubiquitin-mediated protein degradation involves two discrete and successive steps. Firstly, the conjugation of multiple ubiquitin moeities to the protein. Secondly, the degradation of the conjugated protein by the 26 S → proteasome complex together with the release of unbound and reusable ubiquitin. Protein degradation follows a three-tiered enzymatic cascade and involves three classes of proteins: Ubiquitin-activating enzyme E1, ubiquitin-conjugating enzyme E2 and ubiquitin-protein ligase E3.

In mammalian cells a single type of ubiquitin-activating enzyme E1 exists, which carries out all ubiquitin modifications.The activation of ubiquitin involves adenosinetriphosphate (ATP) and results in the formation of an E1-sulphur-ubiquitin high energy thiolester intermediate. Following activation, one of the many ubiquitin-conjugating enzyme E2, transfers ubiquitin from E1 to a member of the ubiquitin-protein ligase family E3. This enzyme then catalyzes the covalent attachment of ubiquitin to the substrate protein, to which subsequently, a poly-ubiquitin chain is added by the progressive transfers of conjugated ubiquitin molecules. This chain presumably serves as a recognition signal for the 26 S proteasome. Multiple forms of E3 enzymes exist, which presumably are responsible for the specificity of protein degradation. As a final step, recycling proteases recognize specifically the C-terminus of ubiquitin and catalyze its release from protein conjugates within cell. Aberrations of this fine-tuned degradation mechanism are thought to be implicated in the pathogenesis of several diseases. Principally, two possibilities of an aberrant degradation mechanism exist: enhanced activity on hand and reduced activity on the other.

Ubiquitin-activating Enzyme, E1

Definition

→ Ubiquitin-activating enzyme E1 belongs to the → ubiquitin pathway enzymes that are involved in protein degradation within eukaryotic cells (from yeast to mammalian cells). Activation involves adenosinetriphosphate (ATP) and the formation of a high-energy thiolester at the C-terminal amino acid glycine; → ubiquitination.

Ubiquitination

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Definition

Ubiquitination is the covalent modification of a protein by conjugation to → ubiquitin. Ubiquitin is a small, 76-residue protein found in all eukaryotes. Conjugates are formed through the ligation of the C-terminus of ubiquitin to the ε-amino groups of protein lysine residues. The major, but not sole, function of ubiquitination is to target proteins for degradation by the → proteasome. A large number of proteins are substrates for this regulatory pathway. Efficient targeting of proteins for degradation usually involves formation of a multiubiquitin chain on the target protein. Such chains are characterized by specific ubiquitin-ubiquitin linkages.

Characteristics

Intracellular proteins are degraded at rates that vary over 100,000-fold. While most proteins are relatively stable, critical regulatory proteins such as oncoproteins and tumor suppressor proteins are often short lived. Examples include → p53, → Mdm2, → receptor tyrosine kinases, β-catenin [→ E-cadherin], → NFκB, → Fos, → Jun, cyclins [→ cyclin D] and inhibitors of cyclin dependent kinases. The degradation of these proteins is subject to complex controls and is in many cases a dominant aspect of their regulation. For example, the cell cycle is driven principally by temporally controlled variations of cyclin levels, which are enforced by sudden periods of cyclin degradation. Exit from mitosis is marked by the deregulation of one cyclin, while other cell cycle transitions follow upon degradation of other cyclins and cyclin dependent kinase inhibitors.

Protein degradation has several advantages as a regulatory mechanism. As compared to regulated gene expession, it is capable of changing the level of a gene product very rapidly. Down-regulation by repressing gene transcription is very slow when the gene product is stable. As compared to phosphorylation, degradation allows a regulatory switch to function irreversibly. Regulation by degradation is a kind of ratchet mechanism, which in the case of the cell cycle or circadian cycle, for example, can prevent the possibility of reverse movement through the cycle.

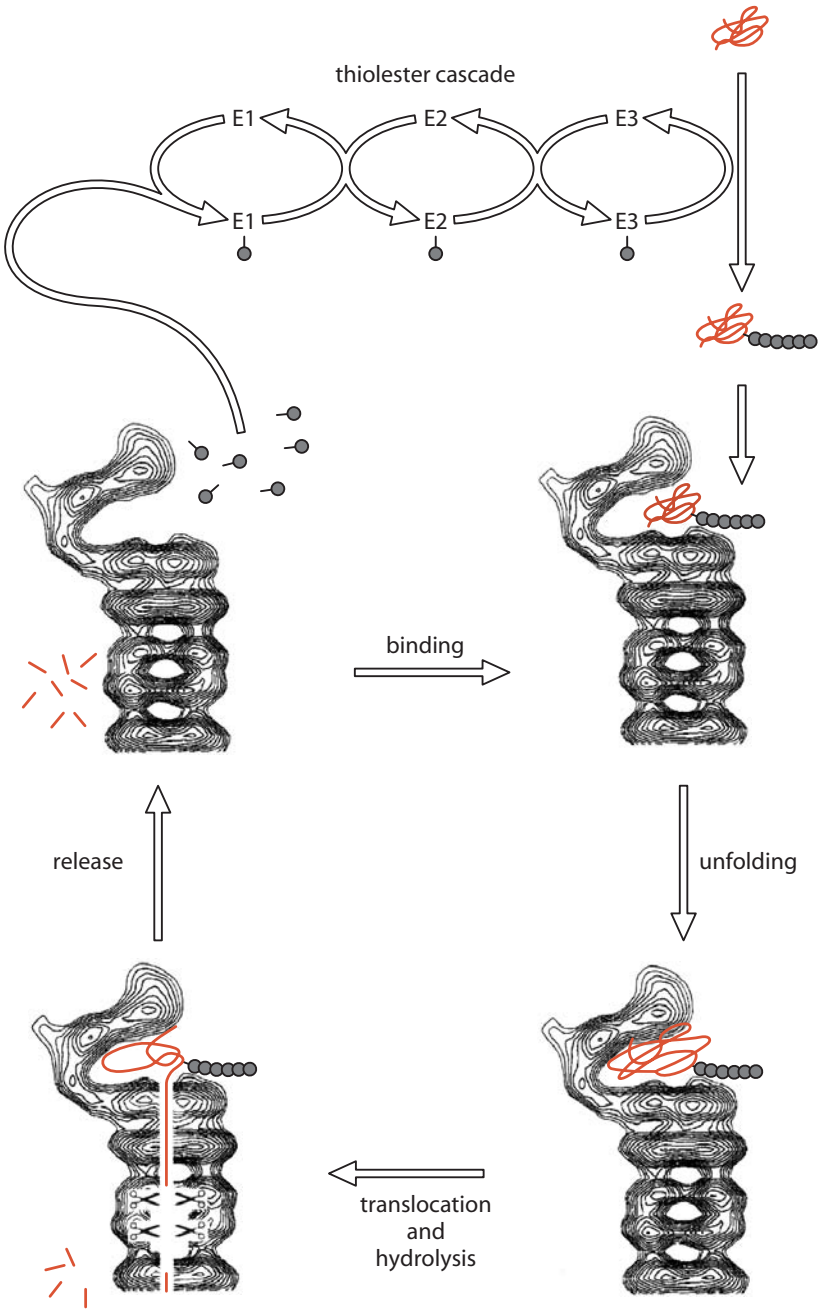
Cellular & Molecular Regulation

Two major systems for protein degradation have been identified in eukaryotic cells:

- the lysosomal pathway
- the ubiquitin-proteasome pathway.

Short lived regulatory proteins, such as those discussed above, are degraded by the ubiquitin-proteasome pathway, with few exceptions. The ubiquitin-proteasome pathway is unusual in that it is a two-step pathway for protein turnover; attachment of the small protein ubiquitin to the substrate protein targets it to be recognized and degraded by the proteasome. Ubiquitin is attached to proteins via its C-terminus, which can form either an isopeptide bond with the ε-amino group of a protein lysine residue, or in rare cases ubiquitin may be conjugated to the α-amino group at the N-terminus of the target protein. Efficient recognition by the proteasome requires multiple ubiquitin molecules to be ligated to the substrate, usually in the form of a multiubiquitin chain. Once bound to the target protein, ubiquitin itself becomes a preferential target of ubiquitination, and thus ubiquitin chains can be assembled on the substrate.

The ubiquitin-proteasome pathway is highly elaborate. The proteasome itself has over 30 subunits arranged in a remarkable self-compartmentalized structure. The active sites of the proteasome are thus inaccessible to the ubiquitinated substrate proteins unless the substrates are unfolded and translocated through an internal channel in the enzyme into its hydrolytic chamber for degradation. The initial attachment of ubiquitin to the substrate protein involves three classes of enzymes, known as E1, E2 and E3. E1 uses the energy of ATP to form a



Ubiquitination. Fig. – The proteasome cycle in protein degradation. Ubiquitin is shown as a large dot, and the peptidase sites are shown as scissors. For simplicity, the 26S particle is shown with a single regulatory particle and the 19S particle is shown without the protein mass that occupies the mouth of the wedge. Degradation of the substrate to oligopeptides is accompanied by the regeneration of free ubiquitin. The cycle begins when ubiquitin is activated at its carboxy terminus by the adenylyl transferase E1, then the activated ubiquitin is passed to an E2 enzyme. The E2 may then pass the activated ubiquitin to an E3 enzyme (ubiquitin-protein ligase). All three enzymes form thiolester bonds to ubiquitin, although in some case the E2 enzyme could directly donate ubiquitin to target proteins. The proteolytic substrate is linked to ubiquitin by an isopeptide bond. Multiple ubiquitin groups are added to the substrate sequentially to form a multi-ubiquitin chain, which finally targets the substrate for degradation. [Figure taken from Ref. 4]

high energy thiolester linkage with the C-terminus of ubiquitin, thus 'activating' ubiquitin for conjugation. E1 then transfers ubiquitin to any of a large number of E2 enzymes, which also bind ubiquitin covalently through a thiolester bond. Ubiquitin is finally donated to the substrate protein, either from the E2 enzyme or from the E3. In either case, the critical substrate recognition component of the machinery is generally the E3 enzyme. Many E3 enzymes are large protein complexes subjected to intricate controls. Substrate recognition by the E3 enzyme can be regulated by phosphorylation of either the substrate or E3 itself.

Ubiquitin conjugates are not always degraded by the proteasome. One alternative fate for such proteins is for the substrate-ubiquitin linkage to be reversed through the activity of any of a large family of de-ubiquitinating enzymes. → De-ubiquitinating enzymes can remove ubiquitin from ubiquitin-protein conjugates, thus sparing the protein from proteasomal degradation. These enzymes also break down abundant multiubiquitin chains that are not attached to any substrate, and produce mature ubiquitin from the precursor forms in which it is synthesized. A second alternative fate for ubiquitin-protein conjugates is that ubiquitinated cell surface proteins may be targeted for endocytosis and eventual degradation via the lysosome rather than the proteasome.

The physiological importance of the ubiquitin pathway has only recently been recognized, and the field is now expanding rapidly. Cancer is one of the many diseases in which the ubiquitin pathway has been implicated, as might expected from a biochemical pathway that has myriad regulatory proteins as substrates. However, ubiquitination is particularly important for cancer because cancer results from aberrant growth regulation, and many growth regulatory factors are unstable substrates of the ubiquitin pathway. For example, the increased degradation rate of a particular tumor suppressor protein could confer a growth advantage on the affected cell over surrounding non-tumor cells. On the other hand, for oncoproteins whose degradation is perturbed, the opposite effect of stabilization and increased accumulation is generally observed. In each case, the physiological effect is to promote cell transformation. Some of the better established examples of the role of protein turnover in cancer are given below.

Clinical Relevance

p53 and HPV-associated cervical carcinoma

→ p53 is a tumor suppressor protein, the levels of which rise rise rapidly after DNA damage. Increased p53 levels result in growth arrest or apoptosis. In normal cells, p53 is degraded through the ubiquitin-proteasome pathway and has a half-life of approximately 30 minutes. p53 accumulation after DNA damage is thought to result principally from its stabilization. p53 is inactivated by mutation or constitutively destabilized in many transformed cells. One example of the latter is that of → human papillomavirus (HPV)-associated anogenital carcinomas. Strains of HPV that confer high risk for cancer encode an E6 protein that targets p53 for degradation. Consequently, p53 levels are very low in cervical carcinomas associated with HPV. E6 acts in cooperation with the cellular factor known as E6-AP to bind p53. E6-AP is a member of a large class of E3 enzymes (the HECT domain E3's) that are distinguished by the ability to form thiolester linkages with ubiquitin, in analogy to E2 enzymes. E6 acts as an ancillary factor to modify the specificity of E6-AP. It appears that the normal pathway of p53 turnover does not involve either E6-AP or a cellular homolog of E6. The oncoprotein Mdm-2, previously recognized as a negative regulator of p53, is apparently a ubiquitin ligase for p53. Mdm2 is overexpressed in many human soft tissue tumors, also occasionally in → neuroblastomas, and elevated Mdm2 levels are associated with poor prognosis of several human cancers.

von Hippel Lindau (VHL) disease

Like *p53*, the von Hippel Lindau (→ *VHL*) gene is a → tumor suppressor gene. In contrast to p53, however, the VHL protein is a component rather than a substrate of the ubiquitin-proteasome pathway. VHL disease affects 1 in 36,000

individuals and is characterized by a range of cancers, including (but not limited to) blood vessel tumors (hemangioblastomas) as well as tumors of the adrenal gland, kidneys, pancreas and lymphatic system. While the complete set of proteins that are stabilized when VHL activity is lost is not yet known, it appears that the major effect of the loss of VHL function is to potentiate angiogenesis and thus allow tumor outgrowth. VHL tumors are richly supplied with blood vessels due to their production of → vascular endothelial growth factor (VEGF). VEGF is overproduced in these cells as a result of their expressing high levels of transcription factors for the *VEGF* gene, HIF-1α and HIF-2α. Under normal conditions these proteins are present at low levels as a result of their rapid degradation via the ubiquitin-proteasome pathway. However, under hypoxic conditions their degradation is inhibited, leading to VEGF induction. Even in the presence of normal oxygen levels, however, the HIF proteins are stabilized in VHL tumors. This stabilization is directly due to the loss of VHL in these tumors; the VHL protein binds HIF protein directly and functions as part of an E3 enzyme, the VBC complex, in the ubiquitination of HIF proteins. This complex is closely related to the SCF complex described below.

β-catenin turnover in colorectal tumors

Aberrant regulation of the Wingless/Wnt signal transduction pathway is a key event in the development of → colon cancer. In the absence of the Wnt signal, glycogen synthase kinase-3β (GSK-3β) is active and phosphorylates β-catenin, causing it to be degraded. Upon Wnt signalling, phosphorylation of β-catenin is inhibited, and β-catenin accumulates and drives proliferation through the activation of downstream transcription factors. The APC (adenomatous polyposis coli) tumor suppressor promotes β-catenin degradation by assisting in its phosphorylation by GSK-3β. Among the most common genetic events leading to colorectal cancer are mutations in the APC gene.

The E3 enzyme that recognizes β-catenin and catalyzes its ubiquitination is known as β-TRCP. β-TRCP is an F-box protein and functions in the context of an SCF complex. In general, the key component in the SCF complexes is the F-box protein, which is the primary, if not always exclusive, mediator of substrate recognition. β-TRCP recognizes β-catenin only when it is phosphorylated by GSK-3β. In some tumors, β-catenin is found with substitutions in the serine residues, whose phosphorylation induces ubiquitination. These β-catenin mutants are stabilized as a result of their inability to be recognized by β-TRCP. Substrate recognition by F-box protein is often dependent on substrate phosphorylation.

Degradation of p27, a CDK inhibitor

Cell-cycle progression is controlled through the activity of cyclin-dependent kinases (CDK enzymes). CDK activity is regulated by two major classes of factors; cyclins and CDK inhibitors. Both cyclins and CDK inhibitors are regulated through the ubiquitin-proteasome pathway (as well as by other mechanisms). Among CDK inhibitors, p27 has been found to be present at significantly reduced levels in common tumors, such as colorectal tumors and in breast cancer. The abundance of p27 is a valuable prognostic marker for epithelial cancers, brain tumors and malignant lymphomas. Reduction of p27 levels has been shown to be due to an increased rate of degradation in epithelial cancers, lymphomas and astrocytic brain tumors. It should be noted, however, that p27 mutations in human cancers are rare. The acceleration of p27 degradation may therefore reflect alterations of the ubiquitination machinery in tumor cells.

The mechanism of p27 ubiquitination is not fully understood, but recent data indicate the S-phase kinase-associated protein 2 (Skp2) and F-box protein functions as the E3 enzyme for p27, in the context of an SCF ubiquitination complex. Recognition of p27 by Skp2 requires phosphorylation of p27 at threonine-187. This phosphorylation event is catalyzed by specific CDK/cyclin complexes such as CDK2/cyclin E, the same kinase complex that p27 inhibits. Given that cyclin E levels fluctuate during the cell cycle, it is not suprising that degradation of p27 is normally under cell cycle control. The lowest rates of p27 phosphorylation and degradation

are found during G1. Both p27 regulators, cyclin E and Skp2, are often found overexpressed in tumors.

Cbl and receptor tyrosine kinase degradation

c-Cbl is encoded by a → proto-oncogene and negatively regulates signalling via cell surface receptor tyrosine kinases, such as receptors for → platelet-derived growth factor, epidermal growth factor and colony-stimulating factor-1. Transforming variants of Cbl have been identified in transforming retroviruses and in pre-B cell lymphomas. These oncogenic forms of Cbl contain mutations affecting the RING finger motif of the protein (a zinc-chelating domain).

RING finger motifs have been implicated in the association of various proteins to E2 enzymes. As such, the presence of a RING finger in a given protein could indicate that it functions as an E3, as an ancillary component of a ubiquitination complex, or even as a substrate for the E2 to which it binds. In the case of Cbl, the RING finger domain has been shown to mediate the association of Cbl to the E2 enzyme known as Ubc4, and to allow Cbl to function as an E3 for receptor tyrosine kinases. The RING finger is essential for ubiquitination and down-regulation of activated receptor proteins. Loss of the RING finger domain is presumably oncogenic because it results in an inactive and dominant negative form of Cbl.

Another domain in Cbl, the SH2 domain, is required for recognition of its substrates. In general, SH2 domains recognize phosphotyrosine residues that are produced upon ligand binding and receptor activation, and thus the involvement of an SH2 domain in receptor ubiquitination may explain the selectivity of down-regulation for active forms of receptor tyrosine kinases.

Summary

In the examples given above, protein turnover pathways that are normally under tight regulation are converted by genetic defects to either constitutively active or inactive states. The protein turnover defects that can lead to cancer are various in nature. The primary defect can lie in:

- E3 enzymes, as in the case of Mdm2, Cbl and VHL,
- an E3-associated ancillary factor, as in the case of E6,
- a component that regulates phosphorylation, as in the case of APC,
- the substrate itself, as for β-catenin.

Given the large number of important substrates for the ubiquitin-proteasome pathway, it is likely that more examples of protein turnover defects underlying cancer will be found.

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Ubiquitin-conjugating Enzyme, E2

Synonyms

- ubiquitin-carrier protein

Definition

→ Ubiquitin-conjugating (UBC) enzyme E2 belongs to the → ubiquitin pathway enzymes that are involved in protein degradation within eukaryotic cells (from yeast to mammalian cells); multiple E2 proteins with distinct functions exist. UBC enzymes transfer ubiquitin from ubiquitin activating enzyme E1 to a member of the ubiquitin-ligase E3 family, to which the substrate protein is specifically bound; → ubiquitination.

Ubiquitin-protein Ligase, E3

Definition

Ubiquitin ligase E3 is a member of the → ubiquitin pathway enzymes that are involved in protein degradation within eukaryotc cells (from yeast to mammalian cells). It catalyses the covalent attachment of the first ubiquitin to the substrate after which a chain of ubiquitin moieties is added. This presumably serves as the recognition signal for the 26 S → proteasome, which then degrades the target protein; → ubiquitination.

UBL1

Synonyms

- → GMP1
- → SUMO-1

Definition

Ubiquitin-like 1; like ubiquitin, UBL1 can be covalently bind to other proteins and alter their behaviour and function. This has been most clearly demonstrated for RanGAP1. Ran is a small GTPase, required for nuclear transport, the mechanism of which can be regulated by binding of UBL1 with RanGAP1. protein maps to the nuclear pore complex (NPC) and to the nuclei. It protects against APO-1/FAS and TNFR1 apoptosis. UBL1 associates also with the human repair proteins → RAD51 and → Rad52 and with the PML component of a multiprotein complex that is disrupted in → acute promyelocytic leukemia (APL). The gene maps to 2q23.

UDP-glucuronosyl Transferase

Definition

UDP-glucuronosyl transferase is a family of enzymes that catalyses conjugation of UDP-glucuronic acid with xenobiotics containing an electron-rich nucleophilic heteroatom (O, N or S), such as those bearing aliphatic alcohols, phenols, carboxylic acids, aromatic and aliphatic amides, and free sulfhydryl groups (→ detoxification).

UGT

Definition

→ UDP-glucuronosyl transferase.

UICC

Definition

Union internationale contre le cancer (International Union Against Cancer).

Ulceration, peptic

Definition

Peptic ulcerations are ulcer lesions of the stomach and duodenum.

Ulcerative Colitis

Definition

Ulcerative colitis is an inflammatory bowel disease recognised as a premalignant condition.

Ultraviolet Light

Definition

Ultraviolet light is high energy light at wavelengths just below those in the visible range. UVB (wavelengths 280-320 nM) irradiation, which penetrates the atmosphere, is believed to be responsible for most of the skin carcinogenesis effects of sun exposure.

Ultraviolet Radiation

Definition

Ultraviolet radiation is electromagnetic radiation with a wavelength between 100 and 400 nm.

Unscheduled DNA Synthesis

Definition

Unscheduled DNA synthesis is any DNA synthesis occurring outside the S phase of the eukaryotic cell.

uPA

Definition

Urokinase-type plasminogen activator (uPA) is an important serine protease involved in the activation of → MMPs.

UPD

Definition

Uniparental disomy (UPD) is defined as the presence of a chromosome pair that derives from only one parent in a diploid individual.

Upstream

Definition

The word upstream identifies sequences proceeding in the opposite direction from expression. For example, the bacterial promoter is upstream from the transcription unit and the initiation codon is upstream of the coding region.

Ureterosigmoidostomy

Definition

Ureterosigmoidostomies are direct anastomosis of ureters to the sigmoid colon, which remain in continuity with the large bowel.

URF

Definition

An open (unidentified) reading frame (URF) is presumed to code for protein, but for which no product has been found.

USF1

Definition

Upstream transcription factor 1 (Usf1), also known as UEF or MLTFI, is a → bHLH protein member of the Usf family of 310 amino acids and 33 kD. The human USF1 or USF gene locus maps at 1q22-q23 and the mouse usf1 gene locus at chromosome 1 (93.50 cM). Usf1-DNA binding requires dimerization with another bHLH protein, either as a homodimer or a heterodimer (Usf1/Usf2).

USF2

Definition

Upstream transcription factor 2 (USF2), also known as FIP, is a → bHLH protein member of the Usf family of 346 amino acids and 36 kD. The human USF2 gene locus maps at 19q13.1 and the mouse usf2 gene locus at chromosome 7 (11.00 cM). Usf2-DNA binding requires dimerization with another bHLH protein, either as a homodimer or a heterodimer (Usf1/Usf2).

USF Family

Definition

Upstream transcription factor (Usf) is a group of → bHLH proteins, comprising Usf1 and Usf2. Usf family members bind DNA as homodimers or heterodimers (Usf1/Usf2). In vivo, the Usf1/Usf2 heterodimer represents over 66% of the Usf binding activity, whereas the Usf1 and Usf2 homodimers represent less than 10%.

Uterine Leiomyoma, cellular and genetic characteristics

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Synonyms

- uterine fibroids
- fibromyomas
- fibromas
- myofibromas
- myomas

Definition

Uterine leiomyomata are benign tumors comprised of smooth muscle cells of the → myometrium and of extracellular matrix components, such as collagen, proteoglycans and fibronectin. Commonly known as fibroids, uterine leiomyomata are the most prevalent pelvic tumor in women.

Characteristics

Uterine leiomyomata are symptomatic in approximately 20 to 25% of reproductive-aged women. However, the prevalence is likely to be much greater (> 70%) as fibroids can be present but asymptomatic. Although uterine leiomyomata rarely (< 0.1%), if ever become malignant, the symptoms are medically and socially significant and include prolonged and profuse uterine bleeding, pelvic discomfort, urinary incontinence, constipation, infertility, recurrent miscarriage and premature labor. Clinical diagnosis is typically made by physical examination and can be confirmed by imaging studies such as ultrasound. Small lesions, however, can go undetected.

Although leiomyomata primarily affect women during childbearing years, the only consistently curative therapy is → hysterectomy. In fact, uterine leiomyomata are the most common indication for hysterectomy in the US, accounting for about 200,000 procedures, or one-third of procedures annually. Effective alternative treatment options are limited. For example, → myomectomy is associated with a high rate of recurrence and medical therapies are not curative and are often ineffective.

Histologically, uterine leiomyomata appear as whorled bundles of smooth muscle cells in well circumscribed masses. Generally, mitosis are rare and appear normal, which is consistent with the benign nature of these tumors. Uterine leiomyomata are classified by their location in the uterus. Tumors within the uterine wall are called intramural fibroids. Tumors located below the outermost uterine layer are termed subserosal fibroids. Alternatively, fibroids that expand toward the innermost uterine layer and distort the uterine cavity are termed submuco-

sal fibroids. Subserosal and intramural locations comprise the majority (95%) of all leiomyomata; submucosal leiomyomata make up the remaining 5%. Tumor location and size correlate with symptoms such as bleeding, pelvic pain and infertility. For example, submucosal fibroids are more often associated with bleeding than are subserosal or intramural tumors.

Despite the major public health impact of uterine leiomyomata, little is known about their etiology. Cellular, molecular, cytogenetic and epidemiological studies have advanced our current understanding of fibroid biology.

Cellular parameters

Until recently, the steroid hormones estrogen and progesterone were considered the most important regulators of leiomyoma growth. There is abundant evidence that sex steroids promote fibroid growth, including the clinical observations that fibroids grow in the presence of high levels of estrogen or progesterone, such as during the reproductive years or during treatment with synthetic progesterones; fibroids regress in the presence of low levels of estrogen or progesterone, such as following menopause or during treatment with gonadotropin releasing hormone agonists (GnRHa) or with antiprogesterone agents such as (RU-486). Furthermore, fibroids have higher estrogen concentrations, bind more estrogen, have more estrogen and progesterone receptors and convert estradiol (a more active form of estrogen) to estrone (a less active form of estrogen) more slowly than does normal myometrium. Other hormones, such as growth hormone and prolactin, are also thought to promote fibroid growth, but their roles are less well defined.

Growth factors, which are small proteins that affect cell growth, recently have been shown to mediate the growth-promoting effects of estrogen and to play an important role in the development of fibroid tumors. Potentially important factors in fibroid growth include transforming growth factor-β, basic fibroblast growth factor, epidermal growth factor, insulin-like growth factor and platelet-derived growth factor. Overall, estrogen, progesterone and growth factors likely promote tumor growth, but only after the initiation of tumor formation. This initiating event remains unknown, although there is evidence to suggest there is a strong genetic component to fibroid development.

Molecular Genetic/ Cytogenetic Characteristics

Uterine fibroids are considered to be independent monoclonal lesions as determined by X-inactivation studies using polymorphisms in either the glucose-6-phosphate dehydrogenase (G6PD) isoenzyme or the androgen receptor genes on the X chromosome.

Although 60% of fibroids studied cytogenetically have a normal karyotype, approximately 40% of fibroids have nonrandom chromosomal abnormalities. Analyses of these leiomyomata with abnormal karyotypes have revealed consistent cytogenetic rearrangements, which can be divided into several subgroups; → translocation between chromosome 12 and chromosome 14, trisomy 12, → deletion on the long arm of chromosome 3 or 7, and rearrangements of the short arm of chromosome 6 or of the long arms of chromosome 10.

Translocation between chromosomes 12 and 14, t(12;14)(q14-q15;q23-q24), is the most common translocation in fibroids and occurs in approximately 20% of fibroids with karyotypic rearrangements. It is observed in many other → mesenchymal tumors including angiomyxomas, breast fibroadenomas, endometrial polyps, hemangiopericytomas, lipomas, pulmonary chondroid hamartomas and salivary gland adenomas. → *HMGIC* is the critical gene involved in chromosome 12 translocations. The specificity of chromosome 14 as a translocation partner for chromosome 12 in fibroids remains to be determined, as compared to a diverse number of partner chromosomes for lipomas.

Rearrangements of the short arm of chromosome 6 (6p21) are present in fewer than 5% of karyotypically abnormal fibroids and include translocations with chromosomes 1, 2, 4, 10, and 14, as well as inversions and translocations with various other chromosomes. *HMGIY* [→ HMG], a gene related to *HMGIC*, is involved

in these rearrangements. Chromosome 6 abnormalities are also consistently present in other benign mesenchymal tumors such as lipomas, hamartomas, and endometrial polyps.

In general, the highest levels of *HMGI* gene expression are seen in tumor cells and during normal development in embryonic tissue, whereas expression is reduced or absent in adult tissues. Studies of chromosome 12 translocations in fibroids and other benign mesenchymal tumors have revealed various mechanisms of *HMGIC* dysfunction, including creation of fusion mRNAs, truncation of *HMGIC* and disruption of *HMGIC* regulatory sequences. Interestingly, rearrangements within the coding region of *HMGIC* are more common in lipomas, and rearrangements outside of the coding region are more common in fibroids. This suggests that dysregulation of *HMGIC* expression, not disruption of coding sequence, is a potential mechanism of fibroid growth.

Interstitial deletion of the long arm of chromosome 7, del(7)(q22q32), is present in 17% of karyotypically abnormal fibroids as well as in other benign mesenchymal tumors, such as lipomas and endometrial polyps. This large area contains a high density of genes, such as *DLX5*, *DLX6*, *COL1A2*, *PCOLCE*, *PMS218*, *ACHE*, *PAI1*, *MUC11*, *MUC12*, *TRIP6*, *CUTL1* and *ORC5L*, thereby complicating identification of critical fibroid gene(s). Although some of these genes influence cell growth, none have been shown to have a definitive role in the pathogenesis of the del(7q) subgroup of fibroid tumors.

Other less common cytogenetic abnormalities in fibroids include rearrangements of chromosomes X, 1, 3 and 10. Rearrangements of the X chromosome include translocations with chromosomes 5 and 12, deletions, and inversions. Rearrangements of chromosome 1 frequently involve ring chromosome formation, as well as translocations with the short arms of chromosomes 2 and 6. Rearrangements of chromosome 3 include insertions, long and short arm deletions and translocations with chromosome 7. Rearrangements of chromosome 10 include loss of one copy of chromosome 10 (→ monosomy 10) and deletions affecting the long arm (especially band q22). To date, no candidate genes on any of these chromosomes have been identified as having a role in leiomyoma formation.

The various types of rearrangements found in fibroids, including translocation, trisomy and deletion, suggest multiple mechanisms of tumor growth. For example, translocations can either up- or down-regulate expression of a gene through juxtaposition of an entire gene sequence next to an ectopic regulatory element. Alternatively, translocations that interrupt gene sequence can result in the formation of novel fusion genes. Trisomies can increase gene expression through increased gene dosage, whereas deletion mutations most often result in loss of gene function. Thus, the different types of chromosomal abnormalities present in fibroids may predict heterogeneous genetic pathways of tumor growth and development.

Of all karyotypically abnormal fibroid tumors, approximately 30 to 40% are mosaic with chromosomally normal cells. These chromosomally mosaic tumors also have been shown to be clonal by X-inactivation analysis, suggesting that cytogenetic abnormalities may be secondary changes in tumor development and that other genetic changes may be primarily responsible for initial tumor growth in genetically susceptible cells.

Genetic epidemiology

A variety of epidemiological studies have assessed whether there might be a genetic basis for uterine fibroids, including ethnic predisposition, twin, and familial aggregation studies. For example, women of African origin experience an approximately three-fold higher incidence of fibroids than women of other racial and ethnic groups, after adjustment for known risk factors, such as obesity and number of births, as well as socioeconomic status and access to health care. Identical twins have twice the rate for hysterectomy as compared to fraternal twins, which is consistent with the expected rates for a genetically influenced trait. In addition, fibroids are at least twice as common in women who have a first degree relative with fibroids than in women who have no relatives with fibroids. This increases about six-fold in

women with early onset of fibroids (< 45 years), an expected observation for a genetically influenced trait. Finally, Reed syndrome (or familial cutaneous leiomyomatosis) is characterized by uterine leiomyomata in association with multiple cutaneous leiomyomata (fibroids of the skin) and is known to be inherited as an autosomal dominant trait with reduced penetrance.

Together, these observations indicate that fibroids have a potentially significant heritable component to their development. Overall, cytogenetic analyses, together with molecular clonality and epidemiological studies, suggest that karyotypic abnormalities in fibroids may be important in the pathobiology of these tumors and may represent secondary somatic changes in genetically susceptible cells. The susceptibility gene(s) that are crucial for fibroid pathogenesis remain to be identified. Ultimately, understanding the mechanisms of fibroid tumor development could lead to innovative, less-invasive treatment options for this significant women's health problem.

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Uterine Leiomyoma, clinical oncology

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Synonyms

- leiomyomata
- fibromyoma
- myoma
- fibroids

Definition

Uterine leiomyomas are benign neoplasms, composed of smooth muscle cells with variable amounts of fibrous stroma.

Characteristics

Leiomyomas are usually found in the myometrium of the uterine corpus; however, they can also originate in the smooth muscle organs. They are composed of smooth muscle and extracellular matrix (collagen, proteoglycan, fibronectin).

Incidence

Uterine leiomyomas are the most common neoplasms of the female pelvis. They occur in approximately 20 to 25% of women of reproductive age, but careful pathologic inspection of the uterus reveals that they are present in more than 80% of women. The neoplasm is more frequently found in the fourth and fifth decades of life and most commonly in patients of African descent. Age-standardized rates by race were 8.9 new cases per 1,000 woman per year for Caucasian women and 30.6 new cases per 1,000 woman per year for black women. Hispanic and Asian women had rates similar to those seen in Caucasian women: 11.0 and 8.0 new cases per 1,000 woman per year, respec-

tively. There is an increased risk of leiomyomas in women with greater → body mass index (BMI), but risk is decreased in women who smoke, who have given birth or who use oral contraceptive pills. Leiomyomas rarely occur before menarche and typically regress after menopause, indicating estrogen as a promoter of growth. Rarely, malignant changes may occur, usually in postmenopausal women. The most common warning sign is rapid enlargement of a fibroid with definitive diagnosis usually made at the time of surgery.

Gross features

Uterine leiomyomas may have solitary nodule or multiple nodules ranging in size from microscopic to huge tumors weighing more than 100 lb. They are firm and well demarcated from the surrounding myometrium. On sectioning, the tumor bulges from the surface and the pseudocapsule, produced by compression of adjacent myometrium, is readily apparent. The surface is smooth and glistening white with a whorled, fasciculated pattern. Variations in this appearance include hemorrhage, hyalinization, hydropic, myxoid or mucinous degeneration, true necrosis and calcification. These variations are important because they may also be seen in leiomyosarcoma. Leiomyomas are characterized by their location in the uterus:

- subserous leiomyoma (just under the uterine serosa),
- interstitial leiomyoma (within the thick myometrium),
- submucous leiomyoma (just under the uterine mucosa).

Microscopic features

Histologically, uterine leiomyomas are bundles of interlacing smooth muscle fibres with varying amounts of collagenous fibrous tissue and few blood vessels. The nuclei have a uniform appearance, mitotic figures are infrequent, and there is never nuclear atypia. The variants of uterine leiomyoma listed in the International Society of Gynecological Pathologists' Classification are:

- cellular leiomyoma,
- epithelioid leiomyoma,
- bizarre leiomyoma,
- lipoleiomyoma.

Symptoms

It is estimated that only 10 to 40% of leiomyomas are symptomatic. Patients may complain of a self-detected mass, abnormal uterine bleeding, pelvic pain or pressure related symptoms.

Bleeding is the most common presenting symptom in uterine leiomyomas. The most frequent presentation includes the development of progressively heavier menstrual flow that lasts longer than the normal duration (menorrhagia). Although menorrhagia can occur in any women with leiomyomas, women with submucous leiomyomas appear to be particularly prone to this complication. Blood loss from this type of menstrual bleeding may be heavy enough to contribute to iron-deficiency anemia.

Another common presenting symptom is pelvic pressure. This is caused by slowly enlarging leiomyomas, which may attain a massive size. Pressure on the bladder produces urinary frequency, urgency and rarely and the inability to void. Constipation may result from pressure on the rectum. Pressure of the uterine leiomyomas on the ureters may cause hydroureter and, on occasion, hydronephrosis.

Acute onset of pain in previously asymptomatic leiomyomas raises the possibility of necrosis, inflammation or torsion of a pedunculated subserous leiomyoma. Prolapse of submucous leiomyoma may present as intense cramping pain, often accompanied by discharge or bleeding. Pain in the low back or legs may reflect alterations in body posture or pressure on lumbosacral nerve trunks.

Uterine leiomyomas can also be associated with other kinds of reproductive dysfunction, including recurrent miscarriage, infertility, premature labor, fetal malpresentation and other complications of labor.

Diagnosis

The diagnosis of such tumors is usually made by the pelvic examination. A leiomyoma may be suspected based on a bimanual examination that reveals an enlarged, firm, non-tender and irregularly shaped uterus. Ultrasonography is the most common method of confirming the diagnosis. A combination of transabdominal and transvaginal ultrasonography should provide information regarding uterine volume, the number of leiomyoma, their location relative to the endometrial stripe, and evaluation of the adnexa. Of other available imaging techniques, magnetic resonance imaging (MRI) may prove to be the most useful but does not improve the findings seen on ultrasonography and has much higher costs.

Treatment

The majority of patients with uterine leiomyomas do not require treatment. If the tumors are stable and slow growing, an annual follow-up including an optimized symptom control is a viable option. Symptomatic patients should usually be offered a trial of conservative management before considering surgery.

→ Gonadotropin-releasing hormone (GnRH) agonists are analogs of gonadotropin-releasing hormone. With continuous administration, they induce a hypoestrogenic pseudomenopausal state. Because uterine leiomyomas are estrogen-dependent benign tumors, this causes shrinkage of these tumors and of the myometrial mass. In addition, treatment with a GnRH agonist induces amenorrhea, allowing women with menorrhagia-induced anemia to increase iron stores and hemoglobin concentrations leading to a technically easier surgery with markedly diminished blood loss. But once the treatment is stopped, the leiomyoma tends to regrow to pre-treatment size. The major use of GnRH agonists is as a preoperative treatment to facilitate surgical procedures, either myomectomy or hysterectomy. This class of drug cannot be used long term (>6 months) because of the attendant risks of prolonged hypoestrogenism, such as osteoporosis and cardiovascular disease. Combinations of GnRH agonists and low doses of estrogen and progesterone (that is, 'add-back' regimens) can minimize the adverse hypoestrogenic effects caused by GnRH agonist treatment.

Androgenic agents (danazol, gestrinone) and progestins (medroxyprogesterone acetate, depomedroxyprogesterone acetate, norethindrone) have also been used to minimize uterine bleeding in women with uterine leiomyomas. However, these kinds of medication do not consistently decrease uterine or leiomyoma volume and their mechanism of action is thought to be the induction of endometrial atrophy. If there is significant endometrial cavity distortion by interstitial or submucous leiomyomas, these agents are often not successful in controlling menorrhagia, because the excessive bleeding is usually related to profound anatomic and vascular distortion.

Hysterectomy is considered to be the definitive treatment for uterine leiomyoma in symptomatic women who have completed childbearing. If the tumors are small, the hysterectomy may be done vaginally, particularly if there is associated pelvic relaxation. The American College of Obstetricians and Gynecologists (ACOG) criteria for hysterectomy for uterine leiomyoma are as follows:

- Presence of 1 to 3 asymptomatic leiomyomas of palpable size abdominally and if they are a concern to the patient.
- Excessive uterine bleeding:
  - profuse bleeding with flooding or clots or repetitive periods lasting more than 8 days;
  - anemia, due to acute or chronic blood loss
- Pelvic discomfort caused by myomas:
  - acute and severe pain;
  - chronic lower abdominal or low back pressure;
  - bladder pressure with urinary frequency not due to urinary tract infection

When women want to preserve childbearing potential, a myomectomy may be performed. Myomectomy has a higher complication rate than hysterectomy. These complications include excess intraoperative blood loss, risk of postoperative hemorrhage, adhesions and bo-

wel obstruction. It is important to inform the patients that there is a 25% to 50% recurrence of leiomyomas after myomectomy. Therefore, a significant number of women undergoing a myomectomy will require a subsequent hysterectomy. Preoperative criteria for myomectomy published by ACOG are as follows:

- failure to conceive or recurrent pregnancy loss;
- presence of leiomyomas of sufficient size or specific location to be a probable factor;
- no other likely explanation for the failure to conceive or for recurrent pregnancy loss.

The use of endoscopic resection will be more widely used for the surgical management of uterine leiomyomas. This technique causes less patient discomfort, less bleeding and has a shorter recovery time. Hysteroscopic resection of submucous leiomyomas can be done using a resectoscope with unipolar cautery loops or with a neodymium:yttrium-aluminium garnet laser (Nd:YAG). The use the Nd:YAG laser has its problems and the operating surgeon must have expertise not only with the hysteroscope but also with the intrauterine laser. Laparoscopic myomectomy and laparoscopically assisted vaginal hysterectomy (LAVH) are safe and reliable treatment options, but can be difficult to perform technically and are not available universally.

Uterine artery embolization is a new, investigational treatment of leiomyomas. The method involves the catheterization of both uterine arteries using a femoral arterial approach. Preliminary studies in women who had large, symptomatic leiomyomas have shown significant improvement in their symptoms. More trials need to be carried out to confirm the efficacy and safety of this surgical treatment.

Cytogenetic changes

Evidence from glucose-6-phosphate dehydrogenase isoenzyme analysis and from polymorphism analysis in the androgen receptor demonstrate that uterine leiomyomas are monoclonal and that each tumor within the same uterus arises independently. Although the classic paradigm suggests that the sex steroid hormones (estrogen and progesterone) are the only important modulators of leiomyoma growth and transformation, it is now clear that chromosomal abnormalities play a role in the pathogenesis of these neoplasms. In uterine leiomyomas, non-random chromosomal changes such as translocations, duplications and deletions have been identified in approximately 50% of tumors studied by cytogenetic analysis. The most frequent abnormalities are translocations between chromosomes 12 and 14, t(12;14)(q14-15;q23-24) and deletions on chromosome 7, del(7)(q22-32). The frequencies of these abnormalities are approximately 20% and 15%, respectively.

Recently, the high-mobility group protein gene *HMGIC* was identified as the target gene affected by the 12q14-15 aberrations. HMGIC is an architectural transcription factor in the nuclear scaffold, a function critical for the correct assembly of stereo-specific transcriptional complexes. The frequent rearrangement of the *HMGIC* gene in uterine leiomyomas suggests that this gene is directly involved in the aberrant growth control observed in these tumors. Gene-targeting experiments indicate that HMGIC plays an important role in mammalian growth and development, since inactivation of the murine *HMGIC* gene results in the pygmy phenotype. In uterine leiomyomas, the mitochondrial aldehyde dehydrogenase (*ALDH2*) gene in 12q24.1, the recombinational repair gene *RAD51B* in 14q23-24, and the cytochrome c oxidase subunit VIc (*COX6C*) gene in 8q22-23 were identified as translocation partners to *HMGIC*. Deletions of chromosome 7 imply that tumor enlargement in some leiomyomas is probably due to loss of tumor suppressor genes.

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V

Variable Region

Definition

The variable region of an immunoglobulin chain is coded for by the V gene. When different chains are compared, they vary extensively as the result of multiple (different) genomic copies and changes introduced during construction of an active immunoglobulin.

Vascular Endothelial Growth Factor

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Synonyms

- VEGF (vascular endothelial growth factor)
- VPF (vascular permeability factor)

Characteristics

VEGF family

Four VEGF family members have been described in mammals, VEGF-A through VEGF-D. VEGF-E, the fifth member of the family is coded by the Orf virus. An additional relative is the placenta-derived growth factor, PlGF. Whereas the VEGFs are potent growth promoting and vascular permeability enhancing factors, PlGF is incapable of inducing permeability. All members exert their biological functions as homodimers. VEGFs act almost exclusively on endothelial cells. VEGF is expressed by almost all cell types with the exception of endothelial cells which express only marginal amounts of the growth factor. Expression is controlled by a number of different mechanisms. Extracellular signals such as growth factors and cytokines are able to induce transcription of the VEGF gene. Activated oncogenes, such as ras, raf, or src, as well as inactivated tumor suppressor genes, such as p53 or von-Hippel-Lindau (VHL), contribute to enhanced transcription. The newly identified p53 analogue p73 in its wildtype form can cause repression of VEGF transcription. Hypoxia, a physiological signal in early embryonic development and a pathophysiological signal in many tumors, causes enhanced production of VEGF mRNA and also stabilizes the VEGF mRNA. The pattern of VEGF expression is strictly controlled by some of these factors in a time- and tissue-specific fashion during embryonic development and physiological angiogenesis. In pathological situations VEGF expression proceeds with no specific control, exceeds physiological concentration and occurs at wrong times and locations.

VEGF-Receptor family

All VEGF family members mediate their signals through a family of distinct high affinity receptors; VEGF-R1 through VEGF-R3. All three VEGF receptors are tyrosine kinases that become stimulated upon ligand binding, with VEGF-R1 tyrosine kinase being much less activated than VEGF-R2 and VEGF-R3. VEGF-R1 is expressed as two distinct forms; the entire transmembrane receptor VEGF-R1 and a soluble variant sVEGF-R1 generated by alterna-

tively spliced mRNA. VEGF-R1 and its soluble variant bind VEGF-A, VEGF-B and PlGF. VEGF-R2 has high affinity for VEGF-A, VEGF-C, VEGF-D and VEGF-E. VEGF-R3 binds VEGF-C and VEGF-D. All three VEGF receptors are exclusively expressed on the surface of endothelial cells. VEGF-R2 is the most important VEGF receptor mediating a proliferative response to endothelial cells. Upon transfection into non-endothelial cells VEGF-R2 becomes autophosphorylated in response to VEGF binding, but is no more mitogenic. This indicates the involvement of cell type-specific signalling mechanisms. The endothelial cell proliferation and survival in response to VEGF requires the association of cell surface adhesive molecules. Activated VEGF-R2 associates with integrins $\alpha_v\beta_3$. VE-cadherin colocalizes with VEGF-R2 and upon stimulation by VEGF becomes associated with VEGF-R2, β-catenin and PI-3-kinase. This leads to activation of PKB/Akt and initiation of a survival signal. Disruption of VE-cadherin leads to prevention of VEGF-mediated cell survival. VEGF-receptor expression is under tight control in embryo development and normal physiology. In pathological situations, such as tumor angiogenesis, VEGF-R1 and VEGF-R2 are transcriptionally upregulated in response to VEGF and thus generate an amplification mechanism to enhance this fatal process.

VEGF in vasculogenesis and physiological angiogenesis

VEGF is widely and abundantly expressed in many tissues during fetal development and has been implicated in the process of vasculogenesis, i.e. the *de novo* formation of the vascular system. Mice deficient for VEGF die at day 8.5-9.0 and show a delayed differentiation and an impairment of both vasculogenesis and angiogenesis, i.e. the sprouting of new capillary vessels from pre-existing vasculature. Similarly, the receptors VEGF-R1 and VEGF-R2 are strongly expressed in the developing embryo. In particular, VEGF-R2 is expressed in the hemangioblasts, the common precursor to both endothelial cells and hematopoetic lineages. VEGF-R1, being non-essential for endothelial development, is required at a later stage of the organisation of the embryonic vasculature. Disruption of the VEGF-R2 gene interferes with endothelial cell development, leading to death of embryos at day 8.5-9.5. Disruption of the VEGF-R1 gene permits endothelial cell differentiation but results in thin walled vessels of larger than normal diameter, and the embryos die at day 9.

In the adult organism, large amounts of VEGF are also found in the female reproductive tissues in association with hormonally-regulated angiogenesis that takes place in the ovary and endometrium at specific stages of the menstrual cycle and in pregnancy. Strong expression of VEGF can be detected in several tissues of the adult in the absence of angiogenesis, particularly kidney, lung, adrenal gland and heart. However, some of these tissues express only low levels or no VEGF receptors which might explain the absence of angiogenesis. As VEGF has been shown to be a survival factor for vascular endothelial cells it might also be that this function, requiring only low levels of VEGF receptors, is important for maintaining vascular homeostasis without angiogenesis to occur.

Clinical Relevance

VEGF in pathophysiological angiogenesis

VEGF, as well as its receptors VEGF-R1 and VEGF-R2, are strongly overexpressed at both the mRNA and protein levels in almost all malignant tumors. Tumor metastases exhibit overexpression of VEGF similar to that found in the primary tumors from which they arose. Elevated VEGF levels have been found in the blood of tumor patients, correlating in many cases with poor clinical prognosis of the disease. Accordingly, the soluble variant of the VEGF-R1 is also found to be elevated in the blood of tumor-bearing patients indicating the presence of activated tumor endothelium in the diseased areas. Thus, VEGF and the soluble variants of VEGF-R1 could be regarded as surrogates for pathological tumor angiogenesis. In addition to the intimate involvement of VEGF and its receptors in tumor angiogenesis, VEGF is also capable of increasing vascular per-

meability. VEGF-induced leakage of plasma from hyperpermeable microvessels results in fluid accumulation within tumors. This is also favoured by the fact that tumors in general lack lymphatic vessels and hence are unable to drain extravasated proteinaceous fluid effectively. This is in particular obvious in brain tumors, showing increased intracranial pressure and in tumors metastasizing to body cavities leading to substantial accumulation of fluid.

VEGF and both of its receptors are also overexpressed in a number of pathological entities that involve angiogenesis, but are not associated with neoplasia. These include diabetic and other retinopathies, rheumatoid arthritis and psoriasis. In all of these examples, overexpression of VEGF and its receptors is accompanied by increased microvascular hyperpermeability and pathological angiogenesis.

VEGF/VEGF receptor system: Therapeutic opportunities

As VEGF and its receptors are intimately involved in the pathology of many diseases, such as cancer, rheumatoid arthritis, diabetic retinopathies, considerable efforts have been made to interfere therapeutically with this signalling system. Monoclonal antibodies against VEGF and the binding domain of its receptor VEGF-R2 have been generated. Animal experiments show efficacy against tumor vascularization, tumor growth and metastases formation. Both antibodies have been humanized and are being evaluated in the clinic. Low molecular weight compounds were developed to inhibit the tyrosine kinase activities of VEGF-R1 and VEGF-R2. These compounds show considerable activity against growth of highly vascularized tumors and also inhibit metastasis formation. Some of these inhibitors also show activity against other non-VEGF receptor tyrosine kinases. Clinical evaluation is being carried out to demonstrate whether the additional non-VEGF receptor related activity is beneficial or not. Furthermore, combination of anti-VEGF strategies together with low dose cytotoxic strategies have revealed synergistic effects in animals. This indicates that anti-angiogenic therapy could be useful to enhance the therapeutic potential of conventional chemo-therapeutic drugs.

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Vascular Permeability Factor

Definition

→ Vascular endothelial growth factor.

Vector

Definition

A vector contains genetic information encoding a gene, plus those sequences required for gene expression and for integration into the host genome where applicable (→ cloning vector). In some situations, the term ‘vector’ denotes the vector sequences in the context of a gene transfer vehicle (viral or non-viral); → gene therapy; → transfection.

VEGF

Definition

→ Vascular endothelial growth factor (VEGF) is an inducer of endothelial cell proliferation and migration. The binding of VEGF to the receptor tyrosine kinases flk1/KDR, flt-1, and flt-4 on vascular endothelial cells promotes their proliferation and leads to vessel formation.

VEGF Receptor

Definition

Vascular endothelial growth factor (VEGF) receptor is an endothelial membrane receptor that interacts with VEGF. VEGF is produced by many tumors and stimulates tumor-associated neo-angiogenesis.

v-ERB-B

Definition

v-erb-B is an epidermal growth factor receptor.

V Gene

Definition

V gene is a sequence coding for the major part of the variable (N-terminal) region of an immunoglobulin chain.

VHL

Definition

VHL is a gene involved in von Hippel-Lindau disease (→ renal carcinoma). Also see → von Hippel-Lindau Tumor Suppressor Gene.

VIPoma

Definition

VIPoma is a gastroenteropancreatic tumor primarily located in the pancreas that produces vasoactive intestinal and other peptides (e.g. somatostatin, neurotensin, helodermin) leading to watery diarrhea, hypokalemia and achlorhydria (WDHA-syndrome).

Viral DNA Polymerase

Definition

Viral DNA polymerase is a virally-encoded polymerase used for replication of the viral genome during natural infection.

Vitamin D_3

Definition

Vitamin D_3 (activated 7-dehydrocholesterol) is essential for maintenance of calcium homeostasis in higher animals. Vitamin D_3 modulates transcription of vitamin D3-responsive genes by binding to its receptor, which in turn interacts with the vitamin D_3-response DNA element.

v-MYC

Definition

v-myc is a viral oncogene that is incorporated by → oncogene transduction into a → retrovirus from the *c-myc* gene of chicken.

Vomeroglandin

Definition

Vomeroglandin is an alternative name for → CRP-ductin. It is specifically expressed by the vomeronasal glands and is suggested to play a role in pheromone perception (→ DMBT1).

von Hippel-Lindau Disease

Definition

Von Hippel-Lindau disease is a rare inherited disorder characterized by a predisposition to develop tumors in multiple organs including brain, spinal cord, eye, adrenal gland, kidney, pancreas and epididymis (→ renal carcinoma).

von Hippel-Lindau Tumor Suppressor Gene

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Definition

The von Hippel-Lindau tumor suppressor gene (*VHL*) is a cellular gene that is required for normal development and differentiation. *VHL* was discovered in families with the hereditary von Hippel-Lindau (VHL) syndrome by virtue of its two-hit mechanism of inactivation, and identified in 1993 following a positional cloning strategy. The *VHL* gene may be subject to mutation either in the germline giving rise to VHL disease or in somatic renal epithelial cells giving rise to sporadic renal clear cell carcinoma. *VHL* mutation patterns suggested two functional domains within the protein pVHL. Current knowledge indicates similarities to the SCF (Skp1-Cul1-F-box protein) ubiquitin ligase complex that targets proteins for degradation. Therefore, pVHL may function as a molecular adaptor in a similar proteolytic pathway.

Characteristics

Molecular features

- Gene located at 3p25-p26 (single copy locus).
- 639 nucleotides in 3 exons (originally reported sequence contained 852 nucleotides with 213 untranslated base pairs at the 5′ end).
- Exon 1 is a CpG island (G + C content is 70%, CpG/GpC > 1).
- TATA-less and CCAAT-less promotor.
- Two transcription initiation codons (amino acid 1 and amino acid 54).
- Follows a two-hit mechanism of inactivation characteristic for tumor suppressor genes.
- Evolutionarily highly conserved.
- No homologies known.
- The full length protein pVHL contains 213 amino acids.
- Known isoforms result from tissue specific and developmentally selective alternative splicing (skipping of exon 2).
- Expressed in a variety of adult and fetal tissues including those of VHL target organs.
- There are two protein binding domains that allow pVHL to function as an adaptor molecule in a proteolytic pathway.
- The gene may be subject to mutations at almost any nucleotide of the 470 bp COOH terminal sequence.
- Phenotypic variation may result from confounding effects of modifier genes.
- No imprinting reported.

Role in diseases - clinical, molecular and cellular characteristics

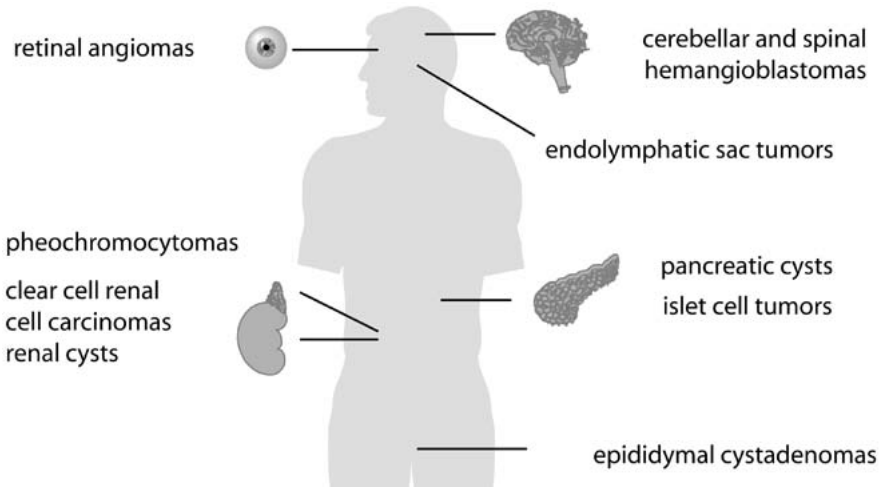
von Hippel-Lindau (VHL) disease is an inherited tumor susceptibility syndrome predisposing gene carriers to a variety of benign and malignant tumors. VHL disease segregates in affected families as an autosomal dominant inherited trait. Penetrance is approximately 90% at the age of 65 years and phenotypic expression is highly variable.

Clinical and molecular features

- Tumors and cysts are frequently bilateral and/or multiple in origin.
- All ethnic groups are involved, there is no sex bias.
- Birth incidence is estimated 1/39,000 (Germany) to 1/53,000 (East Anglia).
- Prevalence is 1/85,000 to 1/31,000.
- Incidence of *de novo* mutations is about 5%.

von Hippel-Lindau Tumor Suppressor Gene. Table

affected organs	clinical symptomes (Fig. 1)	frequency
eyes	angiomatosis retinae	50 – 57%
central nervous system	cerebellar hemangioblastomas	55 – 59%
	spinal hemangioblastomas	13 – 14%
adrenal glands	pheochromocytomas	7 – 19%
kidneys	renal cysts	76%
	clear cell renal cell carcinomas (CCRCC)	24 – 28%
pancreas	pancreatic cysts	22%
	and serous cystadenomas	occasionally
	islet cell tumors usually asymptomatic	infrequent
inner ear	endolymphatic sac tumors	< 10%
epididymis	cystadenomas	10 – 15%
broad ligament	benign adnexal papillary tumors	occasionally
liver	cysts	occasionally



von Hippel-Lindau Tumor Suppressor Gene. Fig. 1 – Lesions associated with von Hippel-Lindau disease (VHL). VHL patients may present with a variety of tumors affecting eye, central nervous system, inner ear, adrenal gland, kidney, pancreas, epididymis. Most frequent tumors include retinal angiomas, and hemagioblastomas of the cerebellum and of the spinal cord which are usually benign. Other benign lesions include pheochromocytomas, renal and pancreatic cysts. Renal clear cell carcinomas are malignant.

- Mean age at diagnosis is 26 years.
- Most severe complications are hemangioblastomas due to unrestricted growth in the confined space of skull or spinal canal, and CCRCC due to metastasis.
- Major cause of death are hemangioblastomas.

Classical clinical definition

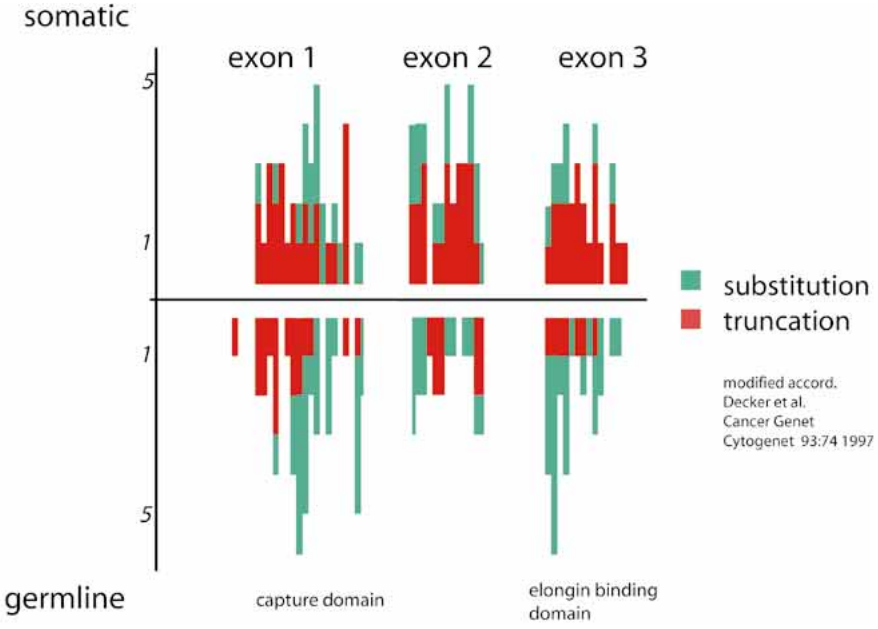
- Known family history of retinal or cerebellar hemangioblastoma: the presence of a single hemangioblastoma or a visceral manifestation, i.e. CCRCC in one patient will define him as a carrier.

- Isolated cases, possibly indicating a *de novo* mutation: two or more hemangioblastomas (spinal, cerebellar or retinal) or a single hemangioblastoma in association with a visceral manifestation, i.e. CCRCC) are sufficient to establish the diagnosis.

It is possible in most cases to determine VHL carrier status by mutation testing of lymphocytic DNA. This can either be performed to assist clinical diagnosis or to establish carrier status presymptomatically in at risk individuals. Based on the presence or absence of pheochromocytoma, VHL disease is phenotypically subclassified into VHL type 1 (without pheochromocytoma, majority of all families) and VHL type 2 (with pheochromocytoma, about 7 – 20% of all families). VHL type 2 syndrome is further subdivided into type 2A (without CCRCC) type 2B (with CCRCC) and type 2C (pheochromocytomas as the only manifestation). In VHL type 2C disease, it is especially important to carefully establish the diagnosis in affected patients since pheochromocytoma is also a manifestation of other inherited syndromes such as multiple endocrine neoplasia type 2 as well as neurofibromatosis type 1.

Germline Mutations

Comprehensive germline mutation analysis allows the detection of nearly 100% of VHL predisposing mutations in gene carriers (Fig. 2). Most mutations (about 80%) are small, affecting one to a few nucleotides, resulting in nucleotide changes, deletions and insertions. These can be readily identified by sequencing. Large genomic and intragenic deletions may be identified by Southern blotting including quantitative Southern blotting, pulsed field gel electrophoresis (PFGE) or/and fluorescence *in situ* hybridization (FISH). In those rare cases where



von Hippel-Lindau Tumor Suppressor Gene. Fig. 2 – *VHL* mutations described in the literature. Germline (lower) and somatic (upper) mutations are listed along a schematic of the *VHL* coding sequence. More than 320 somatic mutations that have been identified in sporadic renal clear cell carcinomas and more than 360 germline mutations that have been identified in *VHL* families are shown. Red color indicates mutations that are predicted to result in protein truncation. Green color indicates mutations that are predicted to result in an amino acid change. In the germline, two main clusters of mutations are in exon 1 and exon 3, indicating functional domains. This distribution of mutations supports the suggested adaptor function of pVHL. Targets are thought to be recognized by interaction with a capture domain (exon1), protein degradation is mediated by interaction with elongins (exon 3).

germline mutations escape detection it is possible that an affected individual may be a mosaic, with some cells carrying the *VHL* mutation and others that do not. Although it may be difficult to find a *VHL* mutation in a mosaic individual, offspring are at high risk to develop the disease. Once the mutation is passed on into the next generation, due to affected germ cells, it should easily be identified in the affected offspring who now will carry the germline mutation in all of their body cells.

Most germline mutations cluster within exon 1 and exon 3 suggesting two functional protein domains. In particular, there is a mutational hotspot affecting codon 167, either changing an arginine to tryptophane or to glutamine. The affected amino acid is located within a 35 residue domain necessary for binding to ElonginC to form the ternary pVHL/ElonginC/ElonginB complex (VCB). Another frequently identified mutation causes a change of histidine to tryptophane at codon 89. However, the frequency of this mutation is due to a founder effect. The affected amino acid is located within a separate remote domain. A *VHL* mutation data collection is available at http://www.umd.necker.fr (C.Beroud, INSERM).

Genotype/Phenotype correlation

There is a difference of frequencies of the types of germline mutations between VHL type 1 and VHL type 2 families. With few exceptions, most VHL type 2-associated germline mutations are of the missense type. The predicted consequence of any missense mutation is a single amino acid change in an otherwise full length protein. Although these mutations seem to cause minor damage to the VHL protein, they have a tendency to compromise pVHL function in a tissue specific manner, which may explain their frequent association with the formation of pheochromocytoma. The spectrum of VHL type 1-associated germline mutations is more diverse and includes large deletions, microdeletions, insertions, nonsense, frameshift and missense as well as splice site mutations. Most of these mutations cause severe damage and most likely loss of VHL function due to destruction of the domain required for VCB complex formation.

Sporadic tumors

There are sporadic equivalents to all VHL tumors. For example, CCRCC is not only a feature of the rare VHL disease but more frequently occurs sporadically in the general population. Likewise, hemangioblastomas frequently develop sporadically.

→ Tumor suppressor genes by definition are subject to a two-hit inactivation mechanism. There are two possibilities to achieve homozygous mutation. For example a germline mutation accompanied by a somatic mutation at the homologous allele will result in a hereditary tumor. Alternatively, two somatic mutations at homologous alleles will result in a sporadic tumor. This accounts for the same tumor suppressor gene being involved in both hereditary and sporadic tumors.

Functional evidence for the tumor suppressor activity of *VHL* comes from transfection experiments that showed inhibition of tumor proliferation upon reintroduction of wildtype *VHL* into the *VHL* $^{-/-}$ tumor cells.

Somatic mutations

Homozygous somatic *VHL* mutations were identified in sporadic CCRCC (up to 80%), sporadic cerebellar hemangioblastomas (up to 25%) but only in a few sporadic pheochromocytomas (<10%). Mutation types and locations in sporadic tumors differ from those in the germline. Most mutations in sporadic CCRCC (Fig. 2) are predicted to severely compromise the VHL protein structure and may therefore cause loss-of-function. About 20% of the cases epigenetic events, such as abnormal methylation of the → CpG island (exon 1), accounts for gene silencing with the predicted consequence of loss of pVHL function. Also, exon 2 is more frequently affected in sporadic tumors than in the germline. In particular, a mutational hotspot was identified in patients with CCRCC, which may reflect possible environmental factors to be involved in tumorigenesis. Altogehter, differences in the *VHL* muta-

tion pattern may reflect the influence of as yet unknown carcinogens, which on their way to become excretable compounds may target the *VHL* gene in kidney epithelial cells at preferential target sites. One such carcinogen was identified to be trichloroethylene, an important industrial solvent.

Somatic *VHL* mutations and alterations are detectable at all stages of tumor growth, however presence of somatic *VHL* alterations may provide a growth advantage to any tumor and therefore indicate a bad prognosis. With the exception of a subgroup of papillary (chromophilic) renal cell carcinomas other pathological entities of renal epithelial tumors, such as chromophobe and the majority of papillary (chromophilc) renal cell carcinomas as well as benign renal oncocytomas, are not associated with *VHL* mutations.

Cellular / functional features

Subcellular localization. pVHL localizes mostly to the cytoplasm at steady state but engages in a dynamic transcription-dependent nucleocytoplasmic shuttle. VHL does not carry a classical nuclear export signal. Therefore, shuttling may be facilitated by a sequence that may function as nuclear export domain.

VHL-ElonginC-ElonginB (VCB) complex formation and transcription elongation control. pVHL binds to ElonginC and ElonginB forming the ternary protein complex VCB. Initially, it was proposed that by binding to the Elongins, pVHL may be involved in the control of transcription elongation. Whereas association of Pol II (RNA polymerase II) with the ternary complex ElonginA/ElonginC/ElonginB allows transcription of downstream genes, the heterotrimer VCB, in which Elongin A is replaced by pVHL, is responsible for the pausing of Pol II at attenuation sites. In normal cells, transcription elongation is controlled by these adverse activities. Mutated forms of pVHL are no longer able to associate with ElonginC/ElonginB, consequently resulting in amplified transcription of genes involved in tumorigenesis.

Recent data of the three-dimensional structure of the VCB complex provided new insight into the spatial distribution of amino acids, the α- and β-domain structure of pVHL, and its interactions with ElonginC/ElonginB. Tumorigenic mutations cluster in two surface patches on the molecule; the 35 residue domain responsible for ElonginC binding and a seperate remote domain suggestive for another macromolecule binding site.

Multimeric complex formation and protein degradation. pVHL forms multimeric complexes with ElonginC and ElonginB, Cul2 and Rbx1. These complexes resemble so-called SCF ubiquitin ligase complexes (Skp1/Cdc53/F-box protein). Thus, by analogy, pVHL is expected to play a role in ubiquitination, a biological process that allows a cell to identify proteins in a timely and specific manner in order to destroy them. Cells that lack pVHL are unable to degrade members of the HIF (hypoxia inducible factor) transcription factor family. The ability of pVHL to ubiquitinate HIF can be prevented by mutations in the β-domain and by mutations at the ElonginC/ElonginB binding site. Whereas pVHL associated ubiquitination activity depends on ElonginC and Cul2 binding via the α-domain, the β-domain is responsible for target capture, stressing the adaptor function of pVHL.

Role in tumor angiogenesis. VHL-related tumors including CCRCC overexpress VEGF (vascular endothelial growth factor), one of the most potent angiogenic factors. This upregulation of VEGF is likely to be responsible for the highly vascular presentation of VHL-related tumors. VEGF expression is under the control of hypoxia inducible transcription factors (HIF). In normal cells, HIF is stable only under hypoxic conditions. Recently, it has been shown that pVHL co-immunoprecipitates with the HIF-1 α-subunit, suggesting that pVHL is necessary for the oxygen dependent proteolysis of the HIF-1 α-subunit. Further, cells lacking pVHL are no longer able to degrade members of HIF. Thus, due to the presence of *VHL* mutations, pVHL lacking cells mimic oxygen deprivation and consequently may unleash HIF and VEGF.

Other up-regulated target genes and role in maintenance of extracellular pH. HIF also controls expression of the carbonic anhydrases CA9 and CA12. These enzymes are involved

in the reversible reaction of carbon dioxide hydration. In VHL tumors and in CCRCC they are overexpressed due to absence of pVHL, suggesting their role in glycolytic acidification of tumors and a role for pVHL to regulate the maintenance of extracellular pH. CA9 is identified by the antigen binding monoclonal antibody mAbG250, that reacts with CCRCC and which is currently under investigation for an application in the treatment of kidney cancer.

Other capture targets and functions. Other likely target proteins for pVHL are transcription factor Sp1, fibronectin and two isoforms of proteinkinase C. Thus, additional proposed functions of pVHL include regulation of the correct assembly of the extracellular matrix and controlled exit from cell cycle.

Animal models. There are *VHL* homologues in rodents (rat and mouse), *Drosophila* and worm. Homology to the predicted human pVHL is 50% in *Drosophila*, 87% in rats and 98% in mice. Homology is highest in the protein binding motifs.

Observations during tracheal development of *Drosophila* suggests that the *Drosophila* homologue of *VHL* (dVHL) plays a role in halting of cell migration at the end of vascular tube outgrowth. Considering a common evolutionary origin of the *Drosophila* tracheal system and the mammalian vasculature, this observation may be helpful to explain the loss-of-function phenotypes in knockout mice and human tumors. In the mouse embryo, loss of pVHL activity may lead to uncontrolled cell movement, causing disruption of major vasculature in the placenta. In the adult human with a completely laid out vasculature, lack of pVHL activity may be involved in extensive cell migration and vessel branching, leading to over-vascularization, as observed in VHL associated tumors.

In homozygously *VHL* deleted knockout mice, embryos die at day 9/9.5 of gastrulation, due to lack of placental vascularization. The heterozygotes do not show any specific phenotype within the observation period of two years. Current scientific efforts focus on the development of a conditional knockout mouse model (deletion of VHL in kidney cells only) to provide a model for the study of kidney tumor initiation and progression and to test gene therapies.

Applications in diagnosis and clinical management

Molecular diagnosis and implications. Patients with VHL disease should be subjected to molecular analysis to determine their family-specific *VHL* germline mutation. Once a mutation has been identified, all family members need to be tested for *VHL* mutation carrier status, that is to identify gene carriers and those who do not carry the mutation. Only family members with a *VHL* germline mutation should then be subjected to clinical diagnostic procedures. This approach significantly reduces the psychological stress of unnecessary clinical screening examinations for the non-carrier. Also, by this approach, costs will be reduced. Affected patients should be tested according to a standard protocol (Maher ER VHL disease, in Hodgson SV. Maher ER (1993): A practical guide to human genetics Cambridge Press pp 157-162, and American Cancer Society (1999)). Good clinical practice includes genetic counseling prior to molecular testing and prior to disclosure of test results. Patient information on all aspects of VHL disease are available online from patient support groups: http://www.vhl.org/ and http://www.hippel-lindau.de/.

Clinical mangement. Molecular testing of VHL disease today is used to sustain clinical diagnosis. In many cases, the disease will be discovered presymptomatically. Early detection allows treatment of lesions prior to the onset of symptoms. For retinal hemangioblastomas, adequate laser treatment significantly reduces the risk of blindness. Cerebellar or spinal hemangioblastomas are benign tumors and need to be removed prior to the onset of neurological impairment. Pheochromocytoma should be treated when symptomatic. Renal lesions require specific attention. Cysts may be critical, as their lining epithelium may be the origin of malignoma. Thus, any cysts in VHL patients should be regarded as potentially malignant, especially when they are accompanied by solid and/or fast growing tumors. With respect to the size and location, nephron sparing surgery is the treatment of choice for renal clear cell carcinoma. The involvement of a variety of different organ systems requires interdisciplin-

ary cooperation of experts from different medical fields for the appropriate management of VHL disease.

First clinical trials have been established to test anti-angiogenetic substances in tumor treatment in VHL.

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VP-16

Definition

VP-16 is an → epipodophyllotoxin used in the treatment of pediatric → acute lymphocytic leukemia (ALL); → etoposide.

VPF

Definition

→ Vascular permeability factor.

V-SRC

Definition

→ Src.

W

WAF1

Definition

→ p21(Waf1/Cip1/Sdi1).

WAGR Syndrome

Definition

WAGR syndrome is the combination of → Wilms tumour, aniridia (lack of iris), genitourinary malformation and mental retardation. This is a congenital malformation syndrome due to mutation of contiguous genes on 11p13. Aniridia, which is dominant, is due to deletion of the *PAX6* gene. Wilms tumour is recessive at a cellular level and is due to deletion of the adjacent *WT1* gene, with tumour formation generally requiring somatic mutation of the remaining *WT1* allele. The genitourinary malformation is a pleiotropic effect of mutation in the *WT1* gene and is dominant. The mental retardation is variable and is believed to be due to deletion of additional genes.

Waldenstrom Macroglobulinemia

Definition

Waldenstrom macroglobulinemia is a low grade B-cell lymphoma which is characterised by the secretion of IgM. The tumours are also called lymphoplasmacytoid lymphoma in the → REAL classification. The absence of CD5 and CD10 expression is useful for its distinction from chronic lymphocytic leukemia.

Werner Syndrome

Definition

Werner syndrome is a rare autosomal recessive disease. It is phenotypically characterized by premature appearance in young adults of features of old age, including gray hair, cataracts, type 2 diabetes and mesenchymal cancers. On the cellular level, it is associated with limited replicative capacity and genomic instability manifested by increased somatic mutations, chromosome loss or deletions and prolonged S-phase. Cells are hypersensitive to DNA-damaging effects of chemicals agents. The disease results from mutation in the *WRN* gene that leads to the formation of a truncated Wrn protein. The Wrn protein is a 1432 amino acid RecQ helicase, which is a member a family of helicases that includes the → *xeroderma pigmentosum* genes *XPB* and *XPD* as well as the → Bloom syndrome gene *BLM*.

Wilms Tumour

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Definition

Wilms tumour (or nephroblastoma) is a childhood embryonal cancer of the kidney. It was named after a German physician, Max Wilms, who described the first large collection of cases of this tumour in a paper published in 1899. The term Wilms tumour used to be applied to other types of childhood kidney cancer (clear cell sarcoma of kidney and malignant rhabdoid tumour of kidney) but these are now recognised as distinct entities with different clinical behaviour and requiring different treatments.

Characteristics

How common is Wilms tumour?

Wilms tumour affects approximately 1 in 10,000 children before their fifteenth birthday, typically at around age 3-4 yrs. 90% of cases will have been diagnosed before the age of 7 yrs. There is a degree of ethnic variation; it is commoner in blacks and relatively rare in Asians. Usually only one kidney is affected, but in 5-8% of cases there are tumours in both kidneys (bilateral disease).

What causes Wilms tumour?

Although the majority of cases of Wilms tumour are 'sporadic' with no obvious cause, it is known that genetic predisposition to Wilms tumour can occur. 1-2% of affected children inherit a defective gene from a parent. There are at least four such 'familial Wilms tumour genes', only one of which has been identified to date. More commonly, in approx 5% of all cases, a child has a developmental defect associated with genetic predisposition to Wilms tumour and sometimes other forms of cancer. The first of these to be defined at a molecular level was the association of **W**ilms tumour with **a**niridia, a lack of development of the iris in the eye. Such children usually have other defects including abnormal **g**enital development and variable mental **r**etardation, and hence the acronym, WAGR syndrome. This defect is due to a chromosomal deletion that removes one copy of the → Wilms tumour gene, *WT1*, and the adjacent *PAX6* gene at 11p13. Loss of one allele of *PAX6* is dominant, but development of the tumour requires loss or mutation of the remaining *WT1* allele in one or more kidney cells (i.e. tumour development is recessive, hence *WT1* belongs to the class of tumour suppressor genes in this context). Heritable mutations in WT1 are also responsible for the predisposition to Wilms tumour associated with abnormal kidney development seen in Denys-Drash syndrome, where children lose large quantities of protein in their urine (nephrotic syndrome) and often have abnormal genital development. Thus, Wilms tumour provides a fascinating example of how normal development of an organ can be intimately linked to cancer predisposition in that organ. Indeed, Wilms tumour can bear an uncanny resemblance to cell types seen during normal embryonic kidney development, hence the term 'embryonal tumour'.

There are an increasing number of children recognised with nephrotic syndrome with underlying germline *WT1* mutations who do not develop Wilms tumour. Hence, it is possible that inheriting a mutation in *WT1* provides only a relatively weak stimulus to developing Wilms tumour (i.e. the gene is of low penetrance).

A second category of genetic predisposition to Wilms tumour occurs in the overgrowth syndromes of childhood, of which Beckwith-Wiedemann syndrome (BWS) is the best recognised. The genetics of BWS are complex, involving several different genes within the 11p15.5 chromosomal locus and the phenomenon of → imprinting, whereby expression of a gene depends from which parent it was inherited. The overall tumour risk is ~10%, of which half are Wilms tumours. It appears that children with early nephromegaly (i.e. overgrowth of the kidneys) or asymmetrical overgrowth (hemihypertrophy) are at greatest risk.

Finally, there is some evidence from case control studies that the risk of Wilms tumour may be somewhat increased by certain parental occupations or exposures. However, the relative risk to the child is usually small, of the order of 2-10 fold.

Clinical characteristics

Wilms tumour is one of the most curable of childhood cancers, even when it has spread beyond the kidney to distant sites. The commonest site of such metastases is the lung, followed by lymph nodes and liver. Wilms tumour rarely metastasises to bone, bone marrow or brain. The treatment consists of chemotherapy with one to three different drugs (usually vincristine, actinomycin D +/- adriamycin) together with surgical excision of the affected kidney. Radiotherapy is also used when there is residual or spilt tumour in the abdomen or metastasis to the lungs. With these regimens, approximately 90% of children with a tumour confined to the kidney (stages I and II) are cured, as are over two-thirds of patients with metastatic disease (stage IV). There is a different philosophical approach to the organisation of treatment between different national and international childhood cancer study groups. The National Wilms Tumour Study Group (→ NWTSG) of North America favours immediate surgical excision of the affected kidney, followed by chemotherapy with or without radiotherapy, according to the tumour extent found at the time of surgery. The approach of the International Society of Paediatric Oncology (→ SIOP) is to use pre-operative chemotherapy to shrink the tumour prior to surgery. This study showed a reduction in the risk of tumour rupture during operation and also reduction in the tumour stage, hence allowing less intensive post-operative treatment. The two groups have comparable cure rates, but the NWTSG approach uses slightly more radiotherapy whereas the SIOP approach uses more anthracyclines (adriamycin). Both these treatments have the potential for long term side effects on growth and fertility and on the heart muscle, respectively. The majority of children with Wilms tumour are cured without the need for either of these agents and are unlikely to suffer any long term sequelae.

Is Wilms tumour one disease?

As with most cancers, various 'prognostic factors' can be recognised in Wilms tumour. The most obvious adverse factor is increasing tumour stage. However, a distinct histological subtype called → Wilms tumour anaplasia carries a poor prognosis, especially when associated with advanced stage disease. Anaplasia is associated with mutations in the *p53* gene, which can occur focally as part of clonal evolution of a tumour. Other molecular characteristics that may be associated with worse outcome are allele loss or loss of heterozygosity for markers on chromosome 16q, 1p and possibly 22q and 11q. Some of these are being tested prospectively in the current NWTS 5 clinical trial, which aims to use molecular characteristics of a tumour to better define risk groups.

Molecular characteristics of Wilm tumour

As soon as the *WT1* gene was isolated in 1990 it became clear that it was not mutated in the majority of sporadic Wilms tumours. Among over 600 published cases analysed for intragenic mutations, *WT1* mutation occurs in only ~10%. In some tumours, mutation of both *WT1* alleles appears to be sufficient for tumorigenesis, in accordance with Knudson's two-hit hypothesis. However, in other tumours, either only one *WT1* allele is mutated or other genetic events are clearly interacting. The molecular biology of the *WT1* protein is fascinating and has led to insights into both tumour and normal development and their inter-relationship. Although it is known that several different genetic loci exist for other Wilms tumour genes, their relative contribution to sporadic Wilms tumour is not yet known. A database of *WT1* mutations is maintained (http://www.umd.necker.fr).

Treatment of relapsed Wilms tumour & future therapeutic possibilities

Although Wilms tumour is one of the most curable of childhood cancers at initial diagnosis,

those cases that relapse carry a much worse prognosis, even with intensive retreatment. Less than a third of relapses are due to the anaplastic variant. Hence, one of the goals of current Wilms tumour clinical studies is to identify factors present at diagnosis that are predictive of outcome. Future treatment intensity could then be stratified according to predicted risk of relapse using molecular characteristics of individual tumours. Another potential avenue is to use knowledge of the biology of the Wilms tumour genes to devise novel therapeutic approaches. In the future this might also lead to preventative strategies for children at increased genetic risk of Wilms tumour.

Heritability and screening

By clinical criteria it appears that less than 10% of children with Wilms tumour have a potentially 'heritable' form of the disease. Approximately 1-2% have a family history of Wilms tumour, a further 1-2% have the WAGR syndrome, ~3% have either Beckwith Wiedemann syndrome or some degree of asymmetrical overgrowth. However, it now appears that there are several genes that may be responsible for familial Wilms tumour and they may be of relatively low penetrance. This means that a proportion of patients with apparently sporadic Wilms tumours may transmit a germline mutation to their offspring predisposing them to Wilms tumour. However, even if the proportion of heritable cases is larger than previously believed, the absolute risk to offspring must be low; only four cases among 362 offspring of 462 survivors of unilateral Wilms tumour have been reported.

Early detection of Wilms tumour has the potential to increase survival and reduce treatment morbidity. Children known to be genetically predisposed to Wilms tumour, such as those with WAGR, Beckwith-Wiedemann or Denys-Drash syndromes, are candidates for screening. This is usually done by regular abdominal ultrasound scanning, although teaching the parents to perform regular palpation of the abdomen is an acceptable alternative. Since Wilms tumours can grow very rapidly, ultrasound screening is recommended at intervals of no greater than 3-4 months. However, there are no definitive clinical trials to determine which screening method or interval is superior for detecting tumours at a low stage (I or II). In the future, if a larger proportion of children are shown to be carriers of one of the familial Wilms tumour genes, then more information should become available on the efficacy of screening. A further benefit of the application of molecular genetics is that children with germline *WT1* mutations appear to be at risk of late renal failure and hence require appropriate monitoring.

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Wilms Tumour Anaplasia

Definition

The term 'anaplasia' in Wilms tumour is used to describe a histological pattern defined as the presence of all of the following three features:

- cells with a nuclear diameter at least three times of adjacent nuclei of the same cell type;
- marked hyperchromatism of these cells, indicative of increased chromosome numbers;
- abnormal mitotic figure.

Anaplasia may be focal or diffuse and is associated with mutation of the p53 gene. Anaplasia is felt to be a marker of cellular resistance to therapy but not of increased tumour aggressiveness as anaplasia in stage I tumours has no adverse prognostic significance.

Wilms Tumour Gene

Definition

Wilms tumor (*WT1)* gene is located at 11p13 and consists of 10 exons spanning approximately 50 kb of genomic DNA. The gene encodes a zinc finger protein that has two alternative splice sites affecting the whole of exon 5 and a three amino acid insertion (KTS) between the third and fourth zinc fingers. The WT1 protein is multifunctional and the various isoforms can act as transcription factors or may be involved in RNA processing or splicing. WT1 is essential for formation of the kidney and gonad in the mouse. There is a genotype-phenotype correlation, with germline missense mutations as seen in Denys-Drash syndrome having a more pronounced effect on genitourinary development than complete deletion of one allele, as seen in → WAGR syndrome. Germline intronic mutations affecting splicing of the KTS linker also act in a dominant fashion on genitourinary development.

WNT

Definition

Wnt is a ligand that binds to the Frizzled family of receptors. Activation of the pathway leads to stabilization of β-catenin [→ E-cadherin] or intracellular calcium release. Wnt1 was first identified as an oncogene in mice.

WNT-signalling

Definition

WNT-signalling is a signal transduction pathway involved in many developmental processes and in the development of tumors. Soluble WNT ligands bind their receptors on the cell surface and acivate signal transduction cascades, resulting either in the stabilization of β catenin and the subsequent activation of TCF transcription factors or in the activation of → GTPases and their downstream effectors.

Wobble Hypothesis

Definition

The wobble hypothesis accounts for the ability of a tRNA to recognize more than one codon by unusual (non-G·C, A·T) pairing with the third base of a codon. As the consequence, the third base of the codon can differ, yet the same amino acid will be built into the protein.

WT1

Definition

→ Wilms tumour gene.

X

X-chromosome Inactivation

Definition

X-chromosome inactivation in females is the inactivation of one copy of the X-chromosome, where most genes on this chromosome are silenced.

Xenobiotic

Definition

Xenobiotic is a chemical that originates from outside the human body. Such chemicals are referred to as foreign compounds and may be synthetic (i.e. made by mankind) or naturally occurring (e.g. generated by microorganisms, plants etc.); → detoxification; xenobiotic biotransformation; xenobiotic metabolism.

Xenograft

Definition

Xenograft is the engraftment of organs, cells or tissues between individuals of different species. Is used to describe a human cancer model that has been developed by introducing human cancer cell lines or tissues into an immunodeficient rodent. Xenografts are often employed as preclinical models to study the efficiacy of tumor therapeutic approaches.

Xeroderma Pigmentosum

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Definition

Xeroderma pigmentosum (XP) is a genetic disease with clinical and cellular hypersensitivity to → ultraviolet (UV) radiation and defective → repair of DNA. XP patients display a marked increase in the frequency of skin malignancies. XP, thus, serves as a model disease to study the relationship between defects in DNA repair and (skin) cancer. It is a skin cancer prone disorder, due to defective → nucleotide excision repair.

Characteristics

Xeroderma pigmentosum (XP) subgroups

The DNA repair pathway that deals with the removal of DNA damage induced by UV radiation is → NER (→ nucleotide excision repair). In 1972 it was demonstrated by using cell fusion techniques that fibroblasts from one patient were able to compensate the repair defect in fibroblasts from another patient. Heterokaryons (cells with nuclei from different donors in a common cytoplasm) were found to exhibit mutual correction of the defective removal of UV radiation-induced DNA damage. Such cells were said to be in different complementation groups. If both nuclei in a heterokaryon have the same genetic defect, then the heterokaryons show defective repair of DNA damage and the patients are in the same → complementation

group. Such studies have revealed the existence of at least 7 complementation groups in XP (XP-A through XP-G). Each complementation group may represent a gene that, if mutated and in homozygous condition, causes XP. In addition, a subgroup exists with the clinical symptoms of XP but with normal NER of UV-induced DNA damage. Patients in this subgroup have a defect in an alternative repair process, viz., in postreplication repair. Such patients are called XP-V (XP variants).

Clinical aspects

XP is clinically characterized by photosensitivity, pigmentary changes, premature skin ageing and a high incidence of skin cancer. In the majority of cases the first symptoms are noticed between 6 months and 3 years after birth. Freckling, sensitivity to sunburn and an increased dryness on sun-exposed skin are usually the earliest manifestations. The first malignant tumors may develop as early as the third or fourth year. Basal cell carcinomas, squamous cell carcinomas and melanomas are common and may be multiple. Besides the skin, the eyes and the nervous system may be affected. In the large majority of patients, photophobia and conjunctivitis are early symptoms. Neurological abnormalities occur in approximately 20% of the cases, which are predominantly patients in the XP-A and XP-D complementations groups. These abnormalities may comprise mental retardation, spacticity, ataxia, dysphasia and areflexia. Two-thirds of XP patients die before 20 years of age from metastases, neurological complications or infections, to which they are also abnormally susceptible. The table summarizes the clinical features of the different XP complementation groups.

Cellular parameters

The two major types of DNA damage induced by UV radiation are CPD (cyclobutane pyrimidine dimers) and [6-4]PP (6-pyrimidine-4-pyrimidone photoproducts). Both CPD and [6-4]PP are formed between two adjacent pyrimidines (cytosine and/or thymine) on a DNA strand. Both lesions lead to a considerable distortion of the three-dimensional structure of the double helix. CPD and [6-4]PP are removed from the genome by NER, which comprises two subpathways:

- GGR (global genome repair)
- TCR (transcription-coupled repair).

The GGR subpathway is responsible for the removal of lesions from the transcriptionally inactive DNA and from the non-transcribed strand of active genes. In the TCR subpathway, the repair machinery is directed preferentially to the transcribed strands of active genes to avoid unrepaired lesions interfering with transcription. Generally, the transcribed strand is corrected up to 5 to 10 times as fast as the non-transcribed strand. As different mutations in the same gene can lead to different levels of impairment, patients within the same complementation group can vary quantitatively in their residual GGR and/or TCR capacity. All NER-defective XP complementation groups are more or less defective in both GGR and TCR, with the exception of XP-C and XP-E, which are defective in GGR only.

Molecular parameters

NER is the process in which damaged DNA is removed and replaced with new DNA using the intact strand as a template. This complex sys-

Xeroderma Pigmentosum. Table – Clinical properties of the separate XP complementation groups.

group	skin cancer	neurological abnormalities	relative frequency
XP-A	+	++	high
XP-B	±	++	very rare
XP-C	+	-	high
XP-D	+	+	intermediate
XP-E	±	-	rare
XP-F	±	-	rare
XP-G	±	++	rare
XP-V	+	-	high

tem involves the concerted action of multiple proteins. The first step in mammalian NER is damage recognition. The XP-C and the XP-E protein play a role in recognition of UV-damaged DNA in nontranscribed DNA. In transcriptionally active DNA, the arrest of transcription at the site of a DNA lesion serves as the damage-recognition signal. Subsequently, the XP-A protein and a protein complex called → TFIIH are recruited to the lesion, and the double helix is opened around the site of damage. It is assumed that in both transcription and NER the function of TFIIH is the unwinding of the double-stranded DNA helix. Both the XP-B and the XP-D protein are part of the TFIIH complex. After damage recognition and partial unwinding of the double helix the actual removal of DNA damage is identical for the GGR and TCR subpathways. The XP-F and XP-G proteins play a role in cutting the DNA on either site of the damage, thereby releasing a 24- to 32-residue oligonucleotide. Subsequently, the gap is filled in by a DNA polymerase and sealed by a DNA ligase.

XP-V cells have normal removal of UV-induced DNA damage from both transcribed and nontranscribed DNA. Cells can tolerate unrepaired damage in their genome and removal of damage does not have to be complete before DNA replication takes place. As CPD and [6-4]PP are effective blocks to the progression of replicative DNA polymerases, cells have developed specialized DNA polymerases that can bypass DNA damage and extend replication forks through damaged sites. One of these DNA polymerase can bypass CPD at thymine-thymine sites and usually correctly inserts two A residues opposite the lesion. In XP-V cells this specialized polymerase is defective. Consequently, in XP-V cells lesions are bypassed by polymerases that insert incorrect residues, leading to a high level of mutations in XP-V cells.

Animal models

Experimental studies on the relationship between NER defects and clinical phenotype are difficult to carry out as XP is a rare disease, which consists of at least 7 NER-defective subgroups. In addition, experimental studies with UV radiation in XP patients can be considered questionable for obvious ethical reasons. Therefore, mouse models for XP have been developed, which are well suited to study the relationship between deficiencies in NER and susceptibility to skin cancer. The first viable animal models for XP were *XP-A*-deficient and *XP-C*-deficient transgenic mice (*XP-A* and *XP-C* knockouts). These animals develop normally, are fertile and do not show signs of a NER-disorder. However, after exposure to UV radiation both animal models strongly mimic the phenotype of humans with XP, i.e., they show a severely increased susceptibility to skin cancer. Neurological disorders, common in XP-A patients, were not found in *XP-A* knockout mice. A direct comparison in skin cancer susceptibility between *XP-A* and *XP-C* knockout mice has shown that *XP-A* knockouts are more cancer prone than *XP-C* knockouts. Hence, skin cancer susceptibility is determined by both GGR and TCR, and defective GGR contributes more prominently to skin cancer development than defective TCR. However, acute UV effects appear to be related primarily to TCR; the minimal dose required to induce a slight sunburn is strongly reduced in *XP-A* knockouts but not in *XP-C* knockouts. Probably blockage of RNA synthesis during transcription, by persistent CPD or [6-4]PP, triggers the influx of pro-inflammatory molecules leading to sunburn.

As parents of XP patients usually are clinically normal, inheritance of XP is considered to be autosomally recessive. Whether carriers of *XP* genes (heterozygotes) have a subtle increase in skin cancer risk can easily be addressed by XP mouse models. Heterozygous *XP-A* animals did not show a higher skin cancer susceptibility than wildtypes, whereas heterozygous *XP-C* animals have been reported to have a higher susceptibility to UV carcinogenesis than their wildtype litter mate controls. The reason for this difference has not yet been elucidated.

Other NER-related syndromes

In addition to XP, two other rare genetic diseases have been associated with a defect in NER. The first is CS (Cockayne syndrome), which comprises at least two complementation groups (CS-A and CS-B). CS patients have a defect in TCR and they exhibit a severe clinical phenotype. CS patients show growth failure, progressive neurological degeneration, retinal degeneration, photosensitive skin and deafness, and most CS patients die at an early age. The second NER-related disease is a photosensitive form of trichothiodystrophy called PIBIDS. This is an acronym for photosensitivity, ichthyosis, brittle hair and nails, intellectual impairment, decreased fertility and short stature. Patients with PIBIDS have mutations in the *XP-B* or *XP-D* gene, which are both components of the TFIIH complex. It has been suggested that specific mutations that preserve the transcription function of TFIIH leads to XP, whereas mutations that also modify the transcription function lead to PIPIDS.

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XIAP

Synonyms

- → BIRC4

Definition

X-linked inhibitor of apoptosis protein; also: X-linked IAP-like protein.

Xiphophorus

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Synonyms

- Gordon-Kosswig melanoma system
- platyfish-swordtail melanoma

Definition

Small aquarium fishes of the genus Xiphophorus are known by the common name of platyfish (*X. maculatus*) and swordtail (*X. helleri*). Introgressive hybridization results in offspring that develop melanoma according to Mendelian principles. This represents the first system, described in 1927, systematically employed for studies of genetic factors in cancer and to show induction of melanoma by ultraviolet light (UV-A).

Characteristics

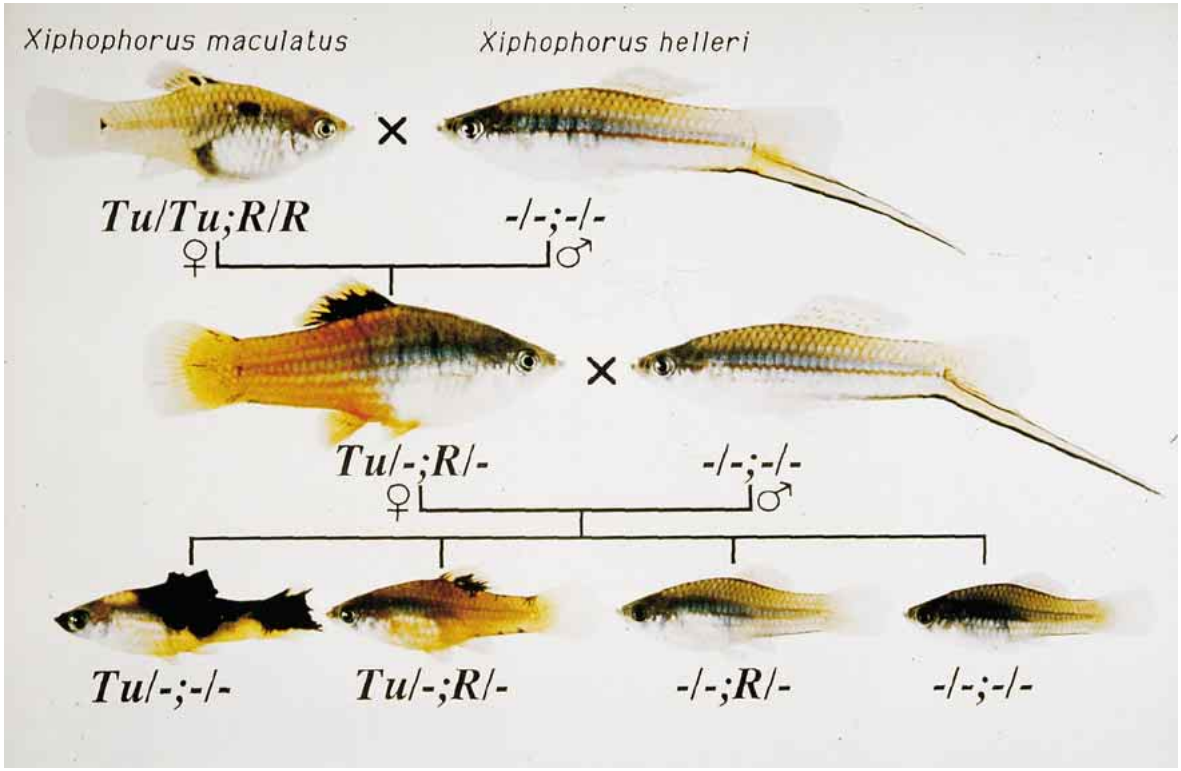
Genetics of melanoma formation

The genetic basis of melanoma formation after introgressive hybridization is explained by the independent segregation of a pigmentation locus (*Sd*), which contains a dominantly-acting oncogene, designated *Tu*, and a trans-acting regulatory gene *R* (also termed *Diff*, *MelSev* or R_{Diff}) that suppresses the oncogenic activity of *Tu* (1, 3, 5, 6) (Fig.). Independent segregation is possible because *Tu* and *R* reside on different

chromosomes. Crossing and backcrossing the fish carrying both *Tu* and *R* (platyfish) to the swordtail results in the progressive replacement of platyfish chromosomes bearing the *R* by swordtail chromosomes lacking *R*. The stepwise elimination of *R* from the hybrid genome allows expression of the *Tu* phenotype, leading to a benign hyperpigmentation in cases where one functional allele of *R* is still present (F_1 and 25% of backcross) or to malignant melanoma in cases where *R* is completely absent (25% of backcross).

The *Xmrk* oncogene

The melanoma oncogene from the *Tu* locus is referred to as *Xmrk* (*Xiphophorus* melanoma receptor tyrosine kinase). It encodes a transmembrane receptor tyrosine kinase (RTK) that is closely related in structure and biochemical features to the epidermal growth factor (EGF) receptor, but constitutes a novel member of the subclass I of RTKs. It arose during evolution by gene duplication from the corresponding proto-oncogene, by which the new copy was fused in the 5′ non-coding region to another (anonymous) sequence. This process generated a novel promoter for the oncogenic version



Xiphophorus. Fig. – Genetic tumors in *Xiphophorus*. The classical cross-breed experiment: a female platyfish (*X. maculatus*), which is homozygous for the X-chromosomal locus *Sd*, encoding the pigment pattern 'spotted dorsal' (small black spots in the dorsal fin composed of a specific type of pigment cells - so-called macromelanophores) is mated to a swordtail (*X. helleri*), which does not have the corresponding locus. The F_1 hybrids show enhancement of the *Sd* phenotype. Backcrossing of F_1 hybrids to *X.helleri* results in offspring that segregate. 50 % did not inherit the *Sd* locus and are phenotypically like the *X. helleri* parental strain. The other 50 % carry the *Sd* locus and develop melanoma. Here, in approximately half of the fish, the severity of melanoma ranges from very benign (phenotype like the F_1 hybrids) to extremely malignant in the others. Highly malignant melanomas become invasive and exophytic, and are fatal to the individual. These melanomas grow progressively, following transplantation into thymus-aplastic 'nude' mice. Symbols below the fish describe their genotypes in respect to the *R* and *Tu*-loci.

(called *ONC-Xmrk* to distinguish it from the proto-oncogenic copy *INV-Xmrk*) of the gene (2).

As a consequence of this rearrangement, the proto-oncogene and the oncogene are subject to different transcriptional regulation. Specific over expression of the *Xmrk* oncogene in the pigment cell lineage of hybrid fish is responsible for melanoma formation. An *R* locus-dependent transcriptional control of the oncogene promoter allows high levels of expression exclusively in pigment cells of certain hybrid genotypes but not in non-hybrids. This explains why the dominantly-acting *Xmrk* oncogene is ineffective in the pure bred parental *Xiphophorus* fish and is a non-hazardous constituent of the genome in natural populations for many generations. This is reminiscent of the situation found for the → *RET* oncogene in humans, where dominantly-acting mutations are transmitted through the germ line and elicit → multiple endocrine neoplasia type 2 (MEN2A, MEN2B). Transcriptional control of *Xmrk* by *R* involves the suppression of an Sp1 transcription factor-mediated constitutive promoter activity in non-melanoma cells and a specific hypomethylation of the oncogene promoter, compared to the proto-oncogene promoter. The *Xmrk* oncogene is necessary for melanoma formation since transposon inactivation of the oncogene in one mutant strain results in loss of melanoma formation. However, over expression of *Xmrk* is not sufficient for tumor induction. Activating mutations in the extracellular domain and a cell type-specific signal transduction machinery downstream of Xmrk are necessary for tumor formation in transgenic fish and for transformation of cells in tissue culture (2).

Signal transduction by *Xmrk* involves components that are generally employed in RTK signalling, such as PLCγ, STAT 5, the PI3 kinase pathway and the Grb/Shc/→ Ras/→ Raf/→ MAP kinase pathway. A specific feature of *Xmrk* is the interaction with the cytoplasmic kinase, fyn, which is a critical constituent of *Xmrk*-mediated cell proliferation. Uncontrolled proliferation of *Xmrk*-transformed cells is due to both the activation of proliferation-associated genes like → jun and → fos and to interference with the microphthalmia-dependent terminal differentiation of pigment cell precursors.

UV-induced tumors

Melanoma in certain hybrid crosses can be induced by UV-B and photoreactivation can reverse this melanoma incidence to background levels. When young backcross hybrids were irradiated with wavelenths from 365-436 nm, melanomas could be induced (4). This led to the appreciation that UV-A, and perhaps the visible light spectrum as well, are important in the etiology of human melanoma. Research on UV-induced melanoma uncovered *CDKN2* as a candidate gene for *R*.

Carcinogen-induced tumors

Besides the propensity to develop melanoma on a hereditary basis, certain backcross strains have a susceptibility to develop cancer after exposure to carcinogens. While fish of wild type strains resist the development of tumors after exposure to N-methyl-N-nitrosourea (MNU) or to X-rays, certain hybrids are highly sensitive and respond to treatment by developing a large spectrum of tumors (3).

Further information

http://www.xiphophorus.org/

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XP

Definition

→ Xeroderma pigmentosum.

XRCC7

Definition

Protein kinase, DNA-activated, catalytic polypeptide.

Y

YAC

Definition

Yeast artificial chromosomes; (YAC → cloning vector) are used to clone DNA fragments 1 Mb or longer.

YAMA

Definition

→ Caspase 3.

Yeast Two-hybrid Assay

Definition

Yeast two-hybrid assay is a method designed to identify protein interactions using the yeast system. Typically, a bait (the protein of interest fused to a DNA-binding domain) is tested against prey (individual protein(s) fused to transcriptional activation domains). When both prey and bait are present in a yeast strain with reporter sites specific to the DNA-binding domain of the bait, the interaction between the two proteins can be identified and measured. When the proteins in the prey and bait interact, they act as a bridge to bring the DNA-binding and transcriptional activation domains close enough together to drive the expression of reporter genes.

Z

ZF87

Synonyms

- → MAZ

Definition

Zinc finger protein 87.

ZIF87

Synonyms

- → MAZ

Definition

Zinc finger protein 87.

ZIP

Definition

ZIP is a (PKC) ζ interacting protein.

Zollinger-Ellison Syndrome

Definition

Zollinger-Ellison syndrome was first described by Zollinger and Ellison in 1955 in two patients with a severe form of peptic ulcer disease, caused by non-β islet cell tumor (→ gastrinoma) of the pancreas. It is is characterized by gastric hyperacidity, multiple peptic ulcers of the stomach, duodenum and jejunum and, in about 40%, diarrhea. The clinical features result from gastrin-producing → NETs (gastrinomas) gastrinomas of the pancreas, the stomach or the duodenum. In about 25%, are part of MEN-1 syndrome.

Zoo Blot

Definition

A zoo blot is a Southern blot containing DNA from a wide range of evolutionary diverged organisms (often from → Drosophila to human DNA). It is used to test evolutionary conservation of DNA sequences.

Zymogen

Definition

A zymogen is the precursor form of a proteinase with absent or limited proteolytic activity. The majority of proteinases are expressed as zymogens that require limited proteolysis to generate the active species. Zymogen activation may involve removal of an auto-inhibitory domain (as in pro→ MMPs), induction of a conformational change that alters active site geometry or generates the substrate binding pocket, or changes in enzyme quarternary structure.

Zymogen Activation Cascade

Definition

The coupling of → zymogen activation reactions into zymogen activation cascades results when an initial event generates an active proteinase that processes a downstream zymogen. The initiating enzyme is often a highly regulated control point, as proteolytic potential is amplified from progression through the cascade. Zymogen activation cascades are not limited to a single mechanistic class of proteinase. For example, activation of the zymogen form of the serine proteinase urinary-type plasminogen activator (uPA, urokinase) leads to activation of the circulating serine proteinase zymogen, plasminogen, to the broad spectrum proteinase, plasmin. Plasmin has been implicated in activation of several pro→ MMPs including prostromelysin-1 (MMP-3). Active MMP-3 can then participate in activation of other proMMPs including procollagenase-1 (MMP-1), promatrilysin (MMP-7), neutrophil procollagenase (MMP-8), progelatinase B (MMP-9) and procollagenase-3 (MMP-13).

Contributors and their Essays

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Aterini, Stefano	Electromagnetic Fields and Cancer
Avet-Loiseau, Herve	Multiple Myeloma
Bale, Sherri	Basal Cell Carcinoma
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Becker, Heinz	Pancreas Cancer, basic and clinical parameters
Ben-David, Yaacov	Erythropoietin
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Bing, Chen	Cachexia
Blow, Julian	Replication Licensing System
Blume-Jensen, Peter	Kit/stem Cell Factor Receptor in Oncogenesis
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Dennler, Sylviane	Smad Proteins in TGFβ Signalling
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Plass, Christoph	Restriction Landmark Genomic Scanning
Platt, Jeffrey L.	Graft Acceptance and Rejection
Poirier, Miriam C.	Carcinogen Macromolecular Adducts
Ponder, Bruce	Multiple Endocrine Neoplasia Type 2
Pool-Zobel, Beatrice L.	Biomarkers
Potten, Christopher S.	Stem Cells and Cancer
Poustka, Annemarie	Deleted in Malignant Brain Tumors 1
Prall, Richard T.	Cholangiocarcinoma
Pritchard-Jones, Kathy	Wilms Tumour
Pui, Ching-Hon	Acute Lymphoblastic Leukemia
Rabes, Hartmut M.	Thyroid Carcinogenesis
Radich, Jerald P.	Minimal Residual Disease
Ramón y Cajal, Santiago	EWS-FLI (ets) Fusion Transcripts
Reddel, Roger	Senescence and Immortalization
Rego, Eduardo M.	Acute Promyelocytic Leukemia (APL)
Renehan, Andrew G.	Stem Cells and Cancer
Ried, Thomas	Interphase Cytogenetics

Contributor	Essay
Rodriguez, Vilmarie	Adoptive Immunotherapy
Ronai, Ze'ev	Stress Response
Roninson, Igor B.	Mitotic Catastrophe
Roninson, Igor B.	P-glycoprotein
Rosen, Eliot M.	Scatter Factor
Ross, Jeffrey S.	HER-2/neu
Ruggiero, Marco	BCL-2
Ruggiero, Marco	Electromagnetic Fields and Cancer
Ruiz-Cabello Osuna, Francisco	HLA Class I
Ruiz-Cabello Osuna, Francisco	MHC
Ruoslahti, Erkki	Homing Peptides
Rusciano, Dario	Adhesion
Russo, Giandomenico	T-cell Leukemia/Lymphoma 1 (TCL1)
Samant, Rajeev S.	BRMS1
Schäfer, Reinhold	RAS Transformation Targets
Schartl, Manfred	Xiphophorus
Schlag, Peter	Fluorescence Diagnostics
Schlag, Peter	Laser Diagnostics
Schlag, Peter	Optical Mammography
Schmitt-Gräff, Annette	Neuroendocrine Tumors
Schönthal, Axel H.	p21 (WAF1/CIP1/SDI1)
Schreiber-Agus, Nicole	Prostate Cancer, basic characteristics and experimental models
Schwab, Manfred	Amplification
Schwab, Manfred	Cancer
Schwab, Manfred	Neuroblastoma
Schwab, Manfred	Oncogene
Sellers, William R.	PTEN
Sen, Subrata	Aneuploidy
Shay, Jerry W.	Telomerase
Shewach, Donna	HSV-TK/Ganciclovir Mediated Toxicity
Shiloh, Yosef	ATM Protein

Contributor	Essay
Shipley, Janet	Rhabdomyosarcoma
Soussi, Thierry	p53 Protein, biological and clinical aspects
Srivastava, Sudhir	Early Detection
Stack, M. Sharon	Matrix Metalloproteinases
Stanbridge, Eric	Tumor Suppression
Steeg, Patricia S	NM23 Metastasis Suppressor Gene
Stein, Stacey	Homeobox Genes and Cancer
Stevenson, Freda	B-cell Tumours
Swanstrom, Ron	Transduction of Oncogenes
Tean Teh, Bin	Multiple Endocrine Neoplasia Type 1
ten Dijke, Peter	Smad proteins in TGFβ Signalling
Testa, Joseph R.	Mesothelioma
Thacker, John	Homologous Recombination Repair
Theodorescu, Dan	Bladder Cancer
Theodorescu, Dan	Prostate Cancer, clinical oncology
Theodoropoulos, Panayiotis A.	Taxol
Thoms, Sven	Autophagy
Tirelli, Umberto	Hodgkin Disease
Tommasino, Massimo	Early Genes of Human Papillomaviruses
Toro, Jorge R.	Basal Cell Carcinoma
Turenchalk, Gregory S.	Lats in Cell-cycle Regulation and Tumorigenesis
Vande Woude, George F.	MET
Vaupel, Peter	Oxygenation of Tumors
Venkitaraman, Ashok R.	Breast Cancer Genes BRCA1 and BRCA2
Verma, Rakesh	Fibronectin
Vleminckx, Kris	Cell Adhesion Molecules
Vlodavsky, Israel	Heparanase
Vogel, Charles L.	Adjuvant Therapy
Vogel, Charles L.	Neoadjuvant Therapy
Vogel, Tilman	Peutz-Jeghers-Syndrome
Von Hoff, Daniel D.	Pancreas Cancer, clinical oncology
von Schweinitz, Dietrich	Hepatoblastoma

Contributor	Essay
Weinstein, I. Bernard	Carcinogenesis
Weiss, Lawrence M.	Hodgkin Disease, clinical oncology
Welch, Danny R.	BRMS1
Welch, Danny R.	KiSS1
Welch, Danny R.	Metastasis Suppressor Gene
Weston, Ainsely	Carcinogen Macromolecular Adducts
Wicking, Carol	Hedgehog Signalling
Williams, Gareth	Cachexia
Wolf, C. Roland	Metabolic Polymorphisms and Cancer Susceptibility
Xu, Tian	Lats in Cell-cycle Regulation and Tumorigenesis
Yang, Xiao Yi	Interleukin-4
Zbar, Berton	Renal Carcinoma
Zhou, Hongyi	Aneuploidy
Zöller, Margot	Progression
Zori, Roberto T.	Cowden Syndrome