L

L&H

Definition

Lymphocytic and/or histiocytic.

► Hodgkin Disease, Clinical Oncology

L&H Cells

Definition

Lymphocytic and histiocytic cells.

L858R

Definition

Somatic mutations of the epidermal growth factor receptor (► EGFR) gene are found in about 25–40% of ► lung cancer patients. These mutations are associated with clinical and radiographic responses to EGFR ► tyrosine kinase inhibitors (TKIs), ► gefitinib and ► erlotinib. Most common is the mutation resulting in arginine for leucine substitution at amino acid 858 (L858R). The EGFR mutation status, especially L858R mutation is correlated with the response to gefitinib and erlotinib.

► Non-Small Cell Lung Cancer

Label-Free Analysis

Definition

A biochemical analysis employing detection method without the need of label such as fluorescence or radioactive tag.

► Surface Plasmon Resonance

Labile Factor

Definition

► Factor V

Lactate

Definition

A three-carbon carboxylic acid also known as 2-hydroxypropanoic acid.

► Warburg Effect

Lactate Dehydrogenase

Definition

LDH is a ubiquitously expressed enzyme. Serum levels of LDH can be used to monitor treatment of testicular

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cancer, ▶ Ewing sarcoma, non-Hodgkin lymphoma, and some types of leukemia. Elevated levels of LDH can also be caused by a number of noncancerous conditions, including heart failure, hypothyroidism, anemia, as well as lung and liver diseases.

▶ Hodgkin Disease
▶ Serum Biomarkers
▶ Testis Cancer

Lactoferricin Antiangiogenesis Inhibitor

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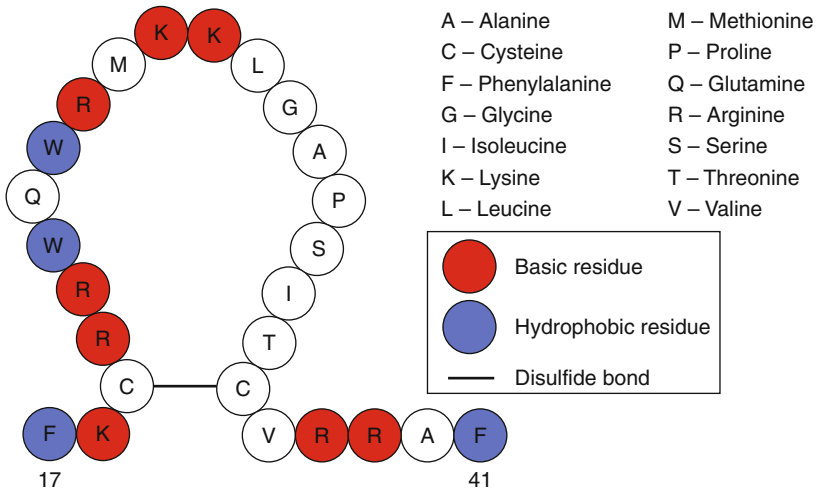
Definition

Lactoferricin is a cationic peptide that is generated by the acid-pepsin hydrolysis of mammalian ▶ lactoferrin present in the secretory granules of neutrophils (polymorphonuclear leukocytes), as well as in exocrine secretions, including milk, tears, and saliva. Interestingly, substantial quantities of lactoferricin are generated in the stomach following the ingestion of lactoferrin-containing milk. Lactoferricin possesses potent antimicrobial, antiviral, immunomodulatory, and antitumor activities. In terms of anticancer activity, the best studied of the lactoferricins is bovine lactoferricin, which consists of amino acid residues 17–41 from the NH_2-terminal region of bovine lactoferrin (Fig. 1). A disulfide bond that forms between cysteine residues located at each end of the peptide creates a looped or hairpin structure. In an aqueous environment, bovine lactoferricin assumes an ▶ amphipathic, twisted β-sheet configuration with clear positively charged and hydrophobic faces. The relatively high proportion of asymmetrically clustered basic amino acid residues (arginine and lysine) and the hydrophobic tryptophan residues that in part comprise bovine lactoferricin are believed to be important for the peptide's biological activity.

Characteristics

The in vitro and in vivo anticancer activity of bovine lactoferricin has been attributed to the selective cytotoxic effect (either by membrane lysis or ▶ apoptosis induction) exerted by this cationic peptide on a broad range of cancer cell types, including leukemias, lymphomas, fibrosarcomas, and various carcinomas. However, recent studies indicate that bovine lactoferricin is also able to inhibit ▶ angiogenesis. A similar, but less potent, antiangiogenic activity has also been reported for bovine lactoferrin. Although ▶ neovascularization is normally tightly regulated by the opposing effects of proangiogenic and antiangiogenic factors, this process becomes dysregulated during tumor growth. The action of proangiogenic factors, in combination with basement membrane degradation by proteolytic enzymes, results in the proliferation, migration, and differentiation of tumor-associated endothelial cells. Neovascularization is an essential step in ▶ tumorigenesis since the new blood vessels provide oxygen and nutrients to rapidly dividing cancer cells and remove metabolic wastes from the tumor ▶ microenvironment. This allows solid tumors to grow in size, as well as promoting the development of metastatic disease. Diffusible, tumor-associated growth factors that stimulate angiogenesis include ▶ vascular endothelial growth factor$_{165}$, ▶ platelet-derived growth factor, heparin-binding epidermal growth factor-like growth factor, and basic ▶ fibroblast growth factor (also known as fibroblast growth factor 2).

Bovine lactoferricin is a potent in vivo inhibitor of vascular endothelial growth factor$_{165}$ and basic fibroblast growth factor–induced angiogenesis in the mouse ▶ matrigel-plug assay. This finding is consistent with the observation that administration of bovine lactoferricin by subcutaneous injection reduces the number of tumor-associated blood vessels in B16-BL6 melanoma-bearing mice. In addition, bovine lactoferricin exhibits a dose-dependent inhibitory effect on the in vitro proliferation of human umbilical vein endothelial cells in response to vascular endothelial growth factor$_{165}$ or basic fibroblast growth factor, as well as inhibiting the in vitro migration of human umbilical vein endothelial cells across transwell membranes in response to vascular endothelial growth factor$_{165}$ or basic fibroblast growth factor. Bovine lactoferricin therefore inhibits endothelial cell



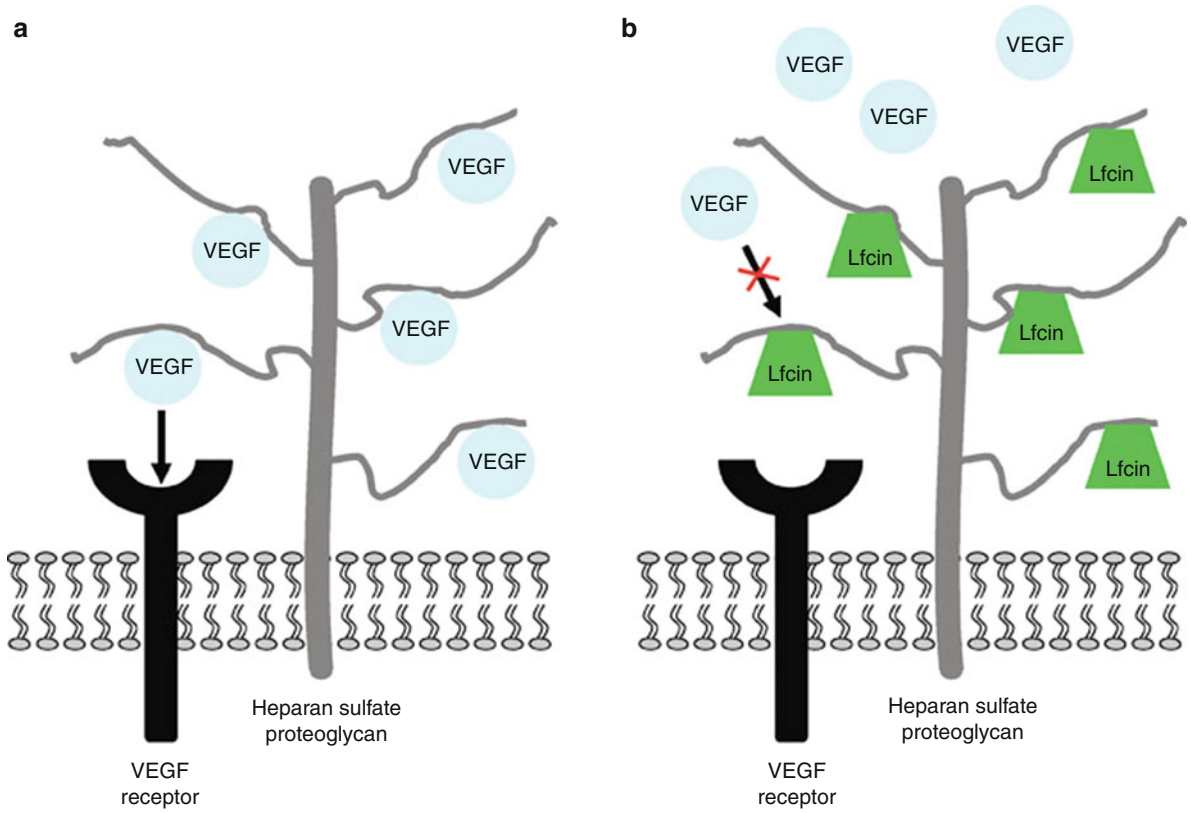
Lactoferricin Antiangiogenesis Inhibitor. Fig. 1 Primary structure of bovine lactoferricin. The amino acid sequence of bovine lactoferricin is indicated by single-letter code (see the accompanying key). Basic and hydrophobic amino acid residues are important for peptide function and are colored *red* and *blue*, respectively. The disulfide bond, indicated by the *solid bar* between cysteine residues at opposite ends of the peptide, forms a loop consisting of 18 amino acids. Numbers indicate the sequence position in bovine lactoferrin

proliferation and migration, which are essential steps in neovascularization. Importantly, bovine lactoferricin does not affect the viability of human umbilical vein endothelial cells, suggesting that the antiangiogenic activity of bovine lactoferricin is independent of its membrane-lytic and apoptosis-inducing activity.

Vascular endothelial growth factor$_{165}$ and basic fibroblast growth factor must first interact with ▶ heparan sulfate proteoglycans on the surface of endothelial cells in order for these proangiogenic factors to bind to and trigger signal transduction through their respective cell-surface receptors. Other heparan sulfate proteoglycan-dependent proangiogenic factors that have been linked to tumor progression include platelet-derived growth factor and heparin-binding epidermal growth factor-like growth factor. Positively charged bovine lactoferricin mediates its inhibitory effect on basic fibroblast growth factor–induced and vascular endothelial growth factor$_{165}$-induced angiogenesis by interacting with negatively charged heparin-like structures on the surface of human umbilical vein endothelial cells, thereby competing with basic fibroblast growth factor and vascular endothelial growth factor$_{165}$ for the heparan sulfate proteoglycans that are required for these proangiogenic factors to bind to and signal through their specific cell-surface receptors (Fig. 2). Although not yet formally proven, it is likely that bovine lactoferricin will have a similar inhibitory effect on angiogenesis induced by other heparin-binding tumor-associated proangiogenic factors. However, ▶ electrostatic interactions alone do not govern the binding of bovine lactoferricin to the heparan sulfate proteoglycans that are involved in cell-surface receptor signaling caused by heparin-binding proangiogenic factors. Thus, a scrambled form of bovine lactoferricin that retains the net positive charge of the native peptide is unable to inhibit the binding of vascular endothelial growth factor$_{165}$ or basic fibroblast growth factor to human umbilical vein endothelial cells. The primary and secondary structure that is conferred on bovine lactoferricin by its amino acid sequence is therefore an important factor in the selectivity of bovine lactoferricin for heparin-like structures that are involved in basic fibroblast growth factor and vascular endothelial growth factor$_{165}$ interactions with their respective receptors on human endothelial cells.

Clinical Perspectives

Starving a solid tumor of its blood supply by preventing or interfering with tumor-induced neovascularization has generated considerable interest



Lactoferricin Antiangiogenesis Inhibitor. Fig. 2 Model of bovine lactoferricin–mediated blockade of heparin-binding growth factor–induced angiogenesis. (**a**) Heparin-binding proangiogenic growth factors such as vascular endothelial growth factor$_{165}$ (VEGF) must interact with heparin-like binding sites on heparan sulfate proteoglycans in order to bind to and signal through receptors on the surface of endothelial cells. (**b**) Bovine lactoferricin (Lfcin) complexes with heparin-like binding sites on cell-surface heparan sulfate proteoglycans, thereby competing with heparan sulfate proteoglycan-dependent proangiogenic growth factors for heparin-like binding sites and preventing proangiogenic growth factor receptor signaling from taking place

as an alternative to conventional chemotherapy for the prevention of tumor growth and metastasis. Indeed, several different inhibitors of angiogenesis are currently being used in the treatment of human cancers. However, the results obtained to date in clinical practice have been less than was hoped for on the basis of preclinical studies, most likely because the current generation of antiangiogenic agents only target a single proangiogenic growth factor receptor, whereas multiple proangiogenic factors are typically associated with tumor-induced angiogenesis. In this regard, the ability of bovine lactoferricin to inhibit neovascularization in response to multiple heparin-binding growth factors may allow bovine lactoferricin to be more effective than current antibody-based antiangiogenic agents for the blockade of tumor-induced angiogenesis. However, the susceptibility of bovine lactoferricin to enzymatic degradation and inactivation through interactions with anionic serum components remains a major obstacle to any future use of this host defense peptide as an antiangiogenic agent. One possible solution is to synthesize an all-► D amino acid analogue of bovine lactoferricin since cationic peptides that consist of all-D amino acids exhibit dramatically increased stability in serum. Alternatively, tumor-targeted ► liposomes might be used to encapsulate and deliver bovine lactoferricin directly to tumor sites while retaining the peptide's ability to mediate antiangiogenic activity. Although preclinical studies have revealed that bovine lactoferricin is a potent inhibitor of angiogenesis, additional research will be needed in order to realize the potential of bovine lactoferricin as a novel antiangiogenic agent for the treatment of human cancers.

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Lactoferrin

Definition

Is an 80-kDa iron-binding glycoprotein belonging to the transferrin family. Lactoferrin has important multifunctional roles in host defense.

► Lactoferricin Antiangiogeneis Inhibitor

LAIR1

Definition

Leukocyte-associated immunoglobulin-like receptor 1.

LAK

► Activated Natural Killer Cells

LAMB

► Carney Complex

Lamellar Phase

Definition

The most common lipid secondary structure, also called the lipid bilayer, which defines most phospholipid membranes found.

► Membrane–Lipid Therapy

Lamellipodia

Definition

Areas at the edge of adherent cells that extend away from the cell body by the pushing of internal actin filaments as they polymerize. Flattened sheet-like structures composed of a cross-linked F-actin meshwork that project from the cell membrane. Often associated with the leading edge of migrating cells.

► Cortactin
► Migration

Laminin

Definition

LM are large, heterotrimeric, cruciform matrix glycoproteins composed of highly homologous α, β, and γ subunits. Five α (α1–5), four β (β1–4), and three γ (γ1–3) chains variably assemble to create 14 known isoforms that convey a variety of important biological functions. Specific LM isoform expression and posttranslational processing can directly influence cellular response to growth factors, intracellular signaling, cell proliferation, susceptibility to apoptosis and migratory capacity. Changes in LM isoform expression in vessel walls have been shown to foster angiogenesis as well as leakage in vessels, rendering them attractive to tumor cells and susceptible to metastatic invasion. Laminins are expressed in both

normal and malignant tissue, but different specific isoforms predominate in each case.

- ▶ Aging and Cancer
- ▶ Laminin Signaling
- ▶ Tissue Inhibitors of Metalloproteinases

Laminin Signaling

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Definition

▶ Laminins are a family of glycoproteins with an apparent molecular weight between 400 and 900 kDa. They are heterotrimers of three subunits, α, β, and γ, held together by disulphide bonds to form triple helical coiled coil in a shape of a cross. Five α chains, three β chains, and three γ chains have been identified, and by combination, they assemble to form over 14 laminin isoforms that have different tissue distributions and functions. Laminins are essential for basement membrane assembly, promote cell attachment and ▶ angiogenesis, induce neurite outgrowth, affect gene expression, and are involved in cell proliferation, migration, and differentiation. Biochemical dissection related some of the laminin functions to specific parts of the glycoproteins. It appears that different parts in the molecules have different effects on cells. Some of these parts are cryptic and interact with cells only after proteolytic cleavage of the laminin molecules. In vitro, most structural and functional studies have been performed on laminin-1 (α1β1γ1).

Characteristics

Laminin Signaling

Laminins activate various ▶ signal transduction pathways. It was shown that ▶ chemotaxis induced by laminin-1 is pertussis toxin sensitive, indicating on the involvement of a pertussis toxin-sensitive G protein in the process, while ▶ haptotaxis seems to involve a different mechanism. It was shown that human osteoclast-like cells selectively recognize laminin isoforms. The cells adhered to laminin-2 but not to laminin-1, and a sharp increase in intracellular Ca^{2+} was detected upon addition of soluble laminin-2 to the cells. Another study showed that laminin-1 induced a rapid and transient mRNA expression of c-fos and c-Jun in PC12 cells and stimulated the DNA-binding activity of the complex of these proteins to the ▶ AP-1 site. In tumor cells, addition of laminin-1 resulted in a time- and dose-dependent activation of phospholipase D (PLD) followed by generation of ▶ phosphatidic acid that is involved in signal transduction events leading to the induction of ▶ MMP-2 and enhanced invasiveness of metastatic tumor cells. This effect of laminin-1 was not seen in normal cells in vitro. Laminins' signaling has been shown to involve kinase/phosphatase cascades since bound laminin-1 and laminin-5 induce protein dephosphorylation in neural cells during process formation. A recent study performed in our laboratory showed that mitogen-activated protein kinases (▶ MAPK) are involved in laminin signaling. Addition of exogenous soluble laminin-1 resulted in a significant decrease in the ▶ phosphorylation (activation) of ERK, JNK, and p38 after 30 min of incubation. This laminin-induced dephosphorylation of all MAPK was dose-dependent and transient. Another study demonstrated that incubation of macrophages with a peptide from the laminin-α1 chain, but not intact laminin-1, triggered protein kinase C-dependent activation of ERK1/2, leading to upregulation of proteinase expression. Several recent studies using laminin-5 have shown activation of ERK1/2 via focal ▶ adhesion kinase (FAK), while other studies have shown activation of Rac1 via phosphoinositide 3 kinase (PI3K). Although some functions may be common to all laminin variants, other may be unique and isoform-specific, depending on the tissue or organ in which they are expressed. In addition, various signal transduction pathways may be activated by different ▶ laminin receptors.

Laminin Receptors

The biological effects of laminins are mediated by numerous laminin receptors that are divided into two major groups: ▶ integrin and nonintegrin receptors (Table 1).

Laminin Signaling. Table 1 Laminin receptors and their additional ligands

Receptor	Ligands
Integrins α1β1	Collagen (I,II,IV), laminin (1, 2)
Integrins α2β1	Collagen (I,II,IV), laminin (1, 2), chondroadherin
Integrins α3β1	▶ Fibronectin, collagen (I), laminin (2, 5, 8, 10, 11), nidogen, epiligrin, perlecan
Integrins α6β1	Laminin (1, 2, 5, 8, 10, 11)
Integrins α6β4	Laminin (1, 2, 5, 10)
Integrins α7β1	Laminin (1, 2, 8, 10)
67 kDa laminin receptor	Laminin[a]
Dystroglycan	Laminin (1, 2), agrin, perlecan
Heparan sulfate	Laminin (1, 2), collagen XVIII

Laminin-4 receptor interactions presumed to be similar to those of laminin-2

[a]Most studies on laminin-1.

Integrins

Integrins are a large family of cell-membrane receptors for extracellular matrix proteins (Table 1). Integrins are heterodimeric combination of various α-subunits with various β-subunits. The ligand specificity for different integrins can be altered depending on the type of divalent cation present, the surrounding lipid environment, and various cell-specific factors. Inside the cell, the short cytoplasmic domains of integrins associate with various cytoskeletal proteins that mediate ▶ integrin signal transduction. At least eight integrins bind laminin; some of them bind additional extracellular matrix components as well. Integrins recognize mainly laminin-α chains and hence determine cell adhesion and response to laminin isoforms.

Two possible integrin-related signal transduction pathways have been identified. First is direct signaling, where binding to integrins by extracellular proteins triggers intracellular signaling events. The second is integrin modulation of mitogen-initiated signaling; in this case, integrin-mediated cell anchorage influences signaling pathways activated by growth factors. In general, integrin direct signaling activates FAK, small GTPases of the ▶ Rho family, and MAPK, resulting in accumulation of highly ▶ phosphorylated proteins and cytoskeletal molecules at the adhesion sites. Binding to integrins is followed by receptor clustering that initiates activation and autophosphorylation of FAK. Tyrosine-phosphorylated FAK can recruit Src family kinases to focal contact sites. This sets up additional tyrosine phosphorylation of proteins such as cytoskeletal proteins and adaptor proteins such as Grb2. Small GTPases of the Rho family (Rho, Rac, and Cdc42) are involved in ▶ integrin signal transduction and affect cytoskeleton arrangement. Rho contributes to cell adhesion to extracellular matrix. Rac and Cdc42, via PI3K, mediate the increase in cell motility and invasiveness induced by the integrin. Some integrins activate MAPK cascades. For example, laminin binding to the integrin α6β4 results in activation of an associated kinase and consequently tyrosine phosphorylation of the β4-subunit cytoplasmic domain, followed by association of the adaptor protein Shc with tyrosine-phosphorylated β4 integrin subunit. Shc is then phosphorylated on tyrosine residues, presumably by an integrin-associated kinase, and combines with the adaptor protein Grb2 which exists in a complex with the ras GTP exchange factor SOS. This leads to Ras activation followed by activation of a kinase cascade consisting of Raf, MEK (MAPK/ERK kinase), and ERK, resulting in increased cell motility and proliferation. In addition, integrin α6β4 activates the JNK cascade, via Rac1, resulting in jun protein expression. Jun associates with fos, whose expression is induced by ERK cascade, to form the AP-1 transcription factor. In human hepatocellular carcinoma cells, laminin-binding integrin α6β1 stimulation resulted in FAK tyrosine phosphorylation, leading to FAK–GRB2 association and ERK cascade activation, which promotes tumor cell migration. Interestingly, aggregation of integrin receptors, even in the absence of ligand occupancy, is sufficient to induce a prompt transmembrane accumulation of at least 20 signal transduction molecules, including Src, Rho, Rac1, Ras, ERK1/2, and JNK.

Nonintegrin Receptors

The 67 kDa laminin receptor is a nonintegrin receptor. A highly conserved 37 kDa protein is the precursor of the receptor, but the exact manner by which it

configures its mature form is not clear. The amino acid sequence of the 37 kDa precursor is extremely well conserved through evolution; however, it corresponds to that of additional proteins, suggesting a multifunctional protein. The cDNA of the 37 kDa precursor is virtually identical to a cDNA encoding the ribosomal protein p40. In addition, the 37 kDa precursor acts as a receptor for cellular prion protein and is involved in the life cycle of prions. It has also been found that the 37 kDa precursor is identical to the oncofetal antigen protein that is expressed by tumors.

The 67 kDa laminin receptor mediates cell attachment to laminin. Colocalization of the 67 kDa laminin receptor with the cytoskeleton constituents α-actinin and vinculin, and the focal adhesion plaque was found. The receptor is involved in several physiological processes such as implantation [56], invasive phenotype of trophoblastic tissue, angiogenesis, T-cell biology, and shear stress–dependent endothelial nitric oxide synthase expression. Increased expression of the 67 kDa laminin receptor correlates with cell proliferation, migration, and ▶ invasion capacity. Clinical data suggest a correlation between 67 kDa laminin receptor expression in tumor cells and tumor progression. Expression of the receptor has been shown to be upregulated in neoplastic cells compared to their normal counterparts and directly correlates with an enhanced invasive and metastatic potential in numerous malignancies. Malignant ▶ mesothelioma is one of the most aggressive human cancers; however, no tumor is less susceptible to distant ▶ metastasis and still associated with such high mortality rates. In a recent study, we wound frequent expression of 67 kDa mRNA but very rare expression of the protein in clinical malignant mesothelioma samples in contrast to metastatic breast or lung carcinomas. These findings suggest that the differences between malignant mesothelioma and carcinomas regarding expression of the 67 kDa laminin receptor may account at least in part for the reduced ability of MM to metastasize to distant organs, due to lack of the signaling mediated by the receptor.

By stable transfection of A375SM melanoma cells, we established lines expressing reduced or elevated 67 kDa laminin receptor. We found that stable antisense-transfected cells that expressed reduced 67 kDa laminin receptor demonstrated significantly less aggressive tumor phenotype, as reflected by their reduced invasiveness through Matrigel, diminished attachment to laminin, and decreased MMP-2 expression and activity. Further, the basal phosphorylation extent (activity) of ERK, JNK, and p38 was significantly higher in cell lines expressing reduced 67 kDa laminin receptor, compared to parental cells. The increase in MAPK phosphorylation in cells expressing reduced 67 kDa laminin receptor was accompanied by a significant reduction in MKP-1 mRNA level and a significant increase in PAC-1 mRNA level. It seems that the 67 kDa laminin receptor induces downregulation of MKP-1 expression that may contribute to the reduced activity (dephosphorylation) of MAPK induced by the receptor, which is followed by an upregulation of PAC-1 expression, possibly as a negative feedback.

67 kDa Laminin Receptor and Integrins

There are studies that indicate an association between the 67 kDa laminin receptor and the α6 integrin subunit that is a part of the laminin-binding integrins α6β4 and α6β1. Biochemical analyses indicate on coimmunoprecipitation of the 67 kDa laminin receptor with the α6 integrin subunit. Specific reduction of the α6 integrin subunit by an antisense was accompanied by a proportional decrease in the cell surface expression of the 67 kDa laminin receptor. Other studies targeting the 67 kDa laminin receptor showed a significant reduction in one of the α6 integrin subunit isoforms. Analysis of α6 integrin subunit and of the 67 kDa laminin receptor in ▶ P-glycoprotein family samples showed no statistical correlation between the two.

Dystroglycan

▶ Dystroglycan consists of two subunits, which are translated from a single mRNA as a propeptide that is proteolytically cleaved into two noncovalently associated proteins. Dystroglycan was originally isolated from skeletal muscle as an integral membrane component of the dystrophin–glycoprotein complex (DGC). The exact function of the entire DGC is not completely determined, but evidence indicates that it confers structural stability to the sarcolemma during contraction. In fact, mutations in components of this complex lead to various types of muscle disorder such as Duchenne muscular dystrophy and limb-girdle muscular dystrophies.

Dystroglycan is also expressed in many other cell types, and it plays important roles outside skeletal muscle. It has been implicated in early mouse development, structure and function of the central nervous system, myelination and nodal architecture of

peripheral nerves, epithelial morphogenesis, cell adhesion, synaptogenesis, and signaling. In addition, several extra- and intracellular proteins are less tightly associated with the DGC, such as nitric oxide synthase [nNOS], dystrobrevin, and laminin-2. Molecules that bind to the cytoplasmic tail of β-dystroglycan include the signaling molecule Grb2, components of the ERK–MAP kinase cascade including MEK and ERK, and rapsyn. Binding of laminin-2 to dystroglycan induces phosphorylation of Grb2 followed by Sos binding. This phosphorylation initiates activation of Rac1 pathway that is further followed by MAPK activation.

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Laminin-Receptor

Definition

Are proteins that bind ▶ laminin and transduce a certain signal into the cell bearing the receptor.

▶ Laminin Signaling

Langerhans Cell

Definition

Langerhans' cells are immature ▶ dendritic cells found in skin containing Birbeck granules and expressing CD1a. They are the most efficient antigen processing and presenting cells of dendritic cell family.

▶ Birbeck Granules
▶ Langerhans Cell Histiocytosis

Langerhans Cell Histiocytosis

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Synonyms

Histiocytosis X

Definition

▶ Langerhans cell Histiocytosis (LCH), previously referred to as Histiocytosis X, is a rare clonal disorder of Langerhans cell proliferation, involving the skin, bone, and other organs. The disease family consists of the syndromes originally described as ▶ eosinophilic granuloma, ▶ Hand–Schüller–Christian disease, and ▶ Letterer–Siwe disease. Modern classification of LCH consists of single-system versus multisystem and unifocal versus multifocal.

Characteristics

Epidemiology

Most patients diagnosed with LCH are children with a peak percentage of diagnoses occurring between 1 and 3 years of age. The incidence of LCH has been estimated to be five cases per million per year in children. It appears to be more common in boys than in girls (1.2–2:1). The incidence of LCH in adults is thought to be one-half of that in children. Development of the disease is usually sporadic; however, the fact that about 1% of patients have relatives with LCH and monozygotic twin pairs are concordant for LCH suggests a genetic predisposition. A higher frequency of malignant disorders has been reported in patients with LCH than in the normal population. Acute lymphoblastic leukemia is the most common malignancy preceding or co-occurring with LCH.

Etiology

Whether LCH is a neoplasm or is reactive in nature has been a controversial issue. Pathological Langerhans cells (LCH cells) are monoclonal, and sometimes show chromosomal deletion or gain, suggesting

a neoplastic etiology. While LCH lesions often regress spontaneously, there is no evidence that LCH cells are immortalized, supporting the possibility of a reactive nature. Although infection may play a role in the development or reactivation of LCH, no well-accepted environmental risk factors are associated with the disease, except for cigarette smoking in adult pulmonary LCH. It has been demonstrated that 77% of adult patients with LCH pulmonary lesions are smokers.

Pathophysiology

LCH lesions not only contain LCH cells but also various inflammatory participants, including T lymphocytes, macrophages, plasma cells, eosinophils, osteoclast-like multinucleated giant cells and neutrophils. These cells stimulate each other to produce abundant cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-4, IL-5, IL-7, IL-10, transforming growth factor (TGF)-ß, ▶ RANKL, and ▶ osteoprotegerin. This cytokine storm plays a role in the proliferation of LCH cells and of other infiltrating cells, and is responsible for the various clinical features of LCH. LCH cells in the bone, lymph node, and some skin lesions contain immature ▶ dendritic cells. These cells do not express CD83 or CD86, but do express intracellular the major histocompatibility complex (MHC) class II antigen. They also express the immature dendritic cell marker ▶ CCR6 and produce the ligand for CCR6 (▶ CCL20/MIP-3α) as well as ▶ CCL5/RANTES and ▶ CXCL11/I-TAC. These ligands may play a role in recruiting eosinophils and CD4 positive T cells into the lesions, respectively. LCH cells show a greater proliferative capacity and a lower antigen presenting capability, suggesting maturation is arrested at an activated state. It is hypothesized that IL-10 as well as TGF-ß could be key factors in the inhibition of maturation of these cells.

Clinical Manifestations

LCH affects a number of different organs, so clinical signs and symptoms may be extremely variable. Patients can present with either single-system or multisystem involvement. Single-system presentations most often occur in the bone with single-site or multifocal involvement, but can also occur in the skin. Most commonly, the initial manifestations include the occurrence of soft tissue mass, bone pain, skin rash, and fever. Laboratory findings include normochromic and normocytic anemia and an elevated erythrocyte sedimentation rate. Elevations in IgM are also common.

The bone is involved in about 80% of patients with LCH. The skull is most often affected followed by the extremities, ribs, spine, and mandible and maxilla. Osteolytic "punched out" lesions with sharp margins are typically seen on X-ray. Bone lesions may be asymptomatic or accompanied with pain and soft tissue swelling, which may cause compression of adjacent structures such as the optic nerve or the spinal cord. The clinical course of LCH when localized solely to the bone is generally benign, and it sometimes resolves spontaneously over a period of months to years. However, it may result in permanent sequelae including the collapse of vertebral bodies, orthopedic deformities, and growth impairment.

Skin involvement is seen in approximately half of patients. Patients present with lesions that are seborrhea-like eruptions on the scalp or an erytematous rash on the trunk, abdomen, and inguinal areas. Ulcerative lesions in the genital or inguinal region may also be present. There may be bleeding into the lesions, even in the absence of thrombocytopenia.

Lymph nodes in the cervical, axillary, and inguinal areas are most commonly affected. Rarely, the nodes can become massive and cause upper airway obstruction.

Ear involvement usually appears as an aural discharge caused by external otitis, which is often associated with the destruction of mastoid. Ossicle or vestibular damage of the middle ear may cause a loss of hearing.

Hepatosplenomegaly occurs in 20% of patients with the infiltration of histiocytes into hepatic sinuses. Various degrees of liver dysfunction may appear, including hyperbilirubinemia, hypoproteinemia, hypoalbuminemia, elevation of γ-GTP, alkaline phosphatase and/or transaminases, ascites, and edema. Histological examination of the liver shows portal infiltrates which can cause bile duct destruction and periportal fibrosis (sclerosing cholangitis), leading to biliary cirrhosis with portal hypertension and ultimately secondary hypersplenism.

Pulmonary involvement in children is usually part of multisystem disease, but in adults, the lung involvement may be solitary and frequently regresses after the cessation of smoking. The lung pathology is associated with tachypnea, dyspnea, cyanosis, cough, pleural

effusion, and recurring pneumothorax. High-resolution computed tomography may reveal reticular or micronodular opacities as well as large nodules and honeycombing. Typical histological findings are alveolar destruction and diffuse interstitial infiltration of histiocytes. Pulmonary fibrosis develops in 10% of patients and can lead to respiratory failure.

Hematopoietic involvement is seen in disseminated LCH and defined by anemia (hemoglobin <10 g/dl in the absence of iron deficiency), leukopenia (leukocytes $<4.0 \times 10^9$/l), or thrombocytopenia (platelets $<100 \times 10^9$/l) with or without bone marrow involvement. In severe cases, serious anemia and thrombocytopenia may develop, often associated with a secondary hemophagocytic syndrome.

Oral mucosa infiltration may appear as ulcerations or swelling of gingiva, resulting in the loss of teeth. Infiltration of the small bowel may occur and cause malabsorption of nutrients. Diarrhea with blood and/or mucus suggests involvement of the colon. Occasionally the pancreas is also involved.

In the central nervous system (CNS), infiltration and dysfunction of the pituitary gland and/or adjacent hypothalamus occurs in about 20% of cases in those with multisystem involvement. The most frequent manifestation is diabetes insipidus (DI), which may precede, co-occur, or follow other symptoms and signs of the disease. DI occurs more often among patients with skull involvement (known as "CNS risk lesions"). Infiltration of the anterior pituitary is less common and may cause growth retardation and panhypopituitarism. These pathologies usually develop in those with DI after a disease course of 10 years. Magnetic resonance imaging (MRI) findings of LCH involvement of the pituitary gland and the hypothalamus are demonstrated by the loss of the physiologic high intensity signal of the posterior pituitary lobe on T1-weighted images. There can also be thickening of the pituitary stalk or a hypothalamic mass.

Progressive, degenerative CNS disease may develop over the years after onset of disease, often when the disease is considered quiescent. CNS involvement causes ataxia, tremor, dysarthria, dysphagia, and hyperreflexia. Changes in personal behavior, judgment, and cognitive function may also develop. In this case, MRI studies using T2-weighted or FLAIR images may reveal bilateral symmetric lesions in the cerebellar white matter and basal ganglia. Histologically, the neurodegenerative LCH is characterized by the presence of CD8 positive T lymphocyte infiltration, microglia activation, gliosis, neuronal and axonal destruction with secondary demyelination. There may be a lack of ▶ CD1a positive LCH cells, as is seen in autoimmune encephalitis. Currently there is no established therapy for LCH-CNS disease.

Pathology and Diagnosis

A pathological examination is indispensable in the diagnosis of LCH. With hematoxylin-eosin staining, LCH cells have a distinctive homogeneously stained pink cytoplasm. The nuclei appear twisted with a longitudinal groove and a small nucleolus, often with a "coffee bean" appearance. Immunohistochemical staining of ▶ S-100 protein and ▶ langerin (CD207) is helpful for detection of LCH cells. In active lesions of the disease, LCH lesions show granulomas caused by the aggregation of LCH cells as well as a number of various inflammatory cells. A definitive diagnosis can be made by either positive staining for CD1a or electron microscopic demonstration of ▶ Birbeck granules in the granulomatous lesional cells. In the later stages of LCH, macrophages are more predominant than LCH cells in the lesions, and xanthomatous and fibrotic changes are characteristic. It is not uncommon that lesions at different stages of disease may be mixed in the same organ simultaneously.

Prognosis

The clinical course of LCH varies quite widely depending on the extent of organ involvement. Multisystem disease can be separated into two categories based on whether or not "risk organs" are involved. Risk organs are defined as the liver/spleen, lung, or the hematopoietic system. LCH may resolve spontaneously in patients with localized, unifocal disease. Patients with single-system disease or without risk of organ involvement have a mortality of less than 5%. Prognosis is worse in children with multisystem and risk organ involvement who often have fatal outcomes despite intensive treatment Mortality rates of 10–50% have been reported. Infants younger than 2 years at diagnosis tend to have risk organ involvement, more often than older children; however, a recent study revealed onset age itself is not a prognostic factor. A major, positive prognostic factor appears to be a favorable response to the first 6 weeks of systemic multi-agent chemotherapy. Patients without an initial response tend to have an extremely high

mortality rate with reports of 15–70%. This is in contrast to that of less than 5% in patients with a good initial response. In adults, lung disease may be a life-threatening complication; it has been reported to contribute to a mortality rate of approximately 25%. Reactivation can occur unpredictably in more than half of patients, even those treated with multi-agent chemotherapy. Reactivated lesions may sometimes resolve spontaneously, but there is an increased risk of permanent sequelae.

Treatment

Treatment of LCH should be planned according to the clinical presentation and the extent of organ involvement.

In single-system LCH, the major aims of treatment are to lessen symptoms and to reduce the chance of permanent sequelae. In the case of a single bone lesion without symptoms, a wait-and-see approach or diagnostic curettage is the standard method of care. Steroids may be used for symptomatic bone lesions. Systemic chemotherapy with vinca alkaloids and corticosteroids for 6 or 12 months could be applied for patients with CNS-risk lesions or multifocal symptomatic bone disease. Radical operation of jaw lesions is discouraged as this often results in disfigurement and loss of teeth. Radiation is rarely used because of the reported increased risk of secondary tumors. When there is skin involvement, only a wait-and-see approach is considered optimal. Alternatively, patients can be provided therapies such as topical corticosteroids or thalidomide. In patients with isolated pulmonary LCH with functional impairment, systemic chemotherapy is indicated to reduce further parenchymal destruction.

In multisystem LCH, the major aims of treatment are to increase survival and to reduce the incidence of late sequelae. Systemic chemotherapy with vinca alkaloids and corticosteroids for 12 months is the most commonly used regimen. In cases with risk-organ disease, more aggressive chemotherapy combined with agents such as cytarabine (Ara-C), 6-mercaptopurine, and methotrexate may be considered. Etoposide (VP-16) is no longer considered a reasonable therapeutic agent because there is no reported significant efficacy, and it has been shown to cause therapy-related acute myeloid leukemia (t-AML).

In patients with refractory progressive disease, myeloablative therapy with a combination of high dose Ara-C and cladribine (2-CdA) is currently being tested. Immunosuppressive therapy with cyclosporin A, anti-thymocyte globulin or anti-TNF agent, and immunomodulation agents like thalidomide, IFN-α, or bisphosphonate are also used on an investigational basis. Allogeneic hematopoietic stem cell transplantation has proved to be efficacious in some cases. Additionally, liver or heart and lung transplants have been performed successfully in patients with end-stage organ involvement.

Late Sequelae

Permanent sequelae are common events in many LCH patients. They are most often the result of the infiltrative nature of the disease itself which causes tissue destruction and granulomatous fibrosis or gliosis of various tissues. Seventy percent of patients with multisystem disease and 25% of single-system disease patients suffer one or more lifelong sequelae, including DI (24%), orthopedic problems (20%), hearing loss (13%), neurologic problems (11%), growth hormone deficiency, loss of teeth, pulmonary fibrosis, and biliary cirrhosis with portal hypertension.

t-AML may develop as a consequence of LCH treatment with chemotherapy, especially following the use of topoisomerase II inhibitors, such as VP-16. The cumulative incidence of t-AML in patients treated with VP-16 for LCH has been estimated to be around 1%. In addition, secondary solid tumors, particularly sarcomas and brain tumors, may develop in irradiated areas.

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Langerhans, Islets of

Definition

Are groups of specialized cells in the pancreas that produce and secrete hormones. Named after the German pathologist Paul Langerhans (1847–1888), who discovered them in 1869, these cells are arranged in groups that Langerhans likened to little islands in the pancreas. There are five types of cells in an islet: alpha cells that produce ▶ glucagon, which raises the level of glucose (sugar) in the blood; beta cells that produce ▶ insulin; delta cells that produce ▶ somatostatin which inhibits the release of numerous other hormones in the body; and PP cells and D1 cells, about which little is known. Degeneration of the insulin-producing beta cells is the main cause of type I (insulin-dependent) diabetes mellitus.

Langerin

Definition

Is a cell surface receptor that induces the formation of the ▶ Birbeck granule.

▶ Langerhans Cell Histiocytosis

LAP

Definition

Latency-associated peptide.

▶ Transforming Growth Factor Beta

Laparoscopic

Definition

Referring to ▶ laparoscopy

Laparoscopy

Definition

Examination of the abdominal and pelvic structures within the peritoneum using an illuminated tubular instrument, which is passed through the abdominal wall via a small incision. It can be used for diagnosis and for certain operations.

▶ Endometriosis

Laparotomy

Definition

A laparotomy is a large incision made into the abdomen. Exploratory laparotomy is used to visualize and examine the structures inside of the abdominal cavity.

Laparotomy may be performed to determine the cause of a patient's symptoms or to establish the extent of a disease. For example, ▶ endometriosis is a disorder in which cells from the inner lining of the uterus grow elsewhere in the body, most commonly on the pelvic and abdominal organs. Endometrial growths, however, are difficult to visualize using standard imaging techniques such as x ray, ultrasound technology, or ▶ computed tomography (CT) scanning. Exploratory laparotomy may be used to examine the abdominal and pelvic organs (such as the ovaries, fallopian tubes, bladder, and rectum) for evidence of endometriosis. Any growths found may then be removed.

Exploratory laparotomy plays an important role in the staging of certain cancers, such as ▶ ovarian cancer. ▶ Staging of tumors is used to describe how far a cancer has spread. A laparotomy enables a surgeon to directly examine the abdominal organs for evidence of cancer and remove samples of tissue for further examination. When laparotomy is used for this use, it is called staging laparotomy or pathological staging. Some other conditions that may be discovered or investigated during exploratory laparotomy include:

- Cancer of the abdominal organs
- Peritonitis (▶ inflammation of the peritoneum, the lining of the abdominal cavity)
- Appendicitis (inflammation of the appendix)

- Pancreatitis (inflammation of the pancreas)
- Abscesses (a localized area of infection)
- Adhesions (bands of scar tissue that form after trauma or surgery)
- Diverticulitis (► inflammation of sac-like structures in the walls of the intestines)
- Intestinal perforation
- Ectopic pregnancy (pregnancy occurring outside of the uterus)
- Foreign bodies (e.g., a bullet in a gunshot victim)
- Internal bleeding

http://www.surgeryencyclopedia.com/La-Pa/Laparotomy-Exploratory.html

Lapatinib

Definition

Is an anticancer drug, a ► tyrosine kinase inhibitor of human ► epidermal growth factor receptor type 2 (HER2, (synonym ► HER2/Neu, also epidermal growth factor receptor (EGFR). Lapatinib is active in combination with capecitabine in women with HER2-positive metastatic breast cancer that has progressed after ► trastuzumab-based therapy.

► Breast Cancer Rationally Designed Therapies

Large Cell Calcifying Sertoli Cell Tumor

Definition

LCCSCT; is a rare type of ► testis cancer.

Large Cell Carcinoma

Definition

About 10–15% of ► lung cancer are this type. It can start in any part of the lung. It tends to grow and spread quickly.

Non-small cell lung cancer is a common disease. It is usually treated by surgery (taking out the cancer in an operation) or radiation therapy (using high-dose x-rays to kill cancer cells). However, chemotherapy may be used in some patients.

The prognosis (chance of recovery) and choice of treatment depend on the stage of the cancer (whether it is just in the lung or has spread to other places), tumor size, the type of lung cancer, whether there are symptoms, and the patient's general health. Patients unsuitable for surgery may be offered curative intent radiotherapy. ► Adjuvant therapy may be given to more advanced resected cases. For late stage cases, chemotherapy with or without palliative radiotherapy are the conventional options, although the long term survival rates are very low.

Large Cell Medulloblastoma

Definition

Variant of medulloblastoma accounting ~5% of cases. Characterized by more abundant cytoplasm than seen in classic medulloblastoma and large areas of necrosis.

► Medulloblastoma

Large Cell Neuroendocrine Carcinoma

Definition

Large cell neuroendocrine carcinoma (LCNEC) is part of the ► neuroendocrine spectrum of ► lung cancer. LCNEC of the lung displays morphologic and ► immunohistochemistry characteristics common to ► neuroendocrine tumors and morphologic features of ► large cell carcinoma.

Large cell neuroendocrine carcinoma is composed of cells with moderate amounts of ► cytoplasm and nuclei that show peripheral clumping of chromatin, a prominent ► nucleolus, and much ► mitosis activity and extensive ► necrosis, as seen in any ► large cell carcinoma. However, the tumor cells are arranged in well-demarcated groups or cords with peripheral palisading. This feature is reminiscent of a ► carcinoid

pattern, and the relationship to carcinoid is strengthened by shared ▶ immunocytochemistry and ultrastructural features of ▶ neuroendocrine ▶ differentiation such as the presence of neural cell ▶ adhesion molecule (▶ CD56), ▶ chromogranin A, ▶ synaptophysin, and scanty dense-core granules.

Certain squamous cell carcinomas also show these neuroendocrine features – these have been termed non-small-cell carcinoma with neuroendocrine features.

Neuroendocrine differentiation can be demonstrated by electron microscopy or immunohistochemistry in 10–15% of non-small-cell carcinomas of the lung despite an absence of morphological neuroendocrine features. Differential diagnosis of large cell neuroendocrine carcinoma includes atypical carcinoid tumor but the organoid pattern of that tumor is not so well developed and the degree of atypia, mitotic activity, and necrosis all far exceed those seen in an atypical carcinoid (which has between 2 and 10 mitoses per 2 mm square [=10 high power fields]). In general, large cell neuroendocrine carcinomas are tumors of middle-aged or elderly cigarette smokers that arise in the central bronchi. Despite the morphological evidence of neuroendocrine differentiation, ectopic hormone secretion is not a feature. Patients with large cell neuroendocrine carcinomas have a significantly worse survival after resection than patients with large cell carcinomas, even in stage I disease. Accurate differentiation of large cell neuroendocrine carcinoma from large cell carcinoma is important because it identifies those patients at highest risk for the development of recurrent lung cancer. The clinical significance of these tumors has yet to be fully evaluated but their recognition is of potential therapeutic significance for their undoubted neuroendocrine nature links them to classic small cell carcinoma and it would be important if their metastases were similarly sensitive to chemotherapy. However, reports available to date regarding their chemosensitivity are contradictory and as yet there have been no large-scale, prospective, controlled trials of small cell chemotherapy for this subgroup of large cell carcinomas or for other non-small-cell carcinomas showing neuroendocrine differentiation. Furthermore, the clinical importance of neuroendocrine differentiation may diminish if a current trend toward treating all inoperable lung carcinomas with aggressive chemotherapy continues.

http://www.histopathology-india.net/large_cell_neuroendocrine.htm

Large Granular Lymphocyte

▶ Activated Natural Killer Cells

Large Tumor Suppressor Gene

▶ Lats in Growth Regulation and Tumorigenesis

Laryngeal Cancer

Synonyms

Laryngeal Carcinoma

Laryngeal Carcinoma

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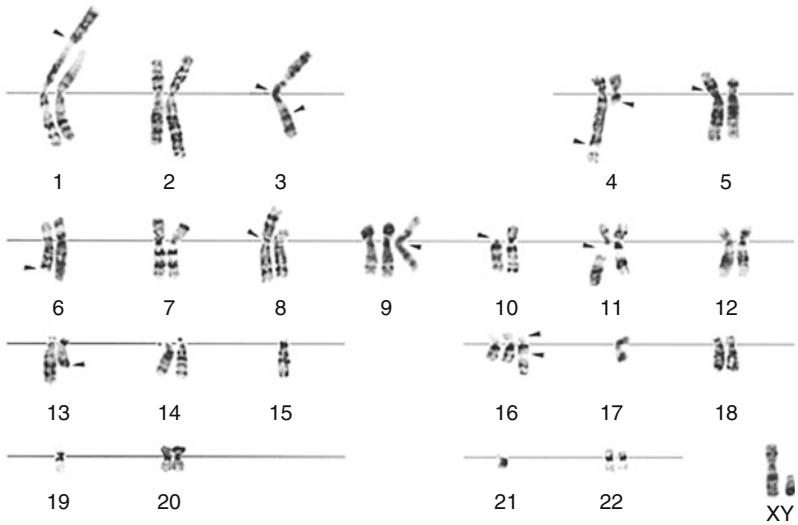
Definition

The vast majority of malignant neoplasms of the larynx arises from the surface epithelium and therefore classified as keratinizing or nonkeratinizing ▶ 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



Laryngeal Carcinoma.
Fig. 1 Representative karyogram from a laryngeal SCC showing complex karyotypes with multiple numerical and structural rearrangements. *Arrowheads* indicate breakpoints in clonal aberrations

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 (▶ Tobacco Carcinogenesis) and alcohol, greatly increases the risk of developing these tumors.

Laryngeal carcinoma infiltrates locally in the mucosa and beneath the mucosa and could metastasize 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.

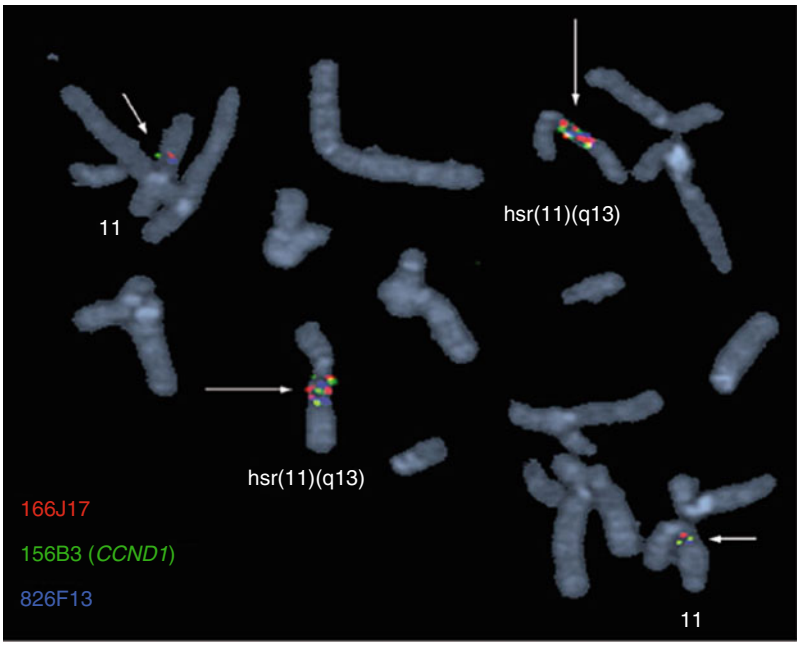
Carcinomas of the supra- and subglottic larynx are more likely to be nonkeratinizing and poorly differentiated, and, in general, they have a more aggressive behavior and tend to ▶ metastasize (Metastasis) early (20–40% of the cases). In contrast, lesions of the true vocal cords are more often moderately to well differentiated, rarely metastasize, and tend to be associated with a better prognosis.

Genetic Changes in Laryngeal Carcinomas

Chromosome Abnormalities in Laryngeal Carcinomas

One hundred and fifteen laryngeal carcinomas with clonal chromosome abnormalities have been reported. In general, the ▶ karyotypes are relatively complex with a nonrandom 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, 2, 3, 4, 5, 7, 8, 11, 12, and 15, with breakpoints clustering to the peri▶ centromeric 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 (▶ Chromosome 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).

A subgroup of laryngeal SCC have had 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 cannot be ruled out that the cytogenetically unrelated clones are united by a



Laryngeal Carcinoma.
Fig. 2 Metaphase plate from a laryngeal SCC hybridized with three bacterial artificial chromosome clones: 166J17 (*red*), 156B3 (*CCND1*, *green*), and 826F13 (*blue*), showing the amplicon of 11q13, including CCND1 locus. *Short arrows* indicate signals in normal chromosome 11, and *long arrows* indicate amplified signals in derivative chromosome 11 harboring hsr

ubmicroscopic, 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 nonneoplastic epithelial or stromal cells in the tumor surrounding.

Fluorescence In Situ Hybridization (FISH)

FISH analysis has recently 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, and that CCND1 (Cyclin D) is always included in the amplicon (Fig. 2). The amplicons mapped vary in size from 3.5 to 4.5 Mb with a core of 1.5–1.7 Mb and often many oncogenes in this region are coamplified, including CCND1, FGF3, FGF4, EMS1, and SHANK2. Another finding is that the amplification of 11q13 is often concomitant with deletion of distal 11q, indicating 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 is 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, suggests that the essential outcome of these rearrangements at the 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

A large scale effort has been devoted to the identification of tumor suppressor gene loci and amplified oncogenes in laryngeal carcinomas. 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 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., FHIT in 3p, CDKN2A in 9p, RB1 in 13q, and TP53 in 17p, have been investigated with regard to

homozygous deletions or inactivating mutations. Extensive analysis of TP53 (p53 gene family) mutation and the alteration of its protein in laryngeal SCC and its precursor lesions have shown that TP53 mutation occurs at an early stage of these neoplasms. Furthermore, correlation studies have shown that overexpression of mutated TP53 predicts poor disease-free and poor overall survival rates in patients with laryngeal SCC. Using various detection techniques such as immunohistochemistry and RT-PCR, it was shown that loss of CDKN24 expression, through either homozygous deletion or promoter hypermethylation, was present in 52–82% of HNSCC including laryngeal SCC. A number of studies have further disclosed that the decreased expression of this gene was associated with poor survival in laryngeal SCC. FHIT was investigated in laryngeal SCC and its precursor lesions. In a recent study, decreased expression of this gene, through deletion or promoter methylation was detected in about 42% of SCC and 23% of dysplasia lesions. Although allelic loss of RB1 was shown in high frequency in laryngeal SCC in a few studies, expression analysis of RB1 revealed inconsistent results in different research groups. Thus, the role of this gene in laryngeal SCC has not been clearly established. Several candidate oncogenes in frequently gained regions, i.e., CCND1 in 11q13, EGFR in 7p, and MYC and PTK2 in 8q have been investigated. 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 patients with laryngeal carcinoma, 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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Larynx Cancer

► Laryngeal Carcinoma

Laser

Definition

Acronym for light amplification by the stimulated emission of radiation; is a coherent light source used to deliver light energy of a single wavelength and high intensity; CALI.

► Chromophore-Assisted Laser Inactivation

Laser Capture Microdissection

Definition

Procedure in which a ► laser beam is used to dissect a patch of cells away from other cells present in a tissue section that has been mounted on a microscope slide.

Laser Diagnostics

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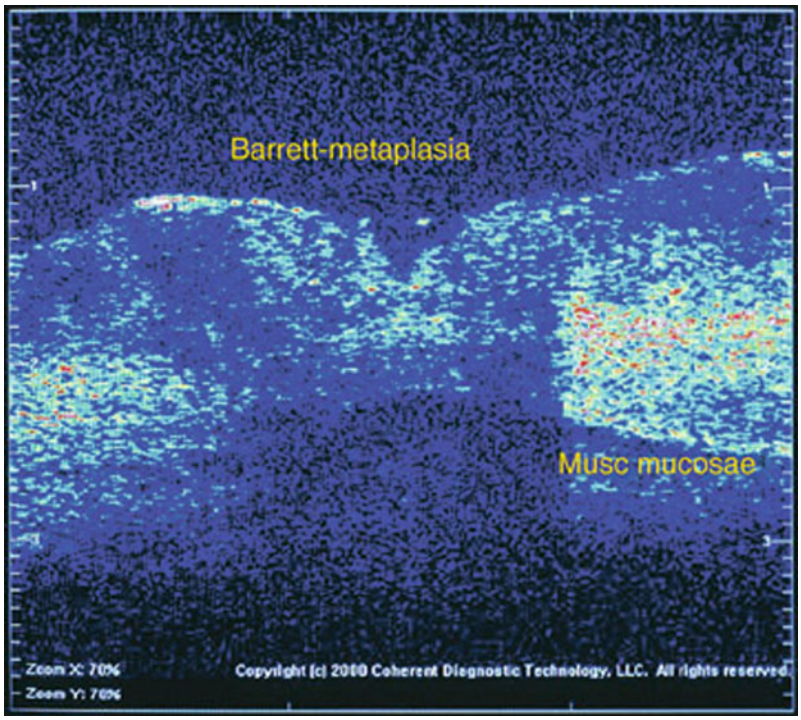
Definition

Laser diagnostics are procedures designed to detect or to differentiate neoplastic tissues based upon various forms of interaction of laser-emitted photons with tissues (Fig. 1).



Laser Diagnostics.
Fig. 1 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)

Characteristics

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 tenfold better than the limits of high-frequency 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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Laser-induced Fluorescence Diagnosis LIFD

► Fluorescence Diagnostics

Latent TGF-β Binding Protein

Definition

Four different gene products. Large glycoproteins that can associate with the extracellular matrices and connective tissues and target small latent TGF-β to tissues.

► Transforming Growth Factor Beta

Lateral Gene Transfer

Synonyms

Horizontal gene transfer

Definition

LGT.

► Circulating Nucleic Acids

Lats in Growth Regulation and Tumorigenesis

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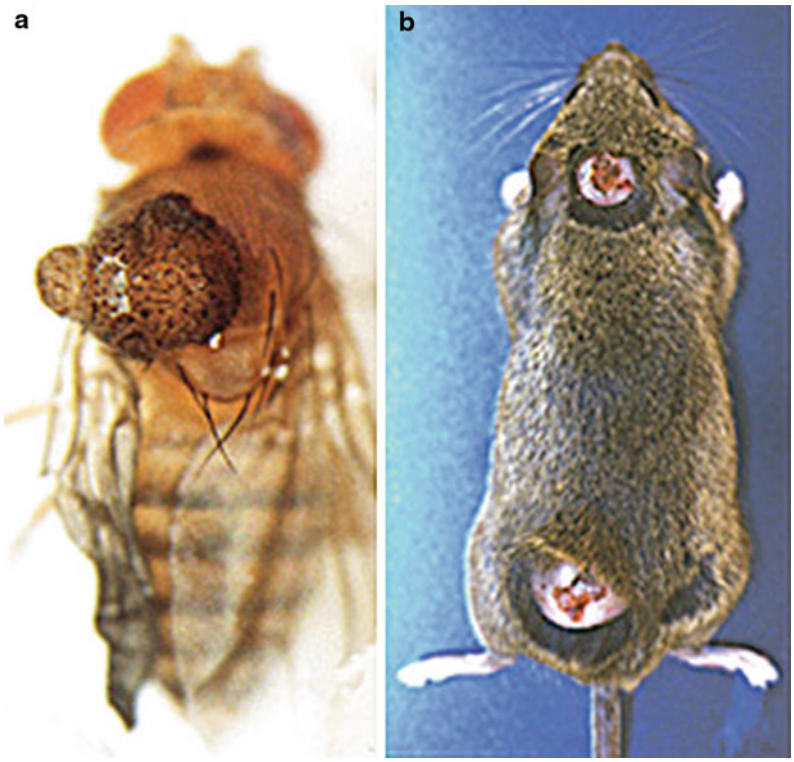
Synonyms

kpm – mammalian LATS2; Large tumor suppressor gene; Warts (wts) – *Drosophila* lats; WARTS (WTS) – mammalian LATS1

Definition

The *lats* (*l*arge *t*umor *s*uppressor) gene, also known as *warts* (*wts*), is a ► tumor suppressor gene originally discovered in the fruit fly *Drosophila melanogaster*. Loss-of-function mutations in the gene cause dramatic overproliferation including developmental defects in ► mosaic tissues within the larval ► imaginal discs (Fig. 1). The *lats* gene encodes a Serine/Threonine protein kinase with a catalytic domain that is highly similar to the human myotonic dystrophy protein kinase. Together with other related members, which include the *Saccharomyces cerevisiae* Dbf2, Dbf20, and Cbk1, *Schizosaccharomyces pombe* Sid2 and Orb6, *Neurospora crassa* Cot1, *Ustilago maydis* UKC1, *Caenorhabditis elegans* Sax-1, *Drosophila* Trc, and mammalian NDR1 and NDR2, they comprise the NDR kinase family, a subclass of the AGC protein kinases.

The *Drosophila* genome contains a single gene for *lats*, whereas there are two homologs, LATS1 and LATS2, in mammals and humans. The genes are functionally conserved since the expression of the human cDNA for either *LATS1* or *LATS2* in the fly rescues the *lats* mutant phenotypes. The overgrowth phenotype of *lats* mutant has been attributed to the coordinated deregulation of both cell cycle progression and ► apoptosis. Recent genetic analyses in *Drosophila* reveal Lats as a key component of the Hippo (Hpo) ► signal transduction pathway (Fig. 2). Mammalian orthologs exist for each component of the pathway and are likely to function in a similar manner. Studies so far indicate that this pathway plays a major role in the developmental regulation of cell proliferation, cell survival, as well as tissue growth and organ size, all of which are key aspects that are also important in tumorigenesis. Whereas knockout mice deficient for *LATS2* are lethal, mice deficient for *LATS1* develop soft-tissue sarcoma, ovarian stromal cell tumors, and pituitary dysfunction (Fig. 1b). In various human cancers, the *LATS1* and *LATS2* genes are found to be mutated or downregulated. In addition, they are able to suppress tumor growth when overexpressed in human cancer cells. Furthermore, cells lacking LATS function display characteristic ► cancer hallmarks such as being multinucleated and having defects in cytokinesis, ► centrosome amplification, and genomic instability. Thus, like their fly counterpart, the *LATS* genes also function as tumor suppressors in mammals.



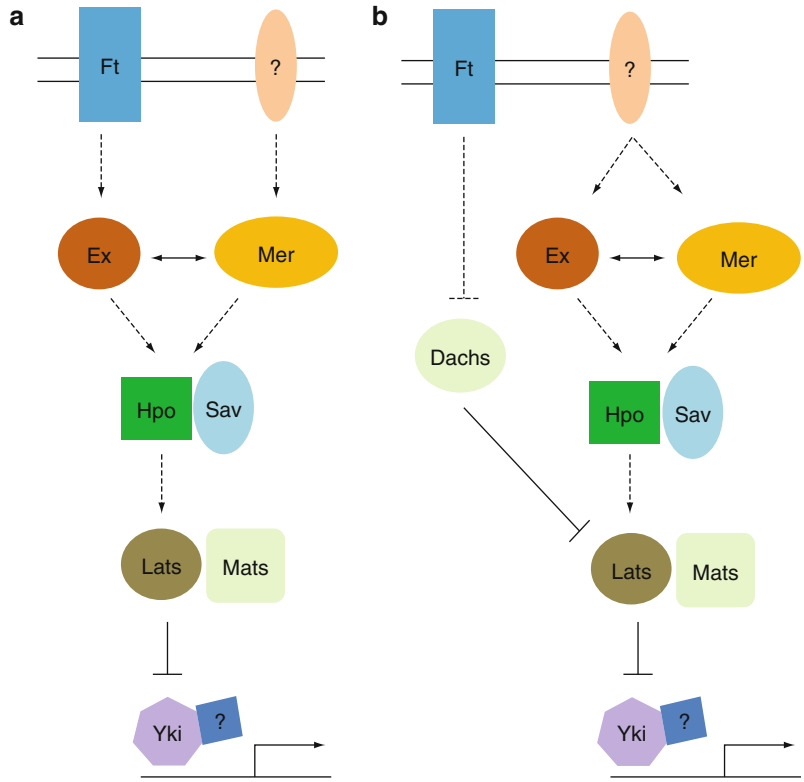
Lats in Growth Regulation and Tumorigenesis.
Fig. 1 Lats in growth regulation and tumorigenesis. Lats has a tumor suppressor function in flies and mice. (**a**) Adult fly showing a clone of *lats* mutant cells which have overproliferated to form a large tumor outgrowth. (**b**) The homozygous LATS1 knockout mouse also develops tumors in the form of a soft tissue sarcoma

Characteristics

The *Drosophila* imaginal disc tissues afford an excellent in vivo context for studying growth, cell proliferation, and survival. The ability to efficiently generate mosaic animals carrying clones of homozygous mutant cells in the developing imaginal discs and to follow their growth over time allow a powerful means to study the effects of mutations in essential genes. Such genetic mosaic approach has been used effectively in genetic screens to look for tumor suppressors or negative regulators of growth, which cause overgrowth when both copies of a gene are inactivated. This inactivation closely recapitulates the in vivo condition in human cancer patients carrying somatic mutant cells.

Using this approach, mutations in the *lats* gene were identified on the basis of the ▶ hyperplastic growth phenotype in mosaic flies. Somatic cells mutant for *lats* overproliferate cell autonomously to form large tumorous overgrowths in a wide variety of adult integumentary tissues (Fig. 1a). The tumors can be as large as one fifth of the body size. Histological examination of the *lats* tumors reveals that they form lobes and folds in the epithelial structures, yet their tissue organization is still characteristically maintained as a monolayer and cell polarity is not affected. Although the mutant tissue is still capable of differentiation, defects are evident in the deposition of extra cuticle and malformed bristles and hairs. On the cellular level, mutant cells appear morphologically irregular such that the apical surface of the cells bulges away from cell body in what is known as "apical hypertrophy." In addition, mutant clones induced in the imaginal discs also appear starkly different from wild-type clones in being rounded and having smooth edges rather than jagged and geometric. Such observation suggests a lack of directional cell division or a modification of cell ▶ adhesion properties. Since apical-lateral junctions are still present in the mutant, *lats* may potentially regulate some functional aspects of the ▶ adherens junctions.

When comparing the growth of clones that are mutant for other *Drosophila* tumor suppressors, such as *lethal(2) giant larvae*, *discs large* or *hyperplastic discs*, *lats* mutant clones exhibit several distinctive behaviors. When clones for other tumor suppressors are induced, the mutant cells appear to be unresponsive to stimulating growth signals. They grow much slower



Lats in Growth Regulation and Tumorigenesis.
Fig. 2 Alternative models of the Hpo-Lats tumor suppressor pathway. (**a**) Components function in a linear fashion. (**b**) Lats plays a central element for alternative inputs from upstream signaling events. On the one hand, Lats stability is regulated by Ft mediated by Dachs bypassing the requirement for Ex function. Alternatively, Lats activity is regulated by Hpo signaling. *Solid lines* indicate observed direct interactions (Adapted from [5])

than wild-type cells and are unable to differentiate. As a result, these cells have a growth disadvantage and are competed away such that very small or no mutant tissues are eventually detected in the adult. Analysis of clone areas and cell numbers in *lats* mosaics, on the other hand, reveals that the mutant cells grow astonishingly faster than wild-type cells in sibling clones. Interestingly, there is no apparent change in the size of *lats* mutant cells, which means there must be a concomitant increase in cellular growth in order to support the faster pace of the cell cycle. Thus, the dramatic expansion of the *lats* mutant clones is not only attributable to an increase in cell number, but also to an increase in tissue mass. This suggests that the deregulation of growth, as well as the inappropriate engagement of the cell cycle, represent key steps where many tumor suppressors may act in tumorigenesis.

The increased rate of proliferation of *lats* mutant cells is due to the shortened phases of the cell cycle which are equally affected. In contrast to proliferative defects seen with other tumor suppressors, *lats* mutant cells do not cycle indefinitely but exhibit a much delayed exit as compared to wild-type cells in the same developmental context. For example, in the developing eye disc, wild-type cells undergo two rounds of cell division and exit the cell cycle after the so-called second mitotic wave (SMW). However, in *lats* mutant clones, cells continue to divide and undergo mitoses at a much later time as indicated by BrdU incorporation and anti-phosphorylated H3 staining of cells posterior to the SMW. Another key finding is that cells within *lats* mutant clones are highly resistant to apoptosis. This is particularly evident during pupal eye development where normally there is a major wave of cell death to remove excess cells that have not been recruited for differentiation. In *lats* mutants, this scheduled cell death is absent leading to the appearance of extra interommatidial cells in the pupal retina. Thus, the effects of increased rate of cell proliferation together with growth and the inhibition of apoptosis contribute to the *lats* dramatic overgrowth phenotype.

Insights into the function of *lats* initially came from the identification of the LATS1 and LATS2 homologs in human and mouse where biochemical studies highlight their roles in the regulation of mitosis and apoptosis. The activity and phosphorylation status of the LATS proteins oscillate in a cell cycle-dependent

manner. Both have been shown to interact with the cyclin-dependent kinase CDC2 and inhibit its kinase activity when they are overexpressed, resulting in cell cycle arrest in ▶ G2/M by LATS1 and in G1/S by LATS2 through the displacement of CDC2. LATS2 has also been shown capable of arresting cells at G1/S by downregulating Cyclin E/CDK2 kinase activity. The localization of the LATS proteins to the mitotic apparatus further points to its role in regulating mitotic events. For example, an interaction with the actin filament assembly factor Zyxin has been detected for LATS1. Recently, LATS1 has been shown to colocalize with and bind to the cytoskeletal protein kinase LIMK1 at the contractile ring during ▶ cytokinesis, the consequence of which is the inhibition of LIMK1-induced cytokinesis defects and phosphorylation of Cofilin. Interestingly, the mechanism of inhibition of both LIMK1 and CDC2 does not appear to involve LATS kinase activity. Nevertheless, consistent with these observations, the removal of LATS1 function leads to defects in mitotic exit and aberrant cytokinesis where the mutant cells are multinucleated, display centrosome amplification, and exhibit ▶ genomic instability. Similar defects are also observed with the expression of a kinase-dead LATS1, which delays mitotic progression through the activation of a spindle assembly check point. These mitotic defects are hallmarks of cancer cells and clearly support the function of the LATS proteins as tumor suppressors.

A role for LATS2 in the regulation of mitosis has also been documented. Its localization to the ▶ centrosome requires phosphorylation by the centrosomal ▶ kinase Aurora-A. LATS2 also functions in a checkpoint pathway to prevent ▶ tetraploidization via p53, which is activated upon binding of LATS2 to MDM2 and feeds back to upregulate *LATS2* expression in G2/M cells. Finally, a recent genetic screen has identified two miRNAs, *miR-372* and *miR-373*, which function as novel ▶ oncogenes in testicular germ cell tumors by downregulating *LATS2* through direct biding to sites in its 3′UTR, the effect of which is to relieve the CDK inhibition by p53.

Although no mitotic defects have been observed in *Drosophila* for *lats* mutants, its role in controlling mitotic events and progression is corroborated by the finding of genetic interactions between *lats*, *cdc2*, and cyclin A. Mutations in either *cdc2* or *cyclin A* can suppress both the lethality and cell proliferation defects of *lats* mutants. Furthermore, the loss of *lats* function leads to the accumulation of cyclin A, supporting the idea that Lats limits cell proliferation by negatively regulating Cdc2/cyclin A activity. The studies above present evidence that may explain in part how mammalian LATS can contribute to tumorigenicity through the regulation of the cell cycle and mitotic events, but they cannot fully account for the effect in *Drosophila* of *lats* mutations on the expansive growth and cell survival. However, further clues do point to an additional role for LATS in regulating apoptosis to suppress growth. For example, the pro-apoptotic proteins Bax and Caspase-3 both have been shown to be upregulated in response to LATS1 overexpression. LATS1 has also been shown to be not only a substrate for the serine protease Omi/HtrA2, but whose activity is also dependent on LATS1 binding. The overexpression of LATS2, on the other hand, can induce cell death through the downregulation of the apoptosis inhibitors ▶ Bcl2 and Bcl-xL.

Thus far, although the kinase activities of LATS1 and LATS2 are clearly required for cell cycle regulation and play a role in tumorigenesis, we know relatively little of the mechanisms by which they act on cell cycle progression, cell growth, and survival. This is hampered by the fact that substrates and effectors of LATS have not been identified, nor do we know what the nature of the upstream regulators is. In recent years, however, work in *Drosophila* has discovered several new tumor suppressor genes that share similar overgrowth phenotypes as *lats*. The first breakthrough was the identification of the *salvador* (*sav*) gene in a genetic mosaic screen for mutations affecting tissue growth. The *sav* gene encodes a WW domain-containing protein and lacks any other enzymatic domains making it likely to serve as a scaffolding protein. Indeed, biochemical assays reveal that Sav protein interacts directly with Lats through the WW region. Mutant clones of *sav* display the same cell autonomous overgrowth phenotype as for *lats* clones. Like *lats*, this is attributed to an increase in cell number and suppression of cell death. These two aspects have been linked to elevated levels of cyclin E and the inhibitor of apoptosis, Diap1, respectively, in mutant clones for either *lats* or *sav*. The mechanism by which *lats* and *sav* regulate these downstream targets at this point has not been elucidated, but they have been used as key signatures in examining other tumor suppressors and genes that might function similarly to *lats*. Of course, as discussed below, these are not the only targets

since the overexpression of *cyclin E* alone does not impart any growth effects, and neither does its coexpression with *Diap1*. Of significance is the ability to coordinate both cell proliferation and cell death that represents a key common feature of the tumor suppressor function of genes such as *lats* and *sav*.

With the discovery of mutations in *hippo* (*hpo*), which also share similar loss-of-function overgrowth phenotypes as *lats* and *sav*, a signal transduction pathway begins to take shape since *hpo* also encodes a Ser/Thr kinase belonging to the Sterile-20/Mst kinase family (Fig. 2). In the budding yeast, the Cdc15 kinase is the closest homolog to Hpo and functions to phosphorylate Dbf2, a *lats*-related kinase. These kinases comprise a signal transduction pathway that regulates mitotic events and cell morphogenesis in the yeast. The conservation of these modular functions suggests that Hpo may also act upstream of Lats. Indeed, Hpo has been shown to phosphorylate and activate Lats in biochemical assays and in tissue culture. This activity requires Sav where it has been suggested to function as a scaffolding protein to bring Lats and Hpo together into an activation complex. The parallel to the yeast modular pathway also makes another prediction where the Dbf2 kinase requires a binding partner, namely Mob1, which has no obvious domains and has been postulated to function analogously to the cyclins for modulating CDK activities. The identification of *Mats* (*Mob as tumor suppressor*) confirms this notion. Mutations in *mats* behave identically to *lats*, *hpo*, and *sav*. Mats has been shown to bind to and modulate Lats intrinsic kinase activity. These four genes, thus, comprise the core components of the Hpo signaling pathway and function to negatively regulate the gene expression of *cyclin E* and *Diap1* (Fig. 2).

Through a yeast two-hybrid screen, Yorkie (Yki) was identified as an interacting protein with Lats, and encodes the *Drosophila* ortholog of the mammalian transcriptional coactivator yes-associated protein (YAP). The overexpression of Yki in clones completely reproduces all aspects of the overgrowth phenotypes of loss-of-function mutations in the Hpo core components, including the upregulation of target genes, which makes Yki a *bona fide* link in the pathway and, significantly, represents a critical transcriptional effector of the Hpo/Lats kinase cascade. Lats has been shown to directly bind and phosphorylate Yki, leading to its inactivation. Yki is capable of activating transcription; however, it lacks a DNA-binding domain, which suggests that it may require a transcription partner or complex to regulate gene expression. As mentioned before, *cyclin E* and *Diap1* are not the only target genes of Hpo signaling since both cannot confer the growth aspect of the pathway. In an overexpression screen, a ▶ microRNA has been found to stimulate imaginal disc growth, as well as to affect both cell proliferation and apoptosis. The microRNA is encoded by the *bantam* gene and has been shown to be a transcriptional target for Yki. However, the growth defect associated with *yki* loss-of-function can only be partially rescued by *bantam* overexpression and, likewise, the overgrowth phenotype of *yki* overexpression can only be partially suppressed by the loss of *bantam* function. The combined effect of *bantam*, *cyclin E*, and *Diap1*, however, still cannot fulfill all of the signaling output of the Hpo pathway as defects in differentiation and cell adhesion, for example, are not accounted for.

How the Hpo pathway is regulated has been an outstanding question until a candidate approach reveals ▶ Merlin (Mer) and Ex (Expanded), which encode related members of the FERM domain-containing protein family, as possible upstream components (Fig. 2). This is suggested by their function as adaptor proteins associated with plasma membrane proteins and the cytoskeleton. Mutations in either gene alone have been shown to have relatively weak effects on growth of imaginal discs. However, since Mer and Ex can heterodimerize and function redundantly, double mutants have characteristic phenotypes of Hpo pathway mutants. Genetic epistasis experiments have placed both genes upstream of *hpo* where their overexpression can influence the phosphorylation of Lats. The mechanism is unknown and additional intervening components remain to be identified since neither Mer nor Ex binds to Hpo. Interestingly, Ex expression is also regulated by Yki, thus, forming a regulatory feedback loop. Despite their membrane localization, Mer and Ex are still intracellular proteins and, therefore, the existence of a receptor for mediating physiological responses to effect Hpo signaling still remains to be seen.

The protocadherin Fat (Ft) recently has been identified as one candidate receptor since loss of its function causes phenotypes similar to *hpo* and *lats* mutants and they all share each other target gene expression. Ft has been known to function as a tumor suppressor, but exactly how it influences growth is not understood. Several studies have placed *ft* genetically upstream

of *ex* and *hpo* in a linear pathway since the over expression of either can bypass the requirement for *ft* (Fig. 2). Furthermore, Hpo and Lats are phosphorylated and activated in response to the overexpression of Ft in cell transfection, which also lead to the repression of Yki-dependent target gene expression. Interestingly, Ft is required for the localization and stability of Ex but not Mer, suggesting that the latter might function in parallel, consistent with the similar phenotypes displayed by *ft mer* and *ex mer* double mutants. Thus, there may be yet another receptor that can regulate Hpo activity through Mer. Although *ft* functions upstream of *hpo* and *lats*, there is an alternative line of evidence to suggest that the pathway may not be linear after all, since Ft can affect the stability of Lats protein specifically and not other downstream components (Fig. 2b). This function is mediated through Dachs, an unconventional myosin downstream of and acts antagonistically to Ft. *dachs* acts genetically upstream of *lats*, and their proteins coprecipitate in biochemical assays. In light of these observations, Lats remains the central element of the pathway but its activity and abundance may be affected differently by various upstream inputs. Nevertheless, further studies are required to resolve the biochemical relationships among the components of the Hpo pathway.

Clinical Relevance

The kinase cascade comprising Hpo and Lats is conserved from yeast to mammals. Many components of this pathway also have orthologs across species and may function in a physiological relevant manner as demonstrated by the ability of some human gene counterparts to rescue the corresponding *Drosophila* mutants. In *Drosophila*, loss-of-function mutations in many components of the Hpo pathway result in overgrowth of ▶ epithelial tissues, which in many ways resemble tumor formation in humans and mammals. Indeed, cells of these imaginal discs possess cellular behavior and properties that reflect closely those of cycling mammalian cells, an especially important consideration when extrapolating mechanistic insights to mammalian models of cancer. Importantly, in most cases cell cycle progression and kinetics in *Drosophila* are punctuated by growth phases characterized by similar G1-S and ▶ G2-M transitions as in mammals. In addition, developing disc tissue structures exhibit a polarized epithelium mirroring mammalian tissues that are highly prone to cancer.

These studies have revealed an emerging tumor suppressor pathway that has profound effects on growth with consequences on body, organ, and cell size as well as on cell number. The phenotypic consequences on cell growth coupled to the coordinated deregulation of both cell proliferation and survival provide a growth advantage to mutant cells in mosaics clones, but also very likely confer such traits to tumor cells. Thus, there is a strong link between these developmental processes and tumorigenesis. Consistent with the overgrowth phenotype in *Drosophila*, knockout mice for LATS1 develop soft-tissue carcinoma, ovarian stromal cell tumors, and pituitary dysfunction (Fig. 1b). Although mutations in either LATS1 or LATS2 have rarely been detected, epigenetic modifications of these genes, for example, downregulation by methylation, have been associated with human breast cancers. Many cancer cell lines derived from human melanoma or mouse mammary gland carcinoma have been shown to carry mutations in genes for *SAV1 (WW45)* and *MATS1 (MOBK1)*. The human ortholog of Mer, ▶ NF2 (▶ Neurofibromatosis 2), gene is notable in the Hpo pathway in being the only one so far that has been discovered as a *bona fide* tumor suppressor. Patients carrying mutations in the NF2 gene develop tumors mainly affecting the central nervous system as found in sporadic tumors as well as in the familial cancer syndrome neurofibromatosis type 2. The loss of NF2 also leads to other tumors such as malignant mesothelioma. From the *yki* overexpression phenotype in *Drosophila*, the mammalian YAP is predicted likely to function as an oncogene. Indeed, the mouse locus at 9qA1, which contains the YAP gene, has been found to be highly amplified in a mouse model of a liver carcinoma as well as mammary cancer. Similar finding has also been found for the human locus at 11q22 where amplification occurs in cancers of the liver, lung, ovary, pancreas, and esophagus.

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LD

► Linkage Disequilibrium

LD_{50}

Definition

Lethal dose 50.

► Preclinical Testing

LDL

Definition

► Low density lipoprotein

LDL Receptor

Definition

► Low density lipoprotein receptor (LDLR); gene family consists of cell surface proteins involved in receptor-mediated ► endocytosis of specific ► ligands.

Lead Chromate

Definition

$PbCrO_4$; A water-insoluble chromium-containing compound consisting of lead, and hexavalent chromium in the chromate anion, that is only very sparingly soluble in water. This compound used to manufacture paint used on aircraft and the yellow line in the middle of streets to demarcate traffic lanes. It gives the yellow and yellowish orange color to paints and pigments. When prepared in small particle sizes (<10 μm), this chromium compound is phagocytosed by mammalian cells and can cause cytotoxicity, chromosomal aberrations, morphological transformation of mammalian cells, and lung tumors when lower animals inhale it.

► Chromium Carcinogenesis

Lead Exposure

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Definition

Lead (Pb) is a heavy, low melting, bluish-gray metal that occurs naturally in various mineral forms in the earth's crust. Lead compounds are substances in which lead is combined with two or more other elements. Organic lead refers to lead compounds which contain carbon, whereas inorganic lead refers to those substances that do not contain carbon and includes metallic lead.

Characteristics

Sources of Lead Exposure

Exposure to lead is predominantly due to ► anthropogenic activity, which has occurred since industrial lead production started millennia ago. The greatest potential for exposure has been experienced by industrial workers, and lead exposure is currently generally well controlled in major lead-using industries such as ► smelting and battery manufacture (Table 1). However, cases of clinical lead poisoning in certain industries still occur and workers using end-products containing lead, such as lead-based paints, continue to be exposed and little to no decreases in lead exposure levels have been observed in certain work environments such as the construction industry. It has been

Lead Exposure. Table 1 Industrial processes and activities with risk of lead exposure

Industrial processes	Activities
Abrasive blasting	Ceramics making
Battery manufacturing	Drinking from a private well
Gas welding and cutting	Enameling
Metal preparation and pouring	Glassblowing
Metal thermal spraying	Home remodeling
Painting (pigments, binders, and biocides)	Ingesting an herbal remedy
Semiconductor manufacturing	Jewelry making
Silk-screen printing	Living in a house with old plumbing or old paint (pre-1979)
Smelting copper or lead	Living near a smelter
Soldering	Lost wax casting
Steel producing	Painting
Welding	Smoking cigarettes
Welding over coatings	Stained glass making

From HazMap: Occupational Exposure to Hazardous Agents (http://hazmap.nlm.nih.gov/)

estimated that about one-fifth of the United States general population has a history of exposure on the job.

Historically, the largest source of environmental lead exposure in the United States has been through inhalation and ingestion of air, dust, soil, water, and food contaminated from the use of lead in pipes, paints, food and drink cans, and gasoline. These uses have been phased out in many developed countries, including the United States, resulting in a decline in blood lead levels by more than fivefold. Nevertheless, segments of the general population continue to be exposed to excessive amounts of lead, especially from lead-based paints and contaminated soil in urban settings with an older housing stock. Additionally, drinking water continues to be an important source of lead exposure.

Lead accumulates in the body and may become biologically available long after the occupational or environmental exposure has ceased. Therefore, lead exposure remains a public health concern worldwide.

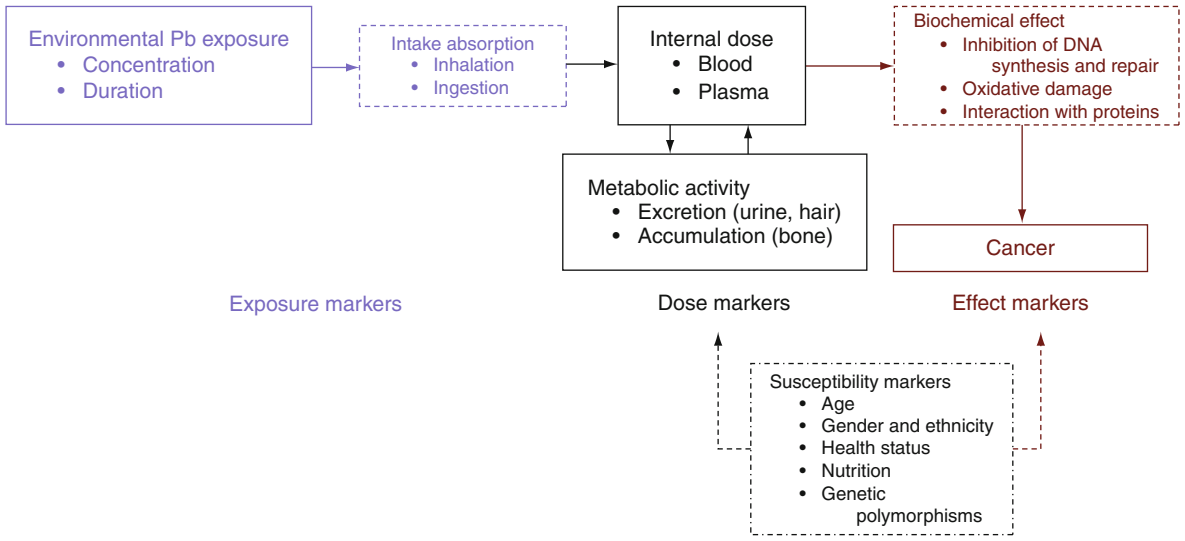
Biological Markers of Lead Exposure

A variety of ▶ biomarkers exists to measure exposure to lead, including lead in urine, blood, hair, nails, teeth, and bone. Blood lead levels are the most common indicator of internal lead exposure in epidemiological studies. However, these levels tend to decline rapidly over time because of a half-life of about 1 month and therefore represent only recent exposures. For children aged <6 years, the U.S. Centers for Disease Control and Prevention (▶ CDC) has defined an elevated ▶ BLL as ≥10 μg/dl. For adults, the highest BLL acceptable by standards of the U.S. Occupational Safety and Health Administration (OSHA) is 40 μg/dl. In the United States, the state-based Adult Blood Lead Epidemiology and Surveillance (▶ ABLES) program tracks laboratory-reported adult BLLs in an effort to reduce the proportion of adults (age 16 or older) who have BLLs of 25 μg/dl or greater, whereas the CDC tracks children's BLLs by using both National Health and Nutrition Examination Surveys (NHANES) and state and local surveillance data.

Lead accumulates in the skeleton and remains there for many years. In children, up to 80% of absorbed inorganic lead is stored in the bone, whereas in adults this figure rises to more than 90%. Organic lead compounds are oxidatively dealkylated in the body, and any inorganic lead produced in this way will be distributed like exogenous inorganic lead. The turnover rate of skeletal lead varies by compartments in bone. The half-life of lead in ▶ cortical bone (e.g., tibia), which constitutes about 80% of skeleton, can be several decades whereas a considerably shorter half-life of only several years has been reported for ▶ trabecular bone (e.g., patella, calcaneus) which constitutes about 20% of the skeleton. While blood lead levels reflect primarily recent exposures (ongoing steady state or recently elevated exposures) and the mobilization of lead from the skeleton back into the circulation, bone lead levels have been shown to accurately reflect accumulated exposure. By providing a biological marker of cumulative dose, bone lead may be used in epidemiological studies of lead carcinogenicity to more accurately evaluate dose–response associations in studies of populations in which lead exposure is occurring through multiple possible pathways. Bone lead levels can be determined with a direct, non-invasive measurement using X-ray fluorescence (▶ XRF) spectroscopy.

Lead Carcinogenesis: Possible Etiologic Mechanisms

Experimental evidence demonstrates that various water-soluble and -insoluble lead compounds can induce ▶ renal carcinoma and ▶ brain tumors (gliomas) in rodents. Lead is known to be toxic to the



Lead Exposure. Fig. 1 Conceptual framework for the association between environmental lead exposure and cancer of the brain and central nervous system

peripheral and central nervous system as well as the kidney. However, there does not seem to be a direct carcinogenic effect of lead since it does not seem to directly result in ▶ DNA damage. Rather, current mechanistic evidence for a role of lead in ▶ carcinogenesis suggests a facilitative role involving inhibition of DNA synthesis and repair, ▶ oxidative stress, and interaction with DNA-binding proteins and ▶ tumor suppressor genes. This facilitative role suggests that lead exposure may interact with other possible risk factors in the etiology of cancer.

A general conceptual framework for the carcinogenic effects of lead exposure is presented in the figure. Individual indicators of susceptibility may influence the nature of this relationship, such as age, gender, and genetic polymorphisms. For example, anemia due to glucose-6-phosphate dehydrogenase (▶ G6PD) deficiency and polymorphisms in the enzyme delta-aminolevulinic acid dehydratase (▶ ALAD) may affect the susceptibility to lead toxicity, including cancer, by affecting the toxicokinetics of lead in the body (Fig. 1).

Lead Exposure and Cancer Epidemiology

The relationship between cancer in human populations and exposure to lead from environmental or occupational sources has been considered in several epidemiological studies. These studies primarily consist of case-control and cohort studies. In ▶ case-control association studies, cases diagnosed with a disease of interest and controls without that disease are asked about lead exposure (on the job, from hobbies, or from living in older homes) at any point in their life (▶ Cancer Epidemiology). In cohort studies, cancer mortality or incidence rates are compared between groups of people with and without elevated lead exposure (based on high-lead jobs compared to the general population or blood lead levels). This literature has been reviewed on several occasions. Most epidemiological studies evaluating the carcinogenic effects of lead exposure have generally suffered from limited statistical power due to a small number of cancer deaths or diagnoses, and the lack of biological measures of exposure. Most studies lack quantitative data on dose–response – one of the key parameters in determining causality – and only few studies divided individuals in high and low exposure groups. Nevertheless, occupational cohort studies have been considered particularly informative with respect to cancer risk because of high documented exposures. These studies were predominantly based on highly exposed battery or smelter workers who were exposed several decades ago (1940s–1970s). These studies have reported on a variety of cancer types, including those of the lung, stomach, kidney, and brain. Most studies show a weak to moderate positive association between lead exposure and cancer, in particular for cancers of the stomach and brain, and to a

lesser extent for lung and kidney cancer. Follow-up studies of general population samples in the United States and case-control studies shed little further light on these associations.

The International Agency for Research on Cancer (IARC) recently concluded that there is limited evidence in humans for the carcinogenicity of inorganic lead compounds, and inadequate evidence for an effect of organic lead compounds on human cancer.

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Lead Generation

Definition

Chemical modification of a structure to increase desired but non-optimized biological activities.

► Lead Optimization

Lead Optimization

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Definition

The synthetic chemical modification of a biologically active molecule to address pharmacokinetic, pharmacodynamic, and toxicologic issues in order to enable its clinical utility.

Characteristics

Drug discovery activities that occur prior to lead optimization, such as the choice of target, assay development, ► high throughput screening, and early molecular optimization and testing, are typically referred to as ► lead generation. Leads typically have been somewhat optimized for their molecular properties (solubility, removal of covalent modifiers, chemical stability), have been subjected to in vitro assays to predict their (► Pharmacokinetic) properties (Caco-2 flux, liver microsomal stability, protein binding, inhibitory activity toward (► Cytochrome P450 isoenzymes) (CYP450s), possess reasonable in vitro and cellular potencies toward the molecular target, and have been subjected to some selectivity measurements (kinase selectivity screen and a broad receptor/transporter selectivity screen).

Lead optimization, the subject of this entry, encompasses chemical modifications of the molecular structure that improve its druglikeness, particularly with respect to issues of potency, selectivity, safety/toxicity, and pharmacokinetic (absorption, distribution, metabolism, excretion) parameters. Efficacy in an animal model of disease also is first evaluated during this phase. This essay focuses on optimization of (► Small Molecule Drug) candidates, though biologics leads are optimized as well.

During (► Preclinical Testing), the candidate drug is further examined to assess its suitability for first-in-human safety trials (Phase 1 clinical trials). Development of medium-scale chemical synthesis, in order to produce (► Good Manufacturing Practices) (GMP) drug substance, is explored. Further assessment of the compounds pharmaceutical properties and formulation work is also undertaken. The initial pharmacokinetic studies are usually repeated in multiple species. This phase also further examines the safety of the candidate drug in nonhuman species. Target candidate drug exposure is then compared to the dose causing overt toxicity in a rodent, dog, or monkey species to evaluate the safety window of the candidate in first-in-human trials. The typical procedure to assess toxicologic risk is based on the compound safety margin which is the ratio of the no adverse event level (NOAEL) in the most sensitive species to the expected therapeutic dose in man.

Chemotherapeutic agents constitute a significant subset of cancer drugs (as well as antibiotics) but are

much less used for treatment of other diseases. Chemotherapeutic drugs rely on selective toxicity for cancer cells, either as a result of favorable differences in distribution or favorable differences in biochemistry.

Physicochemical Optimization

Intravenously administered drugs must have high solubility. Drugs administered via a peroral route should have adequate solubility in order to be able to reach the site of action within the organism. During in vitro testing solubility of a potent compound is usually not an issue, but when animals must be dosed with larger quantities of drug substance in order to measure efficacy or tolerability, absorption may be limited by the failure of the compound to dissolve in the gastrointestinal tract. Thus solubility, permeability, and potency are in dynamic tension during optimization. For instance, high solubility can sometimes overcome low permeability with a drug of moderate to high potency.

Solubility can be measured in a number of ways. One way is to initially dissolve the compound in dimethylsulfoxide, a polar aprotic solvent, which overcomes crystal lattice energy. This is the characteristic of a kinetic solubility method. Some compounds soluble at aqueous 0.5–5% DMSO that would not easily or at all attain dissolution if tested with a thermodynamic method, which entails dissolution initially in a buffered aqueous medium. A thermodynamic method however, is perceived to be more relevant to the in vivo situation and should be evaluated at a range of biologically relevant pHs. There are a number of strategies used to optimize the solubility of sparingly soluble drug candidates. The most important is to introduce solubility directly into the molecule during optimization, through introduction of water solublizing moieties at a permissible part of the structure. Another common approach is the formation of a suitable salt. Almost half of present day drugs are salts, and this strategy may be undertaken very early in the lead optimization process. Also, formulation with appropriate organic solvents, surfactants, and lipids can be explored during lead optimization and during preclinical development.

Besides solubility, there are a number of other physiochemical parameters that begin to be evaluated during the lead optimization phase, namely, chemical stability, morphic states (monomorphic vs. polymorphic), crystallinity, and hygroscopicity.

It is also important during the lead optimization phase to bridge the chasm between the discovery chemistry (large number of compounds synthesized in milligram amounts) and the chemical development (few compounds in kilogram amounts) departments. This can occur via the formation of specialized teams that bridge that scalability gap, or by the presence of chemists in the discovery chemistry departments that work on process improvements, or both.

Pharmacokinetic Optimization

In years past, a substantial percentage of drugs failed in the clinic secondary to poor pharmacokinetic properties. Today, that number is greatly reduced, chiefly as a result of the focus on ADME (absorption, distribution, metabolism, excretion) properties during lead optimization. Ideal ADME properties for a drug candidate depend on the clinical target, but typically encompass good ▶ bioavailability, ▶ systemic clearance, and ▶ volume of distribution that may be expected to yield the desired dosing regimen. A low probability for drug–drug interactions and metabolism-based toxicity is also highly desirable.

Analysis of current orally administered drugs and drug candidates has been a primary guide to correlating physical properties with successful clinical developability. It would be useful to develop models that quantify to some extent known effects of easily calculated molecular properties on absorption. The Lipinski "Rule of Five" states that poor absorption frequently occurs with a molecular weight over 500, a logP over 5, and the number of hydrogen bond donors and acceptors each greater than five. In addition to Lipinski's original rules concerning molecular weight, lipophilicity, and the number of H-bond donors or acceptors; a number of studies have also discussed the negative absorption impact of water complexation by amide bonds, high molecular flexibility, and high polar surface area.

Bioavailability is a term used to describe the rate and extent of absorption of an orally administered drug in comparison to intravenous administration. High oral bioavailability is almost always an important consideration for the development of good drugs. Oral bioavailability is affected by both the extent of absorption into the intestinal cells in the gut lumen as well as the compounds presystemic elimination by both the intestine and the liver. So structure-specific recognition factors do limit oral bioavailability such as

(▶ P-Glycoprotein) (PGP) transport in the gut, CYP450 metabolism, or other enzymatic clearance mechanisms in the gut or liver.

If absolute oral bioavailability is >20% in dogs and rodents then frequently oral bioavailability in humans is >20% as well. However, since there is reasonable correlation between clearance, volume of distribution, and half-life data between rat and human as well, this may be tracked during Lead Optimization. It is widely assumed that monkeys are good predictors of human oral bioavailability but this is frequently not the case. They are a good predictor of the fraction absorbed (Fa), but as they have elevated metabolic clearances and hepatic enzyme activities, the oral bioavailability in monkeys is typically lower.

Consideration must be given to the ability of a compound to undergo in vivo biotransformation as this is an important component of systemic bioavailability and systemic clearance. It is frequently necessary to decrease the rate of hepatic oxidative metabolism and first-pass elimination in a chemical series during optimization. A number of in vitro methods are available to monitor progress. One of the most common and high-throughput entails the use of liver microsomal preparations. These retain activity of key components of enzymes involved in drug metabolism such as cytochrome P450s and ▶ flavin monooxygenases. The drug candidate is incubated in the presence of such preparations, half-life estimation is made and this can be compared to standards with known first-pass eliminations. The compound's metabolites should be identified in human, efficacy, and tolerability species. This can frequently give ideas for means by which the structure can be modified to decrease oxidative metabolism and reduce clearance.

Metabolism of a lead compound may also be evaluated in liver slices or hepatocytes. When a compound induces an enzyme involved in its own metabolism, evidence for which is usually found during multiple-dose pharmacokinetic or efficacy studies, the use of the latter are quite useful. When ▶ Phase II metabolism is important, again the use of liver slices may be more appropriate as well as in vitro glucuronidation assays in microsomal preparations in the absence of NADPH.

Metabolism-based drug interactions comprise one of the major concerns during drug development. If two or more drugs are competing with each other for the same metabolic enzyme, the circulating therapeutic drug concentrations can become elevated and may cause undesirable toxic effects. These are even more clinically important when a drug has a narrow therapeutic index or when the pharmacokinetics is markedly changed. Cytochrome P450 proteins are a critical family of enzymes involved in the metabolism of drugs. Of these the most important are CYP1A2, 2C9, 2C19, 2D6, and 3A4.

CYP3A4 has primarily been associated with in vivo drug–drug interactions leading to the withdrawal of marketed drugs. CYP3A4 is responsible for 25% of total CYP content and has broad substrate specificity. CYP2D6 is responsible for basic amine functionality metabolism, and this is important because of the prevalence of these in marketed drugs. Strong inhibitors <1 μm for 3A4 and 2D6 (cDNA expressed CYPs) are certainly major concerns.

Mechanism-based inhibition of 3A4 (irreversible binding or covalent modification) is also a major concern. Furans, some amines and unsaturated distal groups (double and triple bonds) are common culprits. For instance, the triple bond on ethinylestradiol modifys the heme group, though the CYP protein may be modified as well. Care is required when evaluating compounds, as they may initially appear to be only weak inhibitors of CYP3A4 because the inhibition is time-dependent. High liver distribution can exacerbate this issue, as many drug candidates are highly distributed to liver, where CYP3A4 is found in high concentration.

Clearance is a measurement of the ability of the body to eliminate a drug from the body and is made up of hepatic (metabolic and biliary) and renal clearance. For the majority of compounds hepatic metabolism is a major clearance mechanism, so clearance may be expressed relative to hepatic blood flow in the species in which the pharmacokinetic measurement was obtained. The drug concentration seen in the plasma depends on the amount of drug present in the whole body as well as how extensively it is distributed to various compartments. The volume of distribution is a measure of this. High steady state volumes of distribution are associated with more binding in tissue than in plasma and are found frequently in basic drug candidates, while the reverse is true of a low volume of distribution which is frequently found in association with acidic drugs. Since the half-life of a drug is inversely proportional to the clearance, and directly proportional to the volume of distribution, optimization of these parameters is frequently necessary.

Plasma protein binding can be important when activity is driven by the free fraction of drug in plasma. This may be a significant issue when there is a target within the vasculature and a lead with a small volume of distribution, but may be less of an issue with an extravascular target with a high volume of distribution. In the blood, acid glycoprotein binds weakly basic drugs while albumin, which constitutes 50–60% of total serum protein, binds neutral drugs and weak acids.

Toxicological Optimization

Just as the number of compounds in development that fail for pharmacokinetic issues have decreased, the number of compounds that fail during development for toxicity issues have increased. Even so, Adverse Drug Reactions (ADRs) may account for up to 5% of all hospital admissions and 10–20% of hospital inpatients for marketed drugs.

Toxicity issues are frequently observed during lead optimization either when tolerability is being examined specifically, or when dosing pharmacokinetic or efficacy studies. Toxicity is typically first identified in rats or dogs. Toxicologic effects of a compound frequently do require optimization, especially in backup programs where such issues may be better understood as a result of the identification of toxicology in previously identified drug candidates. ADRs may generally be grouped into two classes. Predictable adverse drug reactions can be predicted based on the pharmacology of the drug and are dose-dependent, idiosyncratic drug reactions are unpredictable, cannot be correlated to the pharmacology of the drug and are typically non dose-dependent. Optimization for predictable toxicity normally occurs via the identification of a (▶ Biomarker) or in vitro readout for the toxicity followed by the development of a structure-activity relationship around the toxicity in order to remove it during optimization.

Idiosyncratic toxicity is more difficult to approach. Hepatotoxicity is the most common idiosyncratic reaction. These effects can be serious (hepatic necrosis) and skin dyscrasias can be as serious as Stevens-Johnson syndrome (toxic epidermal necrolysis). Mechanistically, the hapten hypothesis is that modification of an endogenous protein by a reactive metabolite or directly by a reactive parent drug generates a foreign protein that may lead to an immune-mediated adverse reaction. The alternative danger hypothesis stipulates that rather than the hapten formation triggering the immune system, cellular damage produced by the reactive metabolite generates a danger signal. If cells are resistant to the stress or the toxic metabolites cannot cause cell injury at all times, no idiosyncratic toxicity occurs. This accounts for the interindividual variations in idiosyncratic drug reactions and the nature of differential responses to various reactive metabolites.

The human liver and blood systems are the most active at forming reactive metabolites.

Several enzymes from ▶ Phase I metabolism are recognized as important in generating reactive intermediates such as CYP450s, ▶ myeloperoxidases, ▶ cyclooxygenases, and flavin monooxygenases. CYPs are the most important because electrons are transferred. Phase II metabolism is potentially less of an issue, although there are some reports on acyl glucuronide toxicity (these may bind to proteins with or without cleavage of the glucuronide). A large number of electrophiles (generated from in vitro experiments such as with HLMs) can be trapped as glutathione adducts or N-acetyl cysteine/N-acetyl lysine adduct mixtures. This method is unlikely to produce false negatives, since safe compounds normally do not generate glutathione conjugates.

Cardiovascular safety is another important consideration during lead optimization. Frequently ▶ HERG activity needs to be removed from a compounds list of off-target effects.

This channel is the target of a wide range of agents which increase the risk for ▶ Torsade de Pointes (TdP), as well as many which do not. The growing list of non-antiarrythmic drugs that cause this increased QT interval, and the increasing number of drugs that have been withdrawn from the market because of their ability to trigger TdP, has resulted in increased regulatory scrutiny for this particular safety issue. Unfortunately there is no single cell–based assay, or in vitro heart preparation or animal model that is very strongly predictive for TdP in humans. Not all drugs which prolong the electrocardiographic QT interval cause TdP, nor is there a quantitative correlation between the magnitude of QT interval increases and the arrythmia. Because there is no gold standard test of the torsadogenic potential of a lead, the use of multiple in vitro, ex vivo, and in vivo assays is currently recommended by most experts during the lead optimization phase.

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Leakage Radiation

Definition

Radiation coming from the source outside the useful beam.

► Radiation Oncology

Lean Body Mass

Synonyms

LBM

Definition

Mass of the body, including water, bones, collagen, and muscle, minus fat mass.

► Nutrition Status

LECAM-2

► E-Selectin-Mediated Adhesion in Cancer

Lecithin

Definition

Phosphatidylcholine (PC) is a major class of glycerophospholipids present in all mammalian and some prokaryotic cells, which plays various roles in membrane structure and cellular signaling.

► Lysophosphatidylcholine

Lectin

Definition

They are proteins that interact with a sugar or sugar chain, with carbohydrates in general, but do not include enzymes related to carbohydrate metabolism, such as glycosyltransferase and glycosidase. Lectins include galectin, mannose-6 phosphate receptor, the calnexin/► calreticulin families, and others.

► Cell Adhesion Molecules
► Fucosylation
► Glycobiology

Leiomymatous Harmatoma of the Kidney

► Mesoblastic Nephroma

Leiomyoma

Definition

A benign neoplastic tumor composed of smooth muscle cells with variable amount of fibrous stroma. They appear most commonly in the smooth muscle lining of the uterus (Uterine leiomyoma), the myometrium.

► Fumarate Hydratase
► Uterine Leiomyoma, Clinical Oncology

Leiomyomata

► Uterine Leiomyoma, Clinical Oncology

Leiomyosarcoma

Definition

Belongs to a group of cancers called soft tissue sarcomas. Sarcomas are cancers that develop in the supporting or connective tissues of the body (such as muscle, fat, nerves, blood vessels, bone, and cartilage). They are rare. Leiomyosarcomas are one of the commoner types of sarcomas to occur in adults over the age of 50.

► Non-rhabdomyosarcoma Soft Tissue Sarcomas

Lentigines

Definition

Are benign lesions that occur on the sun-exposed areas of the body. The backs of hands and face are common areas. The lesions tend to increase in number with age, making them common among the middle age and older population. They can vary in size from 0.2 to 2 cm. These flat lesions usually have discrete borders, are dark in color, and have an irregular shape.

Lentiginosis

Definition

Presence of ► lentigenes in very large numbers or in a distinctive configuration. Centrofacial lentiginosis, uncommon autosomal dominant syndrome of small hyperpigmented macules in a horizontal band across the centre of the face at 1 year, increasing in number up to 10 years, and associated with skeletal and neural defects.

► Carney Complex

Lentiginosis polyposa Peutz

► Peutz-Jeghers-Syndrome

Leptin

Definition

Is an appetite suppressing hormone. The World Health Organization has now classified ► obesity as a disease. It is often said that obesity is the biggest health problem facing the developed world today. It causes health problems such as hypertension, type II diabetes, heart attacks and strokes, elevated cholesterol, and many more. Obesity leads to an estimated 30,000 premature deaths each year and it is shortening the lives of people by an average of 9 years. Leptin is an appetite suppressant. It stops one eating too much as well as makes one more active so one burns off more energy. It is produced in fat cells by a specific gene called the obese(ob) gene. Small amounts of leptin are also secreted by cells in the epithelium, stomach, and placenta. The amount of leptin found in people increases as their body fat increases. Leptin acts on receptors in the hypothalamus. The theory is that as one gets fatter one also gets less sensitive to the affects of Leptin. Leptin works on the body in the following ways:

1. Counteracts the effects of neuropeptide Y, which is a feeding stimulant secreted by cells in the gut wall and in the hypothalmus
2. Counteracts the affects of anandamid, which also is a feeding stimulant
3. Promotes the effects of alpha-MSH, which is an appetite repressor, resulting in inhibition of food intake
4. Raises the temperature of the subject so energy expenditure is increased

Leptin also acts directly on the cells of the liver and skeletal muscles where it stimulates the oxidation of fatty acids in the mitochondria. This reduces the storage of fat in those tissues (but not in adipose[fat] tissue). In rare cases the gene that produces leptin or its receptors mutate. This can cause severe obesity and diabetes in certain individuals as well as in certain cases failure to reach puberty. However, most people who are obese do not have a defective ob gene. Extensive clinical trials using recombinant human leptin as a therapeutic agent for treating obesity in humans have been inconclusive

because only the most obese subjects who were given the highest doses of exogenous leptin produced statistically significant weight loss. It was concluded that large and frequent doses were needed to only provide modest benefit because of leptin's low circulating half-life, low potency, and poor solubility. Furthermore, these injections caused some participants to drop out of the study due to inflammatory responses of the skin at the injection site. Leptin of humans has 146 amino acid sequence containing one disulphide bond. Its molecular weight is around 16 kDa. Leptin has 67% sequence identity among diverse species.

Leptomeningeal Carcinomatosis

Definition

Involvment of ▶ leptomeninges through seeding which occurs either by direct spread or via bloodstream. Any cancer can cause this, but adenocarcinoma is most commonly involved. Patients usually have a known underlying malignancy but primary presentation can be with symptoms of meningeal involvement.

▶ Carcinomatosis

Leptomeningeal Disease

▶ Leptomeningeal Dissemination

Leptomeningeal Dissemination

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Synonyms

Leptomeningeal disease; Leptomeningeal metastasis; Leptomeningeal seeding; Neoplastic meningitis; Subarachnoidal spread

Definition

Tumor cell spread into leptomeningeal structures of the brain and/or spine.

Characteristics

Leptomeningeal spreading is a major disease complication in primary central nervous system (CNS) tumors, in solid tumors outside the CNS such as breast, lung, gastrointestinal cancer, melanoma, and as well in hematological malignancies. Leptomeningeal dissemination can occur at initial diagnosis (▶ primary dissemination) or during the course of the disease following initial therapy (▶ secondary dissemination), but it is usually a late manifestation of malignant diseases and almost always associated with active tumor disease. Primary leptomeningeal disease is found mostly in primary brain tumors, sarcomas, neuroblastomas, lymphomas, and leukemia. Secondary dissemination occurs mostly in lung and breast carcinomas, but also in leukemia and lymphomas and primary CNS malignancies. The way of neuroaxis dissemination for primary CNS tumors and tumors outside the CNS is thought to be hematogenous via arachnoid vessels. Primary brain tumors may also disseminate throughout the neuroaxis by direct extension, by spreading along the nerves or by release of tumor cells into the cerebrospinal fluid (CSF). Due to the physiology of the CSF flow leptomeningeal metastases can involve all structures of the neuroaxis.

In brain tumors, leptomeningeal dissemination is well known in primary CNS lymphomas and medulloblastomas, occurring in up to 33% of all patients at the time of initial diagnosis, in germ cell tumors and also glioblastomas. Patients with evidence of CSF spread are considered to have a poorer prognosis compared with CNS-negative patients. In children with high-grade gliomas, the rate of leptomeningeal dissemination is reported to range between 7% and 28% and as with other tumors leptomeningeal spread is a sign of frank tumor dissemination and carries a worse prognosis. In patients with acute lymphoblastic leukemia, initial CNS involvement is found in up to 7.4% of patients depending on the individual risk profile of the patient. Although CNS involvement was reported to be negatively correlated with event-free survival (EFS) in one study, this observation could not be

confirmed by others. The percentage of initial CNS involvement in patients with acute myeloblastic leukemia (AML) ranges between 5.9% and 12.2%, patients with CNS-negative AML do better than those with initial CNS involvement.

Classification of cerebral leptomeningeal metastasis according to Chang's guideline for cerebellar medulloblastoma (Chang 1969):

Metastasis stage	
M0	No evidence for metastasis
M1	Tumor cells in CSF
M2	Gross nodular seeding of brain CSF spaces
M3	Gross nodular seeding of spinal CSF spaces
M4	Extra neural spread

Symptoms

The most common symptoms in patients with proven or suspected leptomeningeal dissemination are headache, nausea, vomiting, fluctuating consciousness, cranial or spinal nerve palsy (palsies), and spinal radicular symptoms. Clinical presentation may fluctuate, because several levels of the neuroaxis are affected simultaneously. Duration of symptoms may vary from several days to weeks or even months. In patients with medulloblastoma, secondary diffuse CSF seeding can lead to sudden neurological deterioration and spinal cord compression. Intracranial hypotension syndrome was described in diffuse meningeal melanomatosis. The symptoms could also mimic cerebral vasculitis, meningitis, or viral encephalitis.

Diagnosis

If leptomeningeal dissemination in a patient with an underlying malignant disease is suspected meningitis and viral encephalitis have to be excluded. Other rare diseases should also be considered such as granulomatous angiitis, histiocytosis, Lyme disease, multiple sclerosis, vasculitis, Wegner's granulomatosis, sarcoidosis, post-liquor puncture changes, and opportunistic infections.

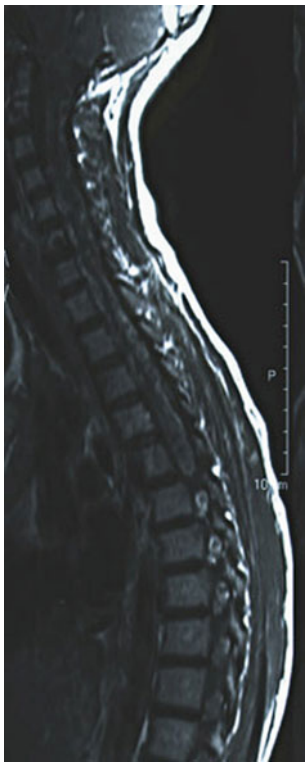
Neuroimaging

In former days conventional myelography and CT myelography improved detection of metastatic disease and can still be used today in cases in which MR imaging is not feasible; it could show nerve root thickening, nodularity, thecal sac irregularity, and spinal cord enlargement. Nowadays magnetic resonance imaging (MRI) is the diagnostic tool of choice in patients with suspected leptomeningeal dissemination of a malignant disease and may show hydrocephalus, pial linear or nodular enhancement, subependymal and/or neural deposits, or dural enhancement; however MRI has low specificity (i.e., meningeal enhancement on MRI does not always indicate metastases). Strong methionin uptake on positron emission tomography (PET) may help to confirm diagnosis even before CSF became positive. The time-point of neuroradiographic evaluations should also be considered: False positive spinal MRI findings of subarachnoidal spread of primary CNS tumors have been described, when MRI studies were performed within 2 weeks after surgery. In medulloblastoma, metastases are generally located along the posterior margin of the spinal cord. More sophisticated MRI techniques such as FLAIR sequences were discussed to be the clue to detect leptomeningeal abnormalities. Contrast- enhanced FLAIR imaging sequences seem to improve the detection rate of leptomeningeal disease compared to routine contrast-enhanced T1–weighted imaging by suppression of signal intensity of normal vascular structures on the surface of the brain and spine. Importantly, in patients with primary brain tumors, MRI imaging of the whole spinal axis should be done as a staging procedure before starting treatment and during follow-up (Fig. 1).

Laboratory Findings

Lumbal puncture for cytologic detection of malignant cells is an important diagnostic feature. In at least 50% an elevated opening pressure can be seen. An elevated protein in CSF is found in 80% of patients, whereas about one-third of the patients have low CSF glucose levels.

Detection of CSF seeding by means of cytopathological analysis of CSF specimens has low sensitivity, since only 15–60% of patients with leptomeningeal metastases have positive results. This could be explained by the paucity of viable tumor cells released in the CSF of patients with minimal disease and the presence of confounding reactive lymphocytes in more than one half of patients with leptomeningeal metastases, especially in patients with lymphoma and leukemia. Patients with low tumor burden who would probably most benefit from treatment are, therefore, more likely to be falsely negative. Several consecutive CSF samples and punctures may be required to identify malignant cells. Since positive CSF cytology correlates with a poor outcome regardless of the time of assessment, CSF cytology was found to be highly predictive



Leptomeningeal Dissemination. Fig. 1 Sagittal T1-weighted postgadolinium magnetic resonance imaging (MRI) in a 12-year-old girl with glioblastoma multiform showing multiple nodular enhancing lesions of the entire spinal cord

for overall survival. In addition, cytologic examination of lumbar CSF was shown to be superior to cytologic examination of ventriculoperitoneal shunt CSF for the detection of leptomeningeal spread in children with primary brain tumors. Of note MRI imaging and cytologic analysis CSF performed simultaneously might increase the detection rate of CSF dissemination.

Another diagnostic tool is the analysis of specific markers for leptomeningeal dissemination such as PSA for prostate cancer, CA-125 for ovarian cancer, and CA153-3 for breast cancer, and of non-specific markers such as beta-glucuronidase, lactate dehydrogenease (LDH), and carcinoembryonic antigen (CEA). These markers are helpful, when clinical symptoms strongly suggest dissemination in patients with negative neuroimaging and negative CSF cytological examination. These markers should be detected in serum and CSF levels and might be used for monitoring (in case of leptomeningeal dissemination: ratio <60:1).

Flow cytometric techniques of CSF in patients with lymphoma or leukemia allow the detection of meningeal dissemination with high sensitivity and PCR analysis of clonal immunoglobulin heavy chain (IgH) seems to have both high specificity and sensitivity.

Treatment

Management of leptomeningeal dissemination has to focus on both, treatment of symptoms and treatment of the malignant disease. ▶ Symptom management includes anticonvulsant treatment in case of seizures and pain medications in case of painful radiculopathy. Corticoids are not very helpful because edema in leptomeningeal metastases is not common.

Because leptomeningeal dissemination is often diagnosed in advanced disease stage, therapy is mainly palliative (except in leukemia and lymphoma, and germ cell tumors), aiming at preserving neurological function and potentially improving quality of life. As to whether the treatment of LM is ▶ palliative or ▶ curative depends on various clinical parameters, particularly on the underlying disease. Treatment of leptomeningeal metastases must be directed to the entire CNS, because leptomeningeal metastasis spreads throughout the neuroaxis by way of CSF flow. Treatment consists mainly of involved field irradiation, systemic, and intrathecal chemotherapy.

Intrathecal chemotherapy could be administered via lumbal puncture or Ommaya reservoir, which is the preferred route of administration due to a better distribution of the drug in the CSF and subarachnoid space. Only few chemotherapeutics are available for intra-CSF drug therapy (methotrexate, cytarabine, etoposide, mafosfamide and thiotepa). These agents were primarily tested in patients with primary brain tumors, leukemia, and lymphoma. Liposomal formulation such as liposomal cytarabine may increase the therapeutic drug level over extended periods of time and are new approaches to enhance efficacy of drugs administered into the CSF. Other drugs are under investigation: darcarbazin, melphalan, and topotecan. Except for hematopoietic neoplasms and to even lesser extent, for breast cancer, intrathecal chemotherapy might be efficacious only in cases, where free tumor cells float within the CSF. It is, however, insufficient to treat solid tumor desposits or even bulky disease. Experimental intrathecal treatment includes immunotherapy (intrathecal administration of monoclonal antibodies, cytokines, or lymphokine activated killer cells) or the use of targeted toxins.

The use of ► systemic chemotherapy is being re-evaluated for the treatment of leptomeningeal disease. It seems to be more effective when it is given as part of the initial treatment. In highly malignant medulloblastoma (and germ cell tumors), CNS-directed therapy is mainly based on craniospinal irradiation, whereas intrathecal chemotherapy with MTX via ventricular access devices is given to young children not amenable to CSI or to patients with leptomeningeal dissemination. In primary CNS lymphomas in which leptomeningeal involvement is common; intrathecal MTX is frequently used in combination with HD MTX. Nevertheless, patients with an initial diagnosis of meningeal leukemia are treated more intensively than CNS-negative patients, i.e., they will receive additionally intrathecal chemotherapy. In addition, intensive systemic chemotherapy may substitute for radiotherapy.

Even today with new radiation techniques radiation is almost strictly avoided in children younger 18 months. Overall in children, the attempt is to minimize radiation and its long-term sequelae by using more intensive chemotherapy. The total dose and fractions depend on tumor entity and age of patients, and could vary between 12 Gy and 30 Gy. In children with ALL prophylactic cranial irradiation is administered to high risk patients to reduce the risk of leptomeningeal disease: Due to improved systemic and intrathecal chemotherapy, doses of radiotherapy could be lowered to 12 Gy and craniospinal irradiation is no longer necessary; whether patients with AML require prophylactic cranial irradiation is a matter of debate. Furthermore radiation is used to stabilize or improve neurologic function, pain, and CSF flow. Additionally, chemotherapy was described to be more effective after radiation. In summary, radiotherapy in leptomeningeal metastases is almost only be used to treat symptomatic areas.

Summarizing the treatment of leptomeningeal dissemination, there is an urgent need to develop new drug-based or radiation-based treatment. For this purpose, new and better surrogate markers for response must be developed to identify clearly the effectiveness of a therapeutic regimen.

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Leptomeningeal Metastasis

► Leptomeningeal Dissemination

Leptomeningeal Seeding

► Leptomeningeal Dissemination

Leptomeninges

Definition

The two innermost layers of tissues that cover the brain and spinal cord. The two layers are called the arachnoid mater and pia mater.

Lesion on Tongue, Lip and Other Areas in the Mouth

► Oral Cancer

LET

Definition

Linear energy transfer represents the average amount of radiation energy lost when traversing a small distance. It has units of energy divided by the short distance (keV/μm).

► Radiosensitization

Letrozole

Definition

Tradename Femara® is used in hormonal therapy of ▶ breast cancer in postmenopausal women. Hormonal therapy (synonym ▶ Endocrine Therapy) interferes with the production or action of particular ▶ hormones in the body. ▶ Hormones are substances produced naturally in the body. They act as chemical messengers and help to control the activity of cells and organs. Letrozole is a member of the group of aromatase inhibitors (▶ Aromatase and Its Inhibitors). Many breast cancers rely on supplies of the hormone estrogen to grow (▶ Estrogenic Hormones). In postmenopausal women, the main source of estrogen is through changing ▶ androgens into estrogen, which is carried out by the enzyme ▶ aromatase. The conversion process is known as aromatisation, and happens mainly in the fatty tissues of the body. Letrozole blocks the process of aromatisation, and so reduces the amount of estrogen in the body.

Letterer–Siwe Disease

Definition

This disease is a fatal and disseminated form of Langerhans cell histiocytosis which is most commonly seen in children less than 2 years old.

▶ Langerhans Cell Histiocytosis

Leucopenia

Definition

A reduction in the circulating white blood cell count to less than 4,000/μL.

▶ Rituximab

Leucovorin

Synonyms

Folinic acid

Definition

Folinic acid is a reduced folate that can assist 5-FU in the inhibition of thymidylate synthase augmenting the toxicity of this drug.

▶ Fluorouracil

Leukemia

Definition

Leukemia is a group of cancers originated from blood cells. It can be divided into different types based on the type of blood cells involved, the stage of differentiation when normal cells become leukemic cells, and the speed of disease development. When leukemia arises from the lymphocyte precursors it is called lymphoblastic leukemia and when it arises from the myeloid precursors it is known as myeloblastic leukemia.

▶ Hematological Malignancies
▶ Minimal Residual Disease
▶ STI-571

Leukemia Diagnostics

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Definition

Leukemia diagnostics comprises the combined application of different methodologies in a standardized fashion, allowing the precise diagnosis, subclassification, and determination of prognostic parameters.

Characteristics

Introduction

The diagnosis of leukemias today is based on a comprehensive approach applying a variety of different methods in combination. While ▶ cytomorphology and cytochemistry have been the mainstay of diagnostics for decades and have been used to differentiate between ▶ acute myeloid leukemia (AML), ▶ acute lymphoblastic leukemia (ALL), ▶ chronic myeloid leukemia (CML), ▶ chronic lymphocytic leukemia (CLL), and ▶ myelodysplastic syndromes (MDS), the current up-to-date approach includes ▶ immunophenotyping/Multiparameter flow cytometry (MFC) as well as ▶ cytogenetics and ▶ molecular genetics to correctly diagnose and subclassify leukemias.

Multiparameter flow cytometry (MFC) is used for immunophenotyping of the leukemic cells which is essential particularly to diagnose and classify lymphatic leukemias. Karyotyping and ▶ fluorescence in situ hybridization (FISH) are applied to detect chromosomal alterations which are considered disease-defining lesions in an increasing number of entities. Different molecular genetic techniques (▶ PCR, ▶ fragment analysis (Genescan), ▶ heteroduplex analysis, ▶ melting point analysis, ▶ nested PCR, ▶ real-time PCR, sequence analysis, ▶ Single stranded conformation polymorphism analysis (SSCP)) allow the identification of leukemia-specific fusion genes and gene mutations and are also an integral part of the diagnostic workup of leukemias.

The basis for establishing this diversified diagnostic approach has been the growing body of insight into the genetics of leukemias, which not only allowed the definition of recurring genetic aberrations but also their strong correlation to distinct disease subtypes with specific clinical characteristics, including prognostic impact. As a consequence, today the decision to treat a patient suffering from a particular leukemia and the specific therapeutic option to select for him or her largely depends on the presence or absence of these genetic alterations.

The monitoring of minimal residual disease (MRD) increasingly gains clinical relevance in patients with leukemias. While conventional methods like cytomorphology can be used to assess the prognostically highly relevant achievement of complete remission, newly developed methods like multiparameter flow cytometry and quantitative real-time PCR allow the exact quantification of the amount of residual malignant cells in patients with complete remission. The level of this MRD in many cases significantly correlates with the further course of the disease and is incorporated into the guidance of a risk-adapted therapy.

The present essay provides, in the first step, an overview of the methods applied in leukemia diagnostics and then focuses on the respective leukemia subentities and their specific diagnostic findings. The content is then summarized by comprehensive algorithms detailing the modern diagnostic workup for the different leukemias.

Methods Applied in Leukemia Diagnostics

Cytomorphology and Cytochemistry

Cytomorphologic analysis and cytochemistry are performed on peripheral blood and bone marrow smears which are air-dried without fixation before staining. A panoptic staining (Pappenheim or May Gruenwald Giemsa) is used for the general assessment of cell characteristics. Cytochemistry applies myeloperoxidase staining and nonspecific esterase staining for the identification of myeloid and monocytic cells, respectively, while the use of Schiff's reaction, acid phosphatase, and chloroacetate esterase (CE) has been substituted by immunophenotyping. Iron staining is applied in the diagnostic setting of MDS.

Immunophenotyping

Immunophenotyping by multiparameter flow cytometry allows the identification, quantification, and characterization of cell populations in peripheral blood or bone marrow samples. Cells are differentiated from each other, based on their light scatter features during their pass across a laser beam (higher cell sizes result in higher forward scatter signal [FSC]; higher heterogeneity of cell content results in higher side scatter signal [SSC]) as well as on their antigen expression patterns. As 1,000–2,000 cells are analyzed per second, the simultaneous analysis of the expression of five antigens in 10,000 cells within seconds is current standard. Populations can be characterized even if their concentration is only around 1%. During the monitoring of minimal residual disease (MRD), 250,000 cells are analyzed.

While the detection of FSC and SSC signals is possible in unmanipulated cells, the detection of antigens requires the use of monoclonal antibodies against

the respective antigens, which are coupled to a fluorescent dye. Five or even more different fluorescent dyes can be routinely used as the light emitted differs in wavelength and is specifically recognized by different detectors. Sophisticated compensation programs, however, are necessary to account for overlaps in the spectrum of the emitted light.

Due to its expression on all peripheral blood and bone marrow at different levels, CD45 represents an ideal antigen for performing a differential count. Thus, monocytes feature the strongest expression of CD45 and may be separated from lymphocytes, which also express CD45 quite strongly, based on differences in the SSC signal. Granulocytes express CD45 at a lower level and feature the strongest SSC signal. Importantly, erythrocytes hardly express CD45 and blasts show a CD45 expression level similar to granulocytes; however, both the latter populations are easily separated from each other, based on their SSC signal (low SSC signal in blasts). Therefore, CD45 gating is useful for the analysis of different cell populations, particularly for the analysis of blasts.

The simultaneous detection of the expression of multiple antigens within one tube allows the comprehensive assessment of antigen expression patterns in different cell populations, which, in comparison to single and dual color approaches, leads to quick and valid results in diagnostics, particularly in the quantification of MRD. The panel of antibody combinations is selected, respectively, in consideration of the suspected diagnosis and may be supplemented based on primary results.

Cytogenetics

Importance of Chromosome Analysis, Indication

Chromosome analysis today is an essential part of the diagnosis in hematologic neoplasias. The results help in establishing the diagnosis. Most importantly, however, chromosome analysis provides prognostic information which is derived from the karyotype of the malignant cells. The neoplasia-associated chromosome aberrations are acquired genetic alterations and are limited to the malignant cells. Thus, the nonmalignant cells in patients with hematologic neoplasias are cytogenetically normal.

Material

For chromosome analysis, bone marrow is preferred to peripheral blood because malignant cells are present at higher percentages and have a higher proliferative activity. If bone marrow cannot be obtained, the cytogenetic analysis may be done on peripheral blood cells. Since viable cells are needed for metaphase cytogenetics, the bone marrow should be shipped into the cytogenetic laboratory within 24 h. The cells must not be frozen.

Conventional Chromosome Analysis Using Banding Techniques

A sufficient number of good quality metaphases is needed for chromosome analysis. Bone marrow cells are arrested in the metaphase by the addition of colchicine either directly after drawing the sample or after short-term cultivation (24–72 h). To maximize the gain of metaphases the leukemic cells can be stimulated by cytokines during cultivation. The addition of hypotonic potassium chloride solution swells the cells and they are fixed in multiple steps by the use of a methanol acetic acid solution. Then the cells are dropped on to glass slides. To allow the unequivocal identification of each chromosome, a banding technique must be applied. The most frequently applied banding techniques are G- (Giemsa-), Q- (Quinacrin-), and R- (reverse) banding. These techniques lead to the appearance of light and dark bands on the chromosomes which are specific for each chromosome and allow an unequivocal identification of each chromosome. To allow a valid report a complete analysis of 20–25 metaphases is required according to the respective international consensus.

Nomenclature – ISCN Cytogenetics

Chromosomes are classified according to their size, the position of the centromere (which divides both arms of the chromosomes), and the characteristic banding patterns. Each ▶ chromosome has a short arm (p) and a long arm (q). Based on the banding pattern, each chromosome is divided into regions and bands, which are numbered from the centromere to the telomere. The internationally accepted cytogenetic system of nomenclature (ISCN: International System of Cytogenetic Nomenclature) allows the exact description of all numeric and structural aberrations in a karyotype formula. The karyotype formula in the first place gives the number of chromosomes followed by the sex chromosomes. Thus, the normal female karyotype is 46,XX and the normal male karyotype is 46,XY.

The numeric chromosome aberrations include monosomies (loss of a chromosome) and trisomies

(gain of a chromosome). Furthermore, the complete set of chromosomes may be multiplied. In normal cells a double set of chromosomes (diploid chromosome set) is present. Three- and fourfold sets of chromosomes are designated as triploid and tetraploid.

The most frequently occurring structural chromosome aberrations are deletions (losses of parts of chromosomes), ▶ chromosomal translocations (exchange of parts of chromosomes between different chromosomes), inversions (twisting of a part of a chromosome by 180°), and isochromosomes (a chromosome consisting of either two short arms or two long arms, while the respective other arms are lost).

In the karyotype formula a gain of a chromosome is indicated by a "+" and a loss of a chromosome is indicated by a "−", e.g., 47,XX,+8 is a trisomy of chromosome 8 and 45,XY,−7 is a monosomy of chromosome 7. There are abbreviations for structural chromosome aberrations which are internationally agreed on, e.g., "t" for translocation and "inv" for inversion: t(8;21)(q22;q22) indicates a break in chromosome 8 at band q22 and a break in chromosome 21 at band q22 as well as an exchange of the fragments between both chromosomes. Different chromosomes and breakpoints in different chromosomes are divided by; in the karyotype formula, while breaks within a chromosome are listed without a separator, e.g., inv(16)(p13q22): breaks occurred in chromosome bands p13 and q22 of a single chromosome 16 with a fragment being twisted by 180°. Another example is del(5)(q13q31): breaks occurred in the bands q13 and q31 of a single chromosome 5 with a loss of the area between q13 and q31.

Chromosome aberrations are designated clonal if an identical structural aberration or a gain of a chromosome is observed in at least two metaphases or if a loss of the same chromosome is observed in at least three metaphases.

Comparative Genomic Hybridization (CGH)

▶ Comparative genomic hybridization allows a comprehensive analysis of the tumor genome with regard to over- and underrepresented DNA sequences (losses and gains of chromosomes, deletions, amplifications). The technique is based on the simultaneous staining of both test DNA of a healthy volunteer and tumor DNA using two different fluorescent dyes. Identical aliquots of both DNA samples are then mixed and hybridized on normal metaphases. Differences in the numbers of copies in different sequences between normal and tumor DNA are detected by the quantification of the ratio of fluorescence intensity (tumor DNA to normal DNA) in each region of the normal metaphase chromosomes. Balanced translocations, inversions, or other aberrations which are not accompanied by a change in the number of copies are thus not detectable by CGH. CGH may play an important role particularly if a chromosome analysis is not possible, e.g., if living tumor cells are not available or if tumor cells do not proliferate in vitro.

Fluorescence In Situ Hybridization (FISH)

The FISH technique relies on the hybridization of DNA probes which identify specific chromosomal structures. Probes can be used which are specific for the centromeric region of particular chromosomes, for genes, or for complete chromosomes. The DNA of both the applied probe and of the patient sample are denaturated, i.e., both DNA strands of the double helix are separated. During the following renaturation, the DNA probes attach to the complementary section of the patient DNA (hybridization). The DNA probes are either directly conjugated to a fluorescent dye or are analyzed using fluorescence-conjugated antibodies. The respective chromosome structures, therefore, are assessable as fluorescence signals.

A significant advantage of the method lies in its applicability not only to metaphases but also to interphase nuclei. A disadvantage is that information is obtained only on chromosomes and genes for which probes are used.

The role of the FISH technique differs between the different leukemia subgroups.

Interphase FISH

Due to the multitude of different chromosome aberrations, which are observed particularly in acute leukemias, a screening based on FISH on interphase nuclei covers only a fraction of potentially present aberrations and, therefore, cannot substitute the classic chromosome analysis. However, if a specific question should be answered, e.g., the detection of the translocation t(15;17)(q22;q12) when acute promyelocytic leukemia is suspected, the FISH technique represents a fast and reliable method, providing a result within 4 h.

In follow-up assessments during therapy, the FISH technique can be used for the detection of residual disease if at diagnosis aberrations have been found by

chromosome analysis for which FISH probes are available. The sensitivity for this method is higher than for the chromosome analysis; however, it is lower than for PCR.

Metaphase FISH

In addition to the probes applicable to interphase nuclei, so-called chromosome painting probes can be applied to metaphases which specifically bind to the complete DNA of a chromosome. This technique is used mainly for the confirmation of the conventional chromosome analysis in difficult cases.

The 24-color-FISH method allows the display of all 22 different pairs of chromosomes as well as of the sex chromosomes in one single hybridization. It is applicable to metaphase chromosomes only and helps in identifying complex structural aberrations.

Molecular Genetics

PCR

PCR is the most frequently applied method to detect molecular genetic changes in leukemia. By using oligonucleotides specific for certain sequences, regions of interest can be amplified and further analyzed. All the following methods are based on this standard method.

RT-PCR

In specific cases, especially when fusion genes are analyzed in which breakpoints are distributed over a wide genomic range, it is advantageous to use cDNA as the template for PCR.

Nested PCR

Using this method, both the sensitivity and specificity of a PCR can be increased. In hematology, it is frequently applied to detect small amounts of molecules, i.e., mainly for the detection of minimal residual disease. For a nested PCR a fraction of a finished PCR is used for a new PCR. Oligonucleotides that hybridize within the first amplificate are used as primers. This additional amplification increases the sensitivity significantly. Depending on the type of mutation and the initial material, one malignant cell in 10^4–10^8 normal cells can be detected. Thus, this is the most sensitive method currently available for most sequences targeted for the detection of ▶ minimal residual disease (MRD). However, due to the high sensitivity, this method also carries the highest risk of contamination. In order to minimize this risk, different precautions must be applied and control reactions must be performed in parallel.

Real-Time PCR

Another method for the amplification and detection of PCR products is the real-time PCR. Different from other methods of detection, this is not an end-point analysis, but the measurement is performed during the phase of PCR when a logarithmic amplification of PCR products occurs. This allows an exact quantification of the target sequences in the material which is assessed. The method is based on the addition of fluorescence-conjugated probes to the specific primers required for the PCR. These probes hybridize during the running PCR with the continuously increasing amplification products and release fluorescence signals, which are detected in an optical device specifically constructed for this approach. Thus, an increase of fluorescence intensity occurs during PCR. The time point (PCR cycle) at which a fluorescence higher than baseline is detected for the first time in a sample correlates with the number of targeted molecules in the sample. The fluorescence intensity of the targeted gene is normalized to a constantly present gene or transcript, based on which the number of malignant cells present in the sample can be calculated. Also, for this method of detection, the application of specific PCR machines equipped with optical devices is needed. Using these machines it is also possible to conventionally detect PCR products; however, the strength of real-time PCR is its capacity for an exact quantification for follow-up analyses.

Mutation Screening

Different methods are available, which allow the screening of defined gene regions for mutations without the necessity of sequencing. Some examples are listed below.

Heteroduplex Analysis As the first step in this analysis, a region with a suspected mutation is amplified by PCR. Since in man two alleles of each locus are normally present, two different PCR products occur. After completion of the PCR, these are denaturated (conversion to single-strand state) and then renaturated. Consequently, four renaturated products are possible: a normal double strand, a mutated double strand, and two heteroduplices, in which one base, respectively, is not paired to the other strand,

i.e., a mismatch is present. These heteroduplices are detectable on specific gels or by a dHPLC device (WAVE).

SSCP Analysis (Single Stranded Conformation Polymorphism Analysis) In this analysis, denaturated single strands are separated using a non-denaturating surrounding by gel or capillary electrophoresis. It takes advantage of the fact that mutated single strands lead to other secondary structures as compared to unmutated ones and thereby have different features within the electric field.

Melting Point Analysis In addition to the classic real-time PCR, a further step is applied. Fluorescence-marked probes, which lie on a potentially mutated area, are slowly melted off the PCR products. In case of a mutation under the probes, a so-called mismatch occurs. Thus, the probes fit less well in the wild type and melt off the PCR product faster.

Fragment Analysis (Genescan)

As an alternative to the gel analysis, PCR fragments can be detected by a fragment analysis which is also called Genescan-analysis. This method is performed using sequencing machines equipped with special software. To allow the detection, one of the two PCR primers is conjugated to a fluorescent dye. The advantage of this method is its capacity to determine the length of the amplificates exactly. In addition, with some limitations, it is possible to quantify the amplificates in comparison with another gene, which is generally the unmutated wild type. The method needs more extensive equipment and resources, due to the use of primers conjugated to fluorescent dyes, as compared to the standard gel analysis. It is frequently used in the context of multiplex PCR reactions, i.e., reactions employing multiple primers with the parallel amplification of up to four different loci. An example of this application is the chimerism analysis after allogeneic stem cell transplantation with microsatellite markers.

Sequence Analysis

The exact delineation of the base sequence of a region of a gene is frequently needed. To allow this, sequencing reactions are performed with nucleotides being integrated during PCR-based reactions. Each of the four different nucleotides is labeled to a fluorescent dye and a dideoxy group, respectively, and leads to a chain stop in the PCR reaction. Subsequently, the products are separated according to their length on a matrix (e.g., a gel or a polymer) and are detected by an optical device allowing the exact determination of the base sequence.

For some gene regions the sequence analysis is feasible as a primary approach in a limited number of patients. In case of a screening of multiple gene regions in one patient or of one gene in many patients, the direct sequencing requires large resources. In this instance, different screening approaches are available for the assessment of mutations in particular gene regions without the need for a full sequence analysis.

Diagnostic Procedures in Leukemia

Diagnostic Workup of AML

Cytomorphology The examination of both bone marrow and peripheral blood smears by cytomorphology and cytochemistry is necessary to diagnose AML according to FAB criteria (Table 1). Based on the FAB (French–American–British) classification, which was established in 1976 and revised in 1985, AML is subdivided into 11 morphologically different groups. Some of these groups correlate with distinct genetically defined forms of AML; however, most of them do not. Increasing insights into the biology of AML as well as the identification of specific chromosome aberrations prompted the WHO to suggest a new classification in 2001, based on biology and cytogenetics.

The FAB classification subdivides AML based on cytomorphology and cytochemistry into different subgroups. With only a few exceptions, the diagnosis of AML requires bone marrow blasts of at least 30% of all nucleated cells; at least 3% of the blasts must react positive for myeloperoxidase. The threshold for blasts is 20% in the WHO classification.

The AML classification suggested by the WHO combines the methods previously used for the FAB classification, i.e., cytomorphology, cytochemistry, and immunophenotyping, with cytogenetics and molecular genetics as well as with clinical parameters (Table 2). The genetic characterization of AML, in particular, not only allows a classification according to the prognosis, but provides the basis for the definition of distinct subgroups with recurrent balanced translocations. Thus, the WHO classification divides AML into four groups:

1. AML with recurrent cytogenetic aberrations
2. AML with myelodysplasia-associated features

Leukemia Diagnostics. Table 1 Definition of subgroups of AML according to FAB, association with genetic aberrations

	FAB-criteria				Association with		
FAB-subtype	Granulopoiesis	Monopoiesis	Erythropoiesis	Immunologic marker	Cytogenetics	Molecular genetics	Frequency
M0	<10% POX <3%	<20%	<50%	Myeloid positive Lymphoid negative			
M1	<10% POX >3%	<20%	<50%		t(8;21)	AML1-ETO	1.7%
M2	>10% maturation	<20%	<50%		t(8;21)	AML1-ETO	12.5%
M3	Hypergranular Auer-rods	<20%	<50%	HLA-DR negative	t(15;17)	PML-RARA	98%
M3v	Microgranular monocytoid nuclei	<20%	<50%	HLA-DR negative	t(15;17)	PML-RARA	
M4	>20%	>20%	<50%				
M4Eo	>20% abnormal eosinophils	>20%	<50%		inv(16)/t(16;16)	CBFB-MYH11	100%
M5a	<20%	>80% immature	<50%		11q23 Aberr.	MLL-Fusion	31%
M5b	<20%	>80% mature	<50%		11q23 Aberr.	MLL-Fusion	17%
M6	>30% of NEC are blasts	Variable	>50%				
M7	>30% megakaryoblasts	Variable	<50%	CD41/CD61 positive			

Bold typical finding, *NEC* non-erythroid cells

Leukemia Diagnostics. Table 2 WHO classification of AML

AML with recurrent cytogenetic aberrations	AML with t(8;21)(q22;q22), AML1(CBF-alpha)-ETO
	Acute promyelocytic leukemia (AML with t(15;17)(q22;q11-12) and variants, PML-RARA)
	AML with abnormal bone marrow eosinophils (inv(16)(p13q22) or t(16;16)(p13;q22), CBFB-MYH11)
	AML with 11q23(MLL)-fusions
AML with multilineage dysplasia	AML following a myelodysplastic syndrome
	AML without antecedent myelodysplastic syndrome
AML and myelodysplastic syndrome, therapy-related	Alkylating agent related
	Topoisomerase type II inhibitor related (some may be lymphoid)
	Other types
AML not otherwise categorized	Minimally differentiated AML
	AML without maturation
	AML with maturation
	Acute myelomonocytic leukemia
	Acute monoblastic or monocytic leukemia
	Acute erythroid leukemia
	Acute megakaryoblastic leukemia
	Acute basophilic leukemia
	Acute panmyelosis with myelofibrosis

3. Therapy-related AML and MDS
4. AML not otherwise categorized

The first group comprises biologically and clinically largely consistent subgroups of AML. The WHO classification thus represents a significant development of the FAB classification. The independent biologic and prognostic impact of groups 2–4, however, has not been demonstrated yet. Importantly, the subdivision of group 4 essentially reflects the FAB classification. A further new definition in the WHO classification is the deletion of MDS-RAEB-t and the reduction of the limit between MDS and AML from 30% to 20% bone marrow blasts.

Immunophenotyping AML is diagnosed on the basis of cytomorphology and cytochemistry. The only exceptions are AML M0 and AML M7 in which immunophenotyping is also necessary. Furthermore, some genetically defined subgroups feature typical although not fully specific immunophenotypes which are therefore used to guide further diagnostics only.

According to the abundance of promyelocytes, the AML M3 shows characteristic findings in the scatter plot and in addition is negative for HLA-DR and has a strong unspecific fluorescence signal. However, some of these findings can be present also in AML M2.

Typically, in AML M2 with t(8;21) there is an aberrant coexpression of CD19 and CD56; this can also be found in some AML without t(8;21).

The same is true for the immunophenotype of AML M4Eo with positivity for CD2 and an asynchronous coexpression of CD15 and CD34 which, however, may be present in other AML subtypes as well.

AML M0 is undifferentiated and lacks myeloperoxidase positivity in cytochemistry. Thus, the diagnosis cannot be made based on morphology alone. Immunophenotyping allows the distinction from ALL based on the expression of CD13, CD33, and CD117 and other myeloid antigens and at the same time a lack of lymphatic antigens or, respectively, a low lymphatic score not sufficient to diagnose a biphenotypic acute leukemia. In half of the cases, an expression of MPO can be detected although cytochemistry shows negativity for myeloperoxidase.

AML M7 is negative for myeloperoxidase in cytochemistry. The expression of CD41 or CD61 is detected flow cytometrically. Due to the frequently occurring myelofibrosis a cytologic examination may not be possible in some cases.

Monitoring of Minimal Residual Disease (MRD)

In patients with AML a significant prognostic impact of immunologically determined levels of MRD has been demonstrated. Due to the similarity of immunophenotypes between AML and normal bone marrow in some cases early work focused on highly aberrant leukemia-associated aberrant immunophenotypes (LAIPs) including mainly the aberrant expression of lymphoid markers (CD19, CD7, CD56) and the asynchronous expression of progenitor cell and differentiation markers (e.g., CD34 + CD117-CD15+).

For MRD levels determined after the achievement of complete remission and completion of consolidation therapy, a significant impact on both relapse-free and overall survival has been demonstrated, which has been largely independent of other prognostic parameters.

Newer studies have aimed at extending the immunologic MRD analysis to patients with less aberrant LAIPs and thus at the applicability of the method to each patient with AML. By the use of a comprehensive panel of monoclonal antibodies this task has been accomplished with a median sensitivity of 0.05%. Further analyses using this approach have confirmed the prognostic impact of MRD levels. Thus, as early as on day 16 of the induction therapy, patients can be divided into two prognostically differing groups based on the MRD level (2-year event-free survival: 53% vs 19%, $p < 0.0001$; 2-year overall survival: 58% vs 43%, $p = 0.0133$). Furthermore, the MRD levels determined after both achievement of complete remission and completion of consolidation therapy demonstrate a similar prognostic impact, which is independent of other parameters.

In patients with AML, thus, multiparametric flow cytometry allows the quantification of MRD in virtually all cases, while molecular techniques allow this in half of the cases. Current studies will determine the role and place of both methods in AML. Table 3 shows a comprehensive panel of antibodies, which can be used to define a LAIP in AML.

Cytogenetics

More than 50 recurrent structural chromosome aberrations have been described in AML. The karyotype of the leukemic blasts is the most important independent prognostic parameter in AML.

Fifty to seventy-five percent in adult AML and 75–85% in childhood AML carry clonal chromosome

Leukemia Diagnostics. Table 3 AML panel for the detection of leukemia-associated aberrant immunophenotypes (LAIP)

Combination	FITC	PE	ECD	PC5	PC7
1	Isotype	Isotype	Isotype	Isotype	Isotype
2	CD64	CD87	CD56	CD4	CD45
3	CD65	CD2	CD13	CD34	CD45
4	CD9	HLA-DR	CD33	CD34	CD45
5	CD11b	CD116	CD117	CD34	CD45
6	CD34	CD56	CD33	CD19	CD45
7	CD15	CD7	CD33	CD34	CD45
8	CD36	CD61	CD235a	CD14	CD45
9	CD4	7.1	CD13	CD14	CD45
10	CD38	CD135	CD90	CD34	CD45
11	CD15	CD133	CD117	CD34	CD45
12	Isotype	Isotype	Isotype	Isotype	Isotype
13	MPO	LF	cCD33	cCD34	cCD45
14	TdT	cCD22	CCD79a	cCD3	cCD45

C cytoplasmic; combinations 12–14: cytoplasmic analysis; 7.1 = antibodies for the detection of the NG2 antigen (associated with 11q23 aberrations)

aberrations. The incidences of the respective chromosome aberrations are age-dependent. However, the prognostic relevance of the karyotype is largely independent of age.

A variety of characteristic chromosome aberrations are known in AML, which define distinct entities with typical morphology and clinical course. The newly defined WHO classification of AML includes cytogenetic aberrations as central classification criteria. Thus, the classification is primarily based on specific cytogenetic rearrangements:

- AML with t(8;21)(q22;q22), AML1/ETO
- Acute promyelocytic leukemia/AML M3/M3v with t(15;17)(q22;q11–12) and variants, PML/RARA
- AML with abnormal bone marrow eosinophils and inv(16)(p13q22) or t(16;16)(p13;q22); CBFB/MYH11
- AML with 11q23(MLL)-anomalies

Based on cytogenetics and pathogenesis AML can be separated into three groups according to the karyotype:

1. AML with normal karyotype (40–45%).
2. AML with balanced chromosome aberrations (20–25%), the most frequent ones being t(8;21)(q22;q22), inv(16)(p13q22)/t(16;16)(p13;q22), t(15;17)(q22;q12), and 11q23-rearrangements involving the MLL gene. Less frequent ones include inv(3)(q21q26), t(6;9)(p23;q34), and t(3;21)(q26;q22).
3. AML with unbalanced karyotype abnormalities (30–40%) including trisomies (e.g., +8, +11, +13, +21), monosomies (e.g., −7), and deletions (e.g., 5q-, 9q-) as well as the large group of complex aberrant karyotypes (three and more chromosome aberrations, but none of the recurrent balanced aberrations).

With regard to prognosis AML is divided into three groups based on the karyotype:

1. Favorable karyotype: t(15;17)(q22;q12), inv(16)(p13q22)/t(16;16)(p13;q22), t(8;21)(q22;q22)
2. Intermediate karyotype: normal karyotype, all aberrations not grouped into 1 or 3
3. Unfavorable karyotype: complex aberrant karyotype, -5/5q-, -7/7q-, 17p aberrations, 11q23/MLL-rearrangements, inv(3)(q21q26), t(6;9)(p23;q34)

For a variety of infrequent karyotype aberrations the prognostic impact has not been clearly defined yet. Significant insights into correlations between genetic alterations and response to therapy led to the approach of increasingly selecting therapy according to the karyotype.

The prognostic relevance of the karyotype is valid within all age groups as well as in both de novo and therapy-associated AML.

Fluorescence In Situ Hybridization

In the diagnostic setting of AML, the FISH analysis is used mainly in addition to the classical chromosome banding analysis. Since the karyotype aberrations occurring in AML are highly heterogeneous, even a large-scale FISH screening on interphase nuclei would cover only a small part of these aberrations. Thus, FISH cannot replace the classic chromosome banding analysis.

However, in targeting a specific alteration, e.g., the translocation t(15;17)(q22;q12) (on the molecular level the rearrangement of the PML and RARA genes) when an acute promyelocytic leukemia is suspected, the FISH technology on interphase nuclei provides a fast and valid result within 4 h. Furthermore, the frequently occurring genetic aberrations, t(8;21)(q22;q22), inv(16)(p13q22) and rearrangements of the MLL gene are used for stratification in the newly defined WHO classification of AML. These alterations are detectable by FISH as are the frequently occurring deletions, e.g., deletions on the long arm of chromosomes 5 or 7, and monosomies (−7) and trisomies

(+8, +11, +13, +21). A large portion of AML with complex aberrant karyotype is detectable by FISH with the help of a medium-scale set of probes.

The so-called chromosome painting using 1–3 or even 24 colors (24-color-FISH) on metaphase chromosomes is applied in addition to the classic chromosome analysis, if the karyotype cannot be fully resolved on the basis of the chromosome analysis after classic banding, which is not uncommon in complex aberrant karyotypes.

FISH can further be used during the course of therapy for the detection of residual disease. It is more sensitive and specific compared to cytomorphology or chromosome banding analysis; however, it is less sensitive compared to real-time PCR and immunophenotyping. FISH has its role in the detection of residual disease, mainly in AML with complex aberrant karyotype, since this subgroup in general lacks genetic alterations detectable by PCR.

Molecular Genetics

Detection of Fusion Genes The detection of fusion genes using RT-PCR plays an important role in the molecular diagnosis of AML. Reciprocal chromosome rearrangements are found in about 25% of all AML. The molecular correlates and fusion genes, respectively, of most reciprocal cytogenetically detectable rearrangements, are known. The most frequently occurring reciprocal translocations t(15;17), t(8;21), and inv(16)/t(16;16) are represented by the fusion genes PML-RARA, AML1-ETO, and CBFB-MYH11, respectively, on the molecular level. Rearrangements of the MLL gene are present in 5% of all AML with more than 50 different translocation partner genes. Furthermore, some reciprocal rearrangements occur infrequently and are present in less than 1% of AML; however, they may be useful for diagnostic purposes and defining prognosis. The most frequent fusion genes in AML are listed here.

The following fusion genes are detectable by RT-PCR (Table 4):

AML1-ETO, AML1-EVI1, BCR-ABL, ETV6-MDS/EVI1, CBFB-MYH11, PML-RARA, RARA-PML, MLL-ENL, MLL-ELL, MLL-AF6, MLL-AF9, MLL-AF10, MLL-AF1q21, MLL-AF17, MLL-MSF, MLL-p300, CALM-AF10, MLL-PTD, FUS-ERG, EFG-FUS, CHIC2-ETV6, NUP98-HOX9, DEK-CAN, MOZ-CBP.

For the fusion genes PML-RARA, AML1-ETO, and CBFB-MYH11 it has been demonstrated that the quantification of their expression at diagnosis is prognostically relevant. For all other fusion genes, the quantification at diagnosis is also useful since this evaluation may be used as the starting point for assessment during follow-up.

Detection of Molecular Mutations In recent years a variety of mutations and small gene rearrangements, which are not detectable cytogenetically, have been described. Nonetheless, they play an important role in molecular diagnosis and estimation of prognosis in AML.

Among these molecular mutations are the partial tandem duplications of the MLL gene (MLL-PTD) and the length mutation of the FLT3 gene. These mutations are found mainly in AML with a cytogenetically normal karyotype and are associated with an unfavorable prognosis. The FLT3-LM is found in 10–15% of all childhood AML and in 20–25% of all adult AML. Furthermore, in another 6–7% point mutations are found within the tyrosine kinase domain of the FLT3 gene (TKD mutations). Thus, with a total of about 30% FLT3 is one of the most frequently mutated genes known so far in AML.

In an additional 10% of all AML, mutations are found in the transcription factors CEBPA and AML1, which play an important role in hematopoiesis. Furthermore, 30% of all AML and 55% of AML with normal karyotype carry NPM1 mutations. Mutations of both CEBPA and NPM1 are considered prognostically favorable.

Mutation	Most frequent subtypes	Frequency (total)	Prognosis
MLL-PTD	Normal karyotype (11%)		
trisomy 11 (20–50%)	6.5%	Unfavorable	
FLT3-LM	Normal karyotype (40%)		
t(15;17) (35%)	23%	Unfavorable	
FLT3-TKD	All AML	6.5–7%	Dependent on additional defects
KITD816	t(8;21) (12%)	1.5%	Unfavorable
KITexon8	inv(16) (10%)	<1%	Unfavorable
NRAS	inv(16) (45%)		
inv(3)/t(3;3)	10%	Intermediate	

(*continued*)

Mutation	Most frequent subtypes	Frequency (total)	Prognosis
KRAS	inv(16); t(8;21) (5–20%)		
(in childhood AML)	<1%		
AML1	M0 (22%), trisomy 21 (30%), trisomy 13 (80%)	5%	Unfavorable
CEBPA	Normal karyotype (18%)	10%	Favorable
NPM1	Normal karyotype (55%)	30%	Favorable

Some point mutations like those of KIT and RAS are not specific for AML; however, they contribute significantly to leukemogenesis, in particular in cooperation with AML1-ETO and CBFB-MYH11. Accordingly, a two-hit hypothesis has been suggested for leukemogenesis. Mutations of tyrosine kinase genes like ABL, FLT3, and KIT as well as RAS mutations are designated type I mutations, which lead to an increased proliferation of hematopoietic cells. Fusion genes and mutations of transcription factors are designated type II mutations, which lead to a stop in differentiation. Only the cooperation of both types of mutations results in the clinically evident acute leukemia.

A variety of methods are available for the detection of these molecular mutations. Among others these comprise conventional RT-PCR (FLT3-LM, MLL-PTD), (FLT3-LM, NPM1), ▶ melting curve analysis (FLT3-TKD, NPM1, NRAS, KITD816), dHPLC/WAVE (FLT3, AML1, CEBPA, NPM1, KIT), and consequently sequence analysis (for all mutations).

In addition to the prognostic relevance in aml, these molecular markers may be used as targets for the PCR-based detection of minimal residual disease.

Monitoring of Minimal Residual Disease (MRD)

The detection of minimal residual disease using PCR-based methods is feasible for all markers with a sensitivity between 1:100 and 1:1,000. The methods mainly applied are conventional and nested RT-PCR, fragment analysis, and WAVE analysis.

An even higher sensitivity (1:10,000–1:1,000,000) is achieved by "real-time PCR." This method is established for the fusion transcripts: AML1-ETO (applicable in 7–10% of all AML cases), CBFB-MYH11 (7–10%), PML-RARA (7–10%), DEK-CAN (1%), different MLL translocations (5%), MLL-PTD (6%), and NPM1 mutations Type A, B, and D (25%). Furthermore, it is possible to build patient-specific real-time PCR assays for the FLT3-LM (23%) and rare NPM1 mutation types (5%).

Diagnostic Workup of ALL

Cytomorphology

Cytomorphology and cytochemistry are used to diagnose bone marrow blasts negative for myeloperoxidase and nonspecific esterase in ALL; however, the exact diagnosis and further subclassification rely on immunophenotyping. The exception is the typical L3-morphology of blast cells in Burkitt's lymphoma and mature B-ALL.

Immunophenotyping

Acute lymphoblastic leukemias (ALL) are grouped into B-precursor- and T-precursor-leukemias, based on the immunophenotype. They are further subdivided according to the degree of maturation of the leukemic blasts into Pro-B-ALL, common-ALL, Pre-B-ALL, and mature B-ALL, and into Pro-T-ALL, Pre-T-ALL, cortical T-ALL, and mature T-ALL, respectively. In general, in case of the respective morphologic findings (negativity for MPO) a B-precursor-ALL is diagnosed if both cCD22 and CD19 are expressed. A T-precursor-ALL is present if c/sCD3 and CD7 are expressed. The definition of ALL with aberrant expression of myeloid antigens as a separate entity is not preferred since these findings in most cases are associated with genetically defined and clinically relevant aberrations. Table 5 provides the antigen expression patterns of the respective entities.

Monitoring of Minimal Residual Disease (MRD)

In T-precursor-ALL the coexpression of cCD3 and TdT is mainly useful as LAIP. In B-precursor-ALL it is the coexpression of CD19 and CD10. Furthermore, in many cases the aberrant coexpression of myeloid antigens as well as the expression of CD34 can be used.

The first area in which the significant prognostic impact of immunologically determined MRD levels has been demonstrated has been after the achievement of complete remission in childhood ALL: the detection of residual leukemic cells was associated with a significantly increased risk of relapse. This association was independent of other prognostic parameters. Further analyses have demonstrated that as early as on day 19 of the induction therapy MRD levels are equally significant with regard to prognosis.

Leukemia Diagnostics. Table 4 Translocations and respective fusion genes in AML

Cytogenetics	Fusion gene	Subtype	Frequency	
			Children	Adults
t(8;21)(q22;q22)	*AML1-ETO*	M2/(M1)	10–15%	8–12%
inv(16)(p13q22)	*CBFβ-MYH11*	M4eo	6–12%	8–12%
t(15;17)(q22;q12)	*PML-RARA*	M3/M3v	8–15%	8–10%
t(6;11)(q27;q23)	*MLL-AF6*	M4/M5a	2–5%	<1%
t(9;11)(p22;q23)	*MLL-AF9*	M5a	8–10%	1–2%
t(10;11)(p13;q23)	*MLL-F10*	M5a	<1%	1–2%
t(11;19)(q23;p13)	*MLL-ENL*	M5a	<1%	<1%
t(11;19)(q23;p13)	*MLL-ELL*	M5a	<1%	<1%
t(3;21)(q26;q22)	*AML1-EVI1*	–	1%	<1%
t(6;9)(p23;q34)	*DEK-CAN*	M1/M2/M4	1–2%	<1%
t(8;16)(p11;p13)	*MOZ-CBP*	M4/M5	<1%	<1%
t(1;22)(p13;q13)	*OTT-MAL*	M7	2%	–
t(7;11)(p15;p15)	*HOXA9-NUP98*	M2	–	<1%
t(10;11)(p13;q14)	*CALM-AF10*	M0/M1/M5	–	<1%
t(16;21)(p11;q22)	*FUS-ERG*		–	<1%

Leukemia Diagnostics. Table 5 Classification of ALL

	B-precursor-ALL				T-precursor-ALL			
Antigen	Pro-B-ALL	c-ALL	Pre-B-ALL	Mature B-ALL	Pro-T-ALL	Pre-T-ALL	Cortical T-ALL	Mature T-ALL
cCD22	+	+	+	+	–	–	–	–
CD79α	+	+	+	+	–	–	–	–
CD19	+	+	+	+	–	–	–	–
CD24	+/–	+	+	+	–	–	–	–
CD20	–/(+)	+/–	+/–	+	–	–	–	–
sIg	–	–	–	+	–	–	–	–
cIgM	–	–	+	+	–	–	–	–
cCD3	–	–	–	–	+	+	+/–	–
sCD3	–	–	–	–	–	–	–/+	+
CD7	–	–	–	–	+	+	+	+
CD5	–	–	–	–	–	+/–	+	+
CD2	–	–	–	–	–	+/–	+	+
CD1a	–	–	–	–	–	–	+	–
CD4	–	–	–	–	–	–/+	+/–	+/–
CD8	–	–	–	–	–	–/+	+/–	+/–
CD10	–	+	+/–	+/–	–/+	–/+	–/+	–
HLA – DR	+	+	+	+	–/+	–/+	–	–
CD34	+	+	+	–/+	–/+	–/+	–	–
TdT	+	+	+	–/+	+	+	+	+/(–)

c cytoplasmic, *s* membrane

These results have been reproduced in adult ALL. Thus, patients with low-level MRD after completion of induction therapy have a longer relapse-free survival. Also in this context, the impact of MRD was independent of other prognostic parameters.

Multiparameter flow cytometry is, compared to molecular techniques, feasible for the quantification of MRD in virtually all patients with ALL. Current trials will define the respective roles of both methods, based on direct comparisons.

Cytogenetics

A large number of chromosomal aberrations with prognostic and therapeutic relevance have been described. On the one hand, ALL are subdivided into ploidy groups based on the karyotype, i.e., according to the number of chromosomes (Table 6). On the other hand, they are grouped according to structural aberrations (Table 7). The most frequent aberration in adult ALL is the t(9;22)(q34;q11) which is associated with a very unfavorable prognosis. With the availability of the tyrosine kinase inhibitor, imatinib, the detection of this Philadelphia-translocation and of its molecular genetic correlate, the BCR-ABL rearrangement, is even more important as treatment with this specific drug improves outcome. In addition, the detection of translocations involving the MLL-gene, which is located on the long arm of chromosome 11, is prognostically important and requires specific therapeutic approaches.

Cases with a t(8;14)(q24;q32), a t(2;8)(p12;q24), or a t(8;22)(q24;q11) – all of which come along with rearrangements of the CMYC gene – have a high chance of cure in case of the application of a specific therapeutic protocol which significantly differs from protocols applied in cases with other ALL subtypes.

Leukemia Diagnostics. Table 6 Frequencies of groups according to ploidy in adult ALL

Group	Frequency (%)
Normal karyotype	26–34
Hypodiploidy <46	2–8
Pseudodiploidy	7–59
Hyperdiploidy 47–50	7–17
Hyperdiploidy > 50	4–9
Nearly triploid	3
Nearly tetraploid	2

Leukemia Diagnostics. Table 7 Frequent chromosomal aberrations in adult ALL

Aberration		Gene	Frequency
t(1;19)(q23;p13)	Pre-B-ALL	E2A-PBX1	3%
t(4;11)(q21;q23)	Pro-B-ALL	MLL-AF4	6%
t(9;22)(q34;q11)	c-ALL	BCR-ABL	25–30%
t(8;14)(q24;q32)	Mature B-ALL	IGH-MYC	5%
t(10;14)(q24;q11)	T-ALL	HOX11-TCR	3%
t(12;21)(p13;q22)	Pre-B-ALL	ETV6-ALL1	<1%
9p	T, Pre-B	p16INK4A	15%
6q	c, Pre-B,T		6%
14q11	T-ALL	TCR	6%

Fluorescent In Situ Hybridization

In the diagnostic setting of ALL FISH analysis is used mainly in addition to the classic chromosome analysis. Since multiple karyotype abnormalities occur in ALL, even an extensive FISH "screening" using interphase nuclei would detect only a part of these abnormalities. Therefore, the chromosome analysis cannot be replaced by FISH.

However, in case of focusing on a specific aberration, the FISH technique on interphase nuclei represents a fast and reliable method, giving the result within 24 h. The genetic aberrations occurring frequently in ALL, i.e., the Philadelphia-translocation t(9;22)(q34;q11) (rearrangement of BCR and ABL on the molecular level), an MLL-rearrangement, or a CMYC-rearrangement, all of which have therapeutic implications, can be identified using FISH on interphase nuclei.

A FISH-screening targeting the most frequent aberrations is useful in particular in cases with no valid result of the chromosome analysis. This should be considered also in cases with a normal karyotype with the selection of probes according to the immunophenotype.

The so-called "chromosome painting" with 1–3 or 24 colors (24-color-FISH) on metaphase-chromosomes is applied in addition to the classic chromosome analysis if the karyotype cannot be fully described after the classic banding approach, e.g., in complex aberrant karyotypes.

Furthermore, the FISH method can be used for the detection of residual disease during the course of therapy. It is more sensitive and more specific than cytomorphology; however, it is less sensitive than real-time PCR and immunophenotyping. The FISH method is used for the detection of residual disease mainly in ALL with unbalanced karyotypes as these cases lack genetic alteration detectable by PCR.

Molecular Genetics

Two types of genetic alterations are present in lymphatic leukemias and lymphomas: Somatic mutations which are involved in the pathogenesis and rearrangements of immunoglobulin genes, and T-cell receptor genes as markers of clonality.

Leukemia Diagnostics. Table 8 Fusion genes in ALL

Cytogenetics	Fusion gene	Subtype	Frequency	
			Childhood	Adult
t(1;19)(q23;p13)	*E2A-PBX1*	Pre-B-ALL	5–6%	3%
t(4;11)(q21;q23)	*MLL-AF4*	Pro-B-ALL	2%	6%
t(11;19)(q23;p13)	*MLL-ENL*	Pro-B-ALL Pre-T	<1%	<1%
t(9;22)(q34;q11)	*BCR-ABL*	c-ALL Pre-B	2–5%	25–30%
t(8;14)(q24;q32)	*MYC-IGH*	Mature B-ALL	3%	5%
t(10;14)(q24;q11)	*HOX11-TCR*	T-ALL	1%	3%
t(12;21)(p13;q22)	*ETV6-AML1*	c-ALL	10–20%	<1%

Leukemia Diagnostics. Table 9 MYC-rearragements in ALL

Cytogenetics	Fusion gene	Subtype	Frequency	
			Childhood	Adult
t(2;8)(p12;q24)	*IGK-MYC*	B-ALL	<1%	<1%
t(8;14)(q24;q23)	*IGH-MYC*	B-ALL	3%	2–4%
t(8;22)(q24;q11)	*IGL-MYC*	B-ALL	<1%	<1%

The molecular diagnostic approach in ALL primarily focuses on the detection of high-risk cases with t(9;22) and t(4;11) in which an RT-PCR for BCR-ABL and MLL-AF4 is performed as a substitute or supplement to cytogenetics and FISH.

In contrast, the ETV6-AML1-rearrangement, which occurs frequently in childhood ALL, and is associated with a favorable prognosis, can be identified exclusively on the molecular level and/or using FISH.

Activation of the MYC gene due to various Ig rearrangements (Tables 8 and 9) is found in 4% of adult ALL representing the leukemic form of Burkitt's lymphoma. The molecular detection is accomplished by Southern blot. The use of FISH makes this diagnostic step simpler and faster; however, it requires the smear of intact cells.

Between 4% and 7% of all T-ALL cases feature rearrangements of the HOX11-gene (Tables 10 and 11). HOX11 codes for a transcription factor. The transforming action of overexpressed HOX11 leads to an immortalization of T-cells.

In cases without fusion genes a characterization of clonal Ig- and TCR-gene-rearrangements should be performed. These can be used as markers for residual disease during the course of therapy. Since the latter alterations are identified in DNA while fusion genes are analyzed in RNA it is important to asservate both DNA and RNA at the time of diagnosis.

Diagnostic Workup of CML

Cytomorphology

Cytomorphology typically reveals hypercellularity in both peripheral blood and bone marrow with an increase of immature myeloid cells as well as of eosinophils and basophils.

Immunophenotyping

MFC is applied in CML only in case of blast crisis in order to delineate the cell lineage, i.e., lymphatic or myeloid.

Cytogenetics

In 90–95% of all patients, a Philadelphia translocation is present on the cytogenetic level: t(9;22)(q34;q11). This is a translocation between the long arm of a chromosome 9 and a long arm of a chromosome 22. The remaining patients carry so-called variant Philadelphia translocations or a normal chromosome set. The variant Philadelphia translocations are divided into simple ones, in which the long arm of chromosome 22 is translocated to another chromosome (and not to chromosome 9), and complex ones, in which further chromosome are involved besides chromosomes 9 and 22. Patients with CML and a normal karyotype carry a BCR-ABL-rearrangement detectable by both FISH and RT-PCR. By applying FISH and using metaphases the BCR-ABL-fusion gene is detected either on chromosome 22 or, less frequently, on chromosome 9. Submicroscopic insertions are discussed as underlying mechanisms which are not detectable by classic cytogenetics. This group of CML is designated Philadelphia-negative BCR-ABL-positive CML. The clinical and hematologic course of patients with classic and variant Philadelphia-translocation does not differ from the course in patients with Philadelphia-negative BCR-ABL-positive CML.

Leukemia Diagnostics. Table 10 T-cell receptor (TCR)-B-rearragements in ALL

Cytogenetics	Fusion gene	Subtype	Frequency Childhood	Adult
t(1;7)(p32;q35)	*TAL1-TCRβ*	T-ALL	<1%	<1%
t(1;7)(p34;q35)	*LCK- TCRβ*	T-ALL	<1%	<1%
t(7;9)(q35)(q32)	*TCRβ – TAL2*	T-ALL	<1%	<1%
t(7;9)(q35;q34)	*TCRβ – TAN1*	T-ALL	<1%	<1%
t(7;10)(q35;q24)	*TCRβ – HOX11*	T-ALL	<1%	<1%
t(7;11)(q35;p13)	*TCRβ – RHOM2*	T-ALL	<1%	<1%

Leukemia Diagnostics. Table 11 T-cell receptor (TCR)-α- and -δ-rearragements in ALL

Cytogenetics	Fusion gene	Subtype	Frequency Childhood	Adult
t(1;14)(p23;q11)	*TAL1-TCRδ*	T-ALL	<1%	<1%
t(8;14)(q24;q11)	*MYC-TCRα*	T-ALL	<1%	<1%
t(10;14)(q24;q11)	*HOX11-TCRδ*	T-ALL	<1%	1–3%
t(11;14)(p13;q11)	*RHOM2-TCRδ*	T-ALL	<1%	2–3%
t(11;14)(p15;q11)	*RHOM1-TCRδ*	T-ALL	<1%	<1%
inv(14)(q11q32.1)	*TCRα – TCL1*	T-ALL	<1%	<1%
inv(14)(q11q32.3)	*TCRα – IGH*	T-ALL	<1%	<1%

During the progression into the accelerated phase or into blast crisis in 75–85% of the patients, additional karyotype aberrations occur. The most frequently occurring aberrations (so-called clonal evolution) are trisomy 8 (+8), isochromosome of the long arm of chromosome 17 (i(17)(q10)), an additional Philadelphia-chromosome (+der(22)t(9;22)), as well as trisomy 19 (+19). The occurrence of additional karyotype aberrations is a prognostically unfavorable parameter and precedes the clinical manifestation of a blast crisis by 2–6 months in most cases.

In CML follow-up, assessments during therapy are prognostically most important with consequences for the further management of the disease. The classic chromosome analysis represents the gold standard for the assessment of a cytogenetic remission. At least 20 metaphases should be analyzed to obtain a valid result. A complete cytogenetic remission (no Ph + metaphases) is differentiated from a "partial" remission (1–32% Ph + metaphases), a "minor" remission (33–66% Ph + metaphases), a "minimal" remission (67–95% Ph + metaphases), and no remission (≥95% Ph + metaphases). The complete and the partial remission are grouped together as "major" remission.

During therapy with imatinib, the occurrence of karyotype aberrations has been observed in some patients in clones lacking a Philadelphia translocation. Most frequently this aberration is the trisomy 8. Further aberrations are monosomy 7 as well as different translocations. The clinical impact of this phenomenon is yet unclear and is being evaluated in clinical trials.

Fluorescence In Situ Hybridization

Using FISH on interphase nuclei, the presence of a BCR-ABL rearrangement can be detected or ruled out within 24 h. The FISH analysis has some advantages as compared to the classic chromosome analysis: Using this method a CML can be diagnosed and monitored even in patients with Philadelphia-negative BCR-ABL-positive CML. Furthermore, it is more sensitive compared to the classic chromosome analysis in detecting residual disease. FISH may be applied on interphase nuclei but also on metaphases. Using the so-called "Hypermetaphase-FISH"-technique, up to 500 metaphases may be assessed for the presence of a BCR-ABL rearrangement, while only 20–25 metaphases are analyzed by the classic chromosome analysis. However, additional cytogenetic aberrations and Ph-negative clones cannot be detected using FISH alone and require conventional chromosome banding analysis.

Molecular Genetics

The molecular correlate of the Philadelphia translocation is the BCR-ABL fusion gene. The diagnosis of

a CML can be made only if the Philadelphia translocation is detected by cytogenetics or by FISH or if the BCR-ABL rearrangement is detected by RT-PCR. Since 5% of all CML cases carry a cryptic BCR-ABL rearrangement that is not detectable cytogenetically, a FISH and/or PCR assessment should be done in parallel to cytogenetics at diagnosis. Furthermore, a PCR-based quantification of the BCR-ABL expression provides a starting value for follow-up assessments.

The PCR-based monitoring of BCR-ABL during therapy is a standard procedure in CML. Independent of the therapy applied, a response to therapy is most effectively assessed by real-time PCR with regard to both quantity of response and sensitivity. In contrast to most other leukemias, follow-up assessment in CML is possible using peripheral blood. It is recommended to do a peripheral blood evaluation every 3 months.

In patients under imatinib therapy, an association between an increase of the BCR-ABL expression and the occurrence of a resistance against imatinib has been described. In 2–5% of all CML cases, a primary resistance against imatinib is present. Many of these resistances are due to point mutations of the ABL gene within the BCR-ABL fusion gene. Other patients acquire secondary mutations in the tyrosine kinase loop of the BCR-ABL fusion protein during therapy with imatinib, which lead to a resistance against the drug. In some cases this resistance can be superseded by an increase of the dose of imatinib or by the combination with IFN-α. Therefore, the early detection of these resistances increasingly carries clinical importance. Mutations have been described in 23 different codons so far. Some of these lead to changes in conformation, while others directly inhibit the binding of imatinib to the activating domain of the tyrosine kinase ABL. The type of mutation therefore provides information on the usefulness of an increase of the imatinib dose. The point mutations can be detected by sequencing the ABL part of the fusion gene.

Diagnostic Workup of CLL

Cytomorphology

The diagnosis of CLL per definition requires a lymphocytosis >5,000/μl, although a chronic elevation of the absolute lymphocyte count in the peripheral blood and the presence of both the typical morphologic findings and the typical immunophenotype are increasingly considered diagnostic. Cytomorphologically, CLL cells are small lymphocytes with a narrow cytoplasm and a dense nucleus without nucleoli. Furthermore, shadow cells of Gumprecht are present.

Leukemia Diagnostics. Table 12 Matutes-score of B-CLL

Characteristics of B-CLL
CD5+
CD23+
FMC7−
sIgM(+)
sCD22(+) or CD79b(+)

Immunophenotyping

CLL features a typical immunophenotype with a coexpression of CD5 and the B-cell-associated antigens CD19, CD20, and cCD79a. A weak surface expression of CD22 and Ig is present, with a clonal light chain restriction. The positivity of CD23 which is commonly present in CLL allows the distinction from mantle cell lymphoma. The latter is further characterized by a strong surface expression of Ig as well as by an expression of CD22 and FMC7. Overall, no single marker allows the diagnosis of CLL. Rather, the comprehensive evaluation of all the antigens assessed and the application of the Matutes-score have been proved useful (Table 12).

The presence of four or five of the findings characteristic for B-CLL was found in a large series in 87% of all B-CLL and in only 0.3% of other B-cell lymphomas. Conversely, only 0.4% of B-CLL and 72% of other B-cell lymphomas had none or only one of these criteria.

The expression of CD38, as well as the cytoplasmic expression of ZAP70, is prognostically important. Both findings are associated with an inferior prognosis.

If CLL/PL is diagnosed by cytomorphology (10–55% prolymphocytes in peripheral blood) often accompanied by a trisomy 12, which occurs more frequently in these cases, the immunophenotype may show a stronger expression of CD20 and of sIg as compared to CLL. Furthermore, there is a weaker expression of CD23 as well as a positivity for CD22 and CD79b.

In contrast to B-CLL, the co-expression of CD5 is not, or only weakly, present in B-PLL (>55% prolymphocytes in peripheral blood), the surface expression of Ig and of CD20 is stronger and CD22 and FMC7 are expressed.

Besides diagnosis, immunophenotyping allows the quantification of minimal residual disease (MRD). The CLL cells typically carry the phenotype CD19 + CD20 + CD79-CD5+ and thereby differ from normal B-lymphocytes. The prognostic relevance of the MRD-level, which can be assessed with high sensitivity, has been demonstrated in multiple studies.

Cytogenetics

The classic chromosome analysis has played only a minor role in the past due to the difficulties in cultivating the cells in vitro. By using FISH analysis, the strong prognostic impact of chromosome aberrations could be demonstrated. Recently, a reliable cultivation of CLL cells has been shown to be feasible and chromosome aberrations have been detected by chromosome analysis at higher frequencies compared to FISH analysis.

Fluorescence In Situ Hybridization

FISH analysis detects the most frequent chromosomal aberrations in CLL in a targeted way. These comprise 13q deletions, trisomy 12, 11q deletion, and 17p deletion. The presence of a 17p deletion or of a 11q deletion indicates a more aggressive course of disease compared to a normal karyotype, while the sole presence of a 13q deletion confers a favorable prognosis. Furthermore, the assessment of t(11;14) is useful for the distinction between CLL and mantle cell lymphoma.

Molecular Genetics

Half of the patients with CLL carry so-called somatic mutations in the variable region of immunoglobulins. The presence of 2% or less mutations in this area of the immunoglobulins as compared to the original DNA sequence is designated "unmutated" while the presence of more than 2% mutations is designated "mutated." The unmutated status is associated with an unfavorable prognosis even in early stages of the disease.

Diagnostic Workup of MDS

Cytomorphology

During the cytomorphologic examination at least 200 bone marrow cells and 20 megakaryocytes should be evaluated. Dysplastic findings should be present in at least 10% of the cells. A particular diagnostic role is played by the so-called pseudo-Pelger neutrophils, ringed sideroblasts, mikromegakaryocytes, and augmented blasts. These morphologic aberrations correlate in part with clonal markers and show a low inter-observer variability. This is true particularly for the prognostically favorable and therefore clinically relevant 5q-syndrome. The assessment of hypogranulation in neutrophils should not be the only diagnostic criterion for dysplasia. Accordingly, an early stage of refractory anemia (RA) with cytopenia in only one lineage is often difficult to diagnose and requires the assessment of follow-up samples. With regard to the differentiation between hypoplastic MDS and aplastic anemia, it is important to notice that dysplastic findings in erythropoiesis may be present also in the latter. They therefore play no diagnostic role in this instance, unlike dysplastic findings in the other lineages and augmented bone marrow blasts. PNH should be considered as differential diagnosis. With regard to the separation of MDS and AML a cut-off of 20% bone marrow blasts has to be used according to the presently applied WHO classification.

Immunophenotyping

Multiparameter flow cytometry allows the qualitative assessment of dysplasia in the different cell lineages, granulopoiesis, monopoiesis, and erythropoiesis through the detection of aberrant antigen expression patterns. Furthermore, the quantification of blasts can be done with a high correlation to cytomorphologic findings. The flow cytometric findings are of particular diagnostic value in cases difficult to judge by cytomorphology. Further studies will define the role of multiparameter flow cytometry in comparison to cytomorphology and cytogenetics with regard to both diagnostics and prognostication.

Cytogenetics

In the context of diagnostic assessment of MDS the chromosome analysis plays a significant role by detecting karyotype aberrations typical for MDS and particularly so in cases difficult to judge by cytomorphology.

The typical aberrations, which are also considered for the prognostically highly relevant IPSS (Tables 13 and 14), include loss of Y chromosome, del(5q), del (20q), as well as complex aberrations and aberrations of chromosome 7. In cytomorphologically defined borderline cases a distinction between AML and MDS can

Leukemia Diagnostics. Table 13 IPSS, basis

	Points				
	0	0.5	1	1.5	2
% Bone marrow blasts	5	5–10		11–20	21–30
Karyotype	Favorable	Intermediate	Unfavorable		
Cytopenias	0/1	2/3			

Karyotype favorable: normal, -Y sole, del(5q) sole, del(20q)

Karyotype unfavorable: complex aberrant (≥3 aberrations), aberrations of chromosome 7

Leukemia Diagnostics. Table 14 IPSS, prognostic grouping

Points	0	0.5–1.0	1.5–2.0	≥2.5
Risk group	Low	Int-1	Int-2	High

be accomplished by the detection of t(8;21)(q22;q22), t(15;17)(q22;q11-12), or inv(16)(p13q22)/t(16;16)(p13;q22) which define an AML, respectively.

Fluorescence In Situ Hybridization

FISH analysis may be used in case of lack of adequate material for cytogenetics, e.g., in case of a dry tap, smears may be obtained from a bone marrow biopsy and analyzed by FISH. The analysis includes probes targeting the aberrations most relevant for determining the prognosis: -Y, del(5q), del(20q), aberrations of chromosome 7, del(17p).

Molecular Genetics

In contrast to AML there are no specific molecular markers for MDS. In case of rare reciprocal translocations, fusion gene–specific PCRs can be applied which, however, do not play a major role in routine diagnosis. Some of the AML-specific mutations like the partial tandem duplication of MLL (MLL-PTD), FLT3 length mutations (FLT3-LM), RAS mutations, as well as mutations of AML1 and CEBPA may be present in MDS with high blast counts. They are indicative of a progress of MDS to AML. Similarly, an increasing expression of WT1 represents a marker for the progress of MDS.

Diagnostic Algorithms in Leukemia Diagnostics

Based on the data provided above, algorithms for the diagnostic workup of leukemias have been proposed. These are outlined below and should be applied and evaluated in the context of large clinical trials. Leukemia diagnosis has undergone steady development and is expected to become even more refined within the next few years, particularly taking into consideration gene expression profiling with microarrays, which may be incorporated.

Diagnostic Algorithm in AML

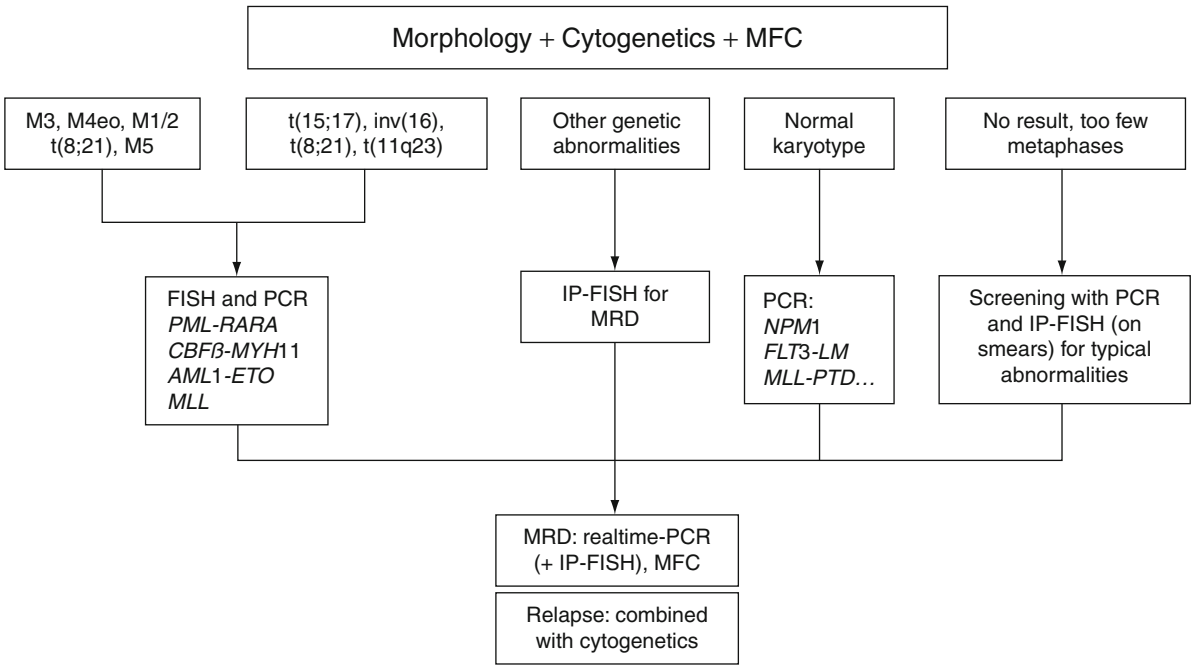
In AML (Fig. 1) a combination of cytomorphology, cytochemistry, immunophenotyping, and cytogenetics should be applied in the first step. Results of cytomorphology and cytochemistry guide the selection of antibody panels to be used for immunophenotyping. Results of these three methods guide the selection of culture conditions for cytogenetics.

In case of specific cytomorphologic findings, the respective genetic alterations should be analyzed by FISH and PCR (AML M3/M3v: t(15;17)/*PML-RARA*; AML M4eo: inv(16)/*CBFB-MYH11*; AML M1/2 with characteristic long Auer rods: t(8;21)/*AML1-ETO*; AML M5a: 11q23/*MLL* rearrangements). The combined analysis of genetic alterations by both FISH and PCR provides a maximized diagnostic security as well as information on variant translocations or submicroscopic deletions, which are only detectable by interphase FISH.

Based on cytogenetic results, FISH analysis using specific probes is applied for numerical (e.g., +8, −7) and structural aberrations (e.g., 5q-, 7q-). Cases with complex aberrant karyotype can further be investigated by 24-color FISH. FISH may be used in infrequent cases without cytogenetic result to identify the most frequent and prognostically relevant aberrations.

RT-PCR is used, according to cytogenetic results, for the detection of fusion genes as well as for the analysis of *FLT*3-LM, *MLL*-PTD, or *NPM*1.

Follow-up assessment during complete remission should be performed by cytomorphology, FISH, PCR, and MFC, whenever a specific marker has been identified at diagnosis. The latter two highly sensitive methods are particularly useful for the quantification of the prognostically important MRD levels.



Leukemia Diagnostics. Fig.1 Algorithm for diagnosis and follow-up in AML

Diagnostic Algorithm in ALL

Cytomorphology and immunophenotyping should be applied in the first step in ALL (Fig. 2).Cytomorphology identifies acute leukemia negative for peroxidase and reveals cases suspicious for Burkitt's lymphoma/mature B-ALL. MFC allows the diagnosis of ALL as well as the subclassification into different B-precursor ALL and T-precursor ALL classes.

Cytogenetics follows the results of MFC with specific culture conditions for B- and T-precursor ALL.

FISH analysis for BCR-ABL and MLL rearrangements follows MFC in case of B-precursor ALL.

FISH analysis for CMYC rearrangements follows cytomorphology in case of findings typical for Burkitt's lymphoma as well as MFC in case of mature B-ALL.

In childhood B-precursor ALL, FISH analysis for ETV6-AML1 is applied.

In case of cytogenetics not yielding a result, FISH and PCR are applied for the detection of BCR-ABL and MLL rearrangements.

Real-time PCR is applied for MRD monitoring, targeting either fusion transcripts or IgH receptor-/T-cell receptor rearrangements. MFC is also used for MRD monitoring.

A complete reevaluation should be performed at relapse.

Diagnostic Algorithm in CML

The diagnosis of CML is made by a combined approach of cytomorpholgy, cytogenetics, FISH, and PCR, identifying characteristic peripheral blood and bone marrow features as well as the t(9;22) and the BCR-ABL fusion gene (Fig. 3).

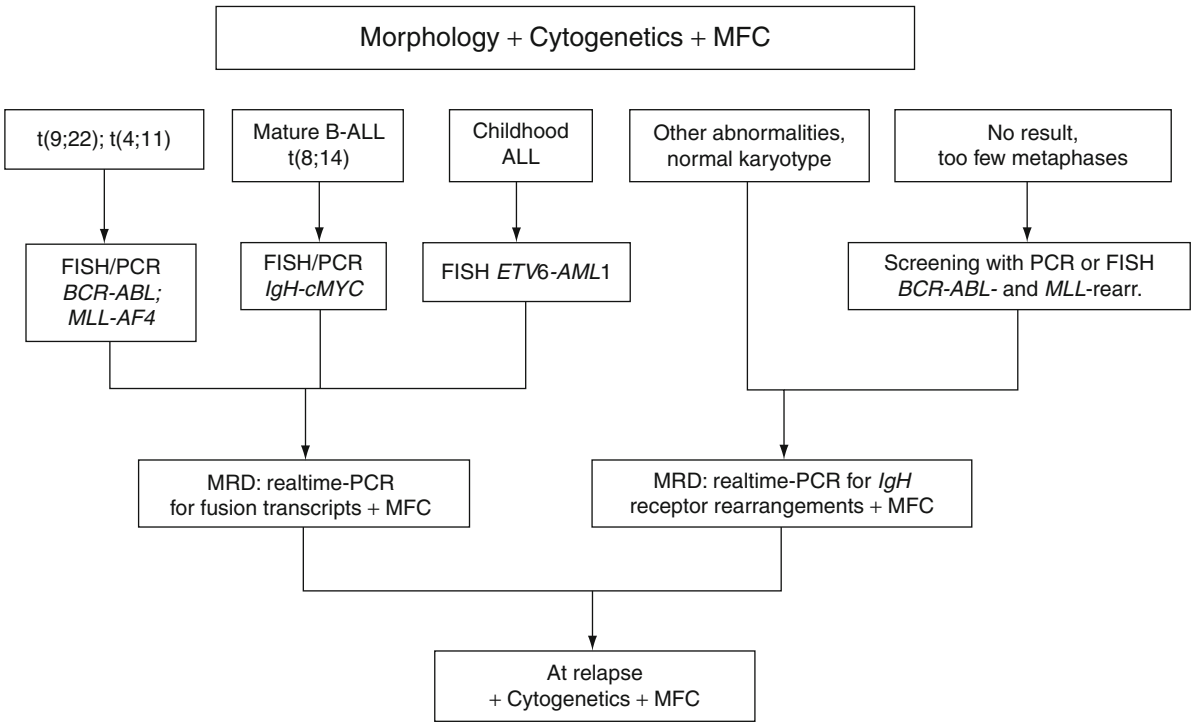
During therapy all methods are applied in combination to quantify the amount of residual disease as well as additional chromosomal abnormalities.

In case of failure or suboptimal response, an analysis for BCR-ABL mutations conferring resistance to imatinib should be performed.

Immunophenotyping should be performed in blast crisis to delineate the lineage (lymphatic or myeloid).

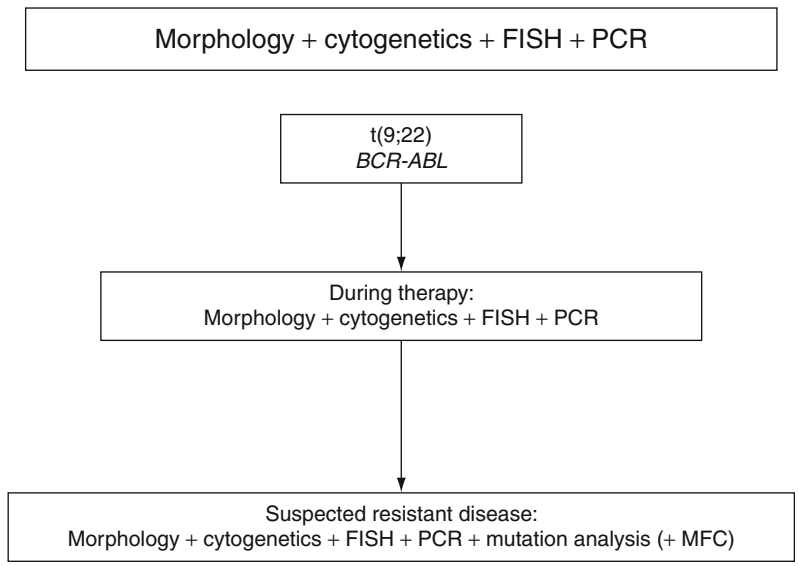
Diagnostic Algorithm in CLL

Cytomorphology and immunophenotyping should be applied in the first step in CLL (Fig. 4). Cytomorphology identifies mature lymphocytosis. MFC allows the diagnosis of CLL and its discrimination from other indolent lymphomas according to the Matutes score.



Leukemia Diagnostics. Fig. 2 Algorithm for diagnosis and follow-up in ALL

Leukemia Diagnostics. Fig. 3 Algorithm for diagnosis and follow-up in CML

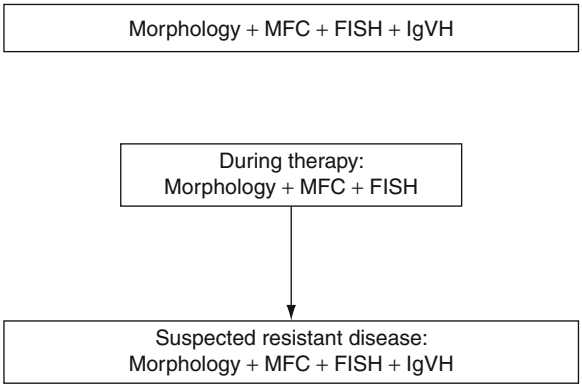


In addition, MFC is used for the determination of the expression of both CD38 and ZAP-70.

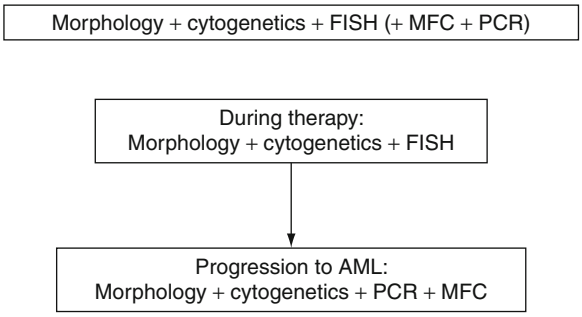
FISH analysis is performed to identify the most common and prognostically important chromosomal abnormalities, i.e., 6q-, 11q-, trisomy 12, 13q-, and 17p- as well as t(11;14). A cytogenetic analysis is capable of identifying additional chromosomal abnormalities which may yield further prognostic information.

Molecular genetics is applied to determine the IgVH mutational status.

Monitoring during therapy is performed by a combination of cytomorpholgy, MFC, and FISH.



Leukemia Diagnostics. Fig. 4 Algorithm for diagnosis and follow-up in CLL



Leukemia Diagnostics. Fig. 5 Algorithm for diagnosis and follow-up in MDS

Diagnostic Algorithm in MDS

The diagnosis of an MDS is based, besides the presence of cytopenias, on cytomorphology (Fig. 5). Cytogenetics is used to identify chromosomal abnormalities which subclassify MDS and yield prognostic information. In cases of equivocal findings in cytomorphology cytogenetics can assure the diagnosis. Immunophenotyping may be used to identify aberrant antigen expression patterns typical for MDS. PCR may be performed in MDS.

During the course of therapy, which may be limited to supportive measures, cytomorphology in combination with cytogenetics and FISH should be applied.

In case of progression to AML, cytomorpholgy, cytogenetics, PCR, and MFC should be applied.

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Leukemia Inhibitory Factor

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Synonyms

Cachexia-inducing agent; CNDF; D-factor; DIA; DRF; Hepatocyte stimulating factor-III; HILDA; HSF-III; LIF; MLPLI

Definition

Leukemia Inhibitory Factor (LIF) is a cytokine belonging to a family of ▶ interleukin-6 related cytokines that signal through gp130 protein coupled receptors and whose inducible production (e.g., due to hypoxia) can occur in ▶ hypoxia-inducible factor-1 manner in multiple tissues. LIF is a pleiotropic cytokine that affects several biological processes, and its current name implicating "a leukemia inhibitory factor" has merely historical meaning.

Characteristics

LIF

LIF was purified from Krebs-II ascites tumor cell line and then cloned from a murine T-lymphocyte cDNA

library and originally identified as a factor that inhibits the proliferation of undifferentiated and highly clonogenic murine myeloid leukemic cell line M1 and induces its differentiation into macrophages. At the molecular level human and murine LIF is a glycoprotein with a 180-amino acid single 4-α-helix polypeptide chain with a conserved disulfide bond. Natural occurring LIF is heavily glycosylated, showing an apparent molecular weight of 32–62 kDa, depending on the source. It is accepted that the absence of glycosylation appears not to affect its biological activity. Human and mouse LIF share 78% sequence homology. Human LIF can activate mouse cells, but vice versa mouse LIF cannot activate human cells.

LIF-Receptor

LIF interacts with the heterodimeric human LIF-receptor (LIF-R) comprising a 190 kDa LIF-binding α-chain (130 kDa mouse) and a 130 kDa signal-transducing β-chain (gp130). LIF receptors have been identified on several cells, including monocytes, liver, neural cells, muscle cells, primordial germ cells, placenta, and embryonic stem cells. Therefore LIF-R is a member of the gp130 superfamily of receptors. Accumulated data that the LIF-binding α-chain shows some level of "molecular promiscuity" and can be also part of the type II receptor for ▶ oncostatin M (OSM), and part of the receptors for ciliary neurotropic factor (CNTF) and carditrophin-1 (CT). Thus LIF in addition to LIF-R can also signal for example through the OSM receptor.

LIF Overexpression and LIF Knockouts

Prolonged over expression of mice to LIF leads to ▶ cachexia coupled with a hypermotile state, overgrowth of bone tissue, and osteoblasts in the bone marrow cavities, loss of spermatocytes from the seminiferous tubules of the testis, absence of corpora lutea in the ovaries, a reduced number of Purkinje cells in cerebellum, thymus atrophy, and an involution of the pancreas. Mice with LIF knocked-out ($LIF^{-/-}$) are viable; however, they show abnormalities of the hippocampus and an accumulation of olfactory receptor neurons. Interestingly $LIF^{-/-}$ mice are unable to get pregnant, which is explained by a crucial role of secreted LIF in the uterus during the process of blastocyst implantation.

Pleiotropic Effects of LIF

LIF plays an important role in several biological processes in embryogenesis, tissue regeneration, metabolism, and cancer ▶ metastasis. It activates in normal cells Jak-STAT-3, MAPKp42/44, and PI-3K-AKT signaling pathways and in murine embryonic stem cells (ES) LIF also regulates expression of Oct3/4. Biological effects of LIF are controlled by circulating soluble LIF-R that blocks the action of any LIF in circulation. At the cellular level LIF signaling is negatively controlled by ▶ suppressors of cytokine signaling (SOCS).

LIF was identified as a factor that maintains totipotency of murine ES and is crucial for blastocyst implantation in mice. LIF is also one of the factors that promote dedifferentiation of murine primordial germ cells into embryonic germ cells. In humans LIF has no effect on totipotency of human ES cells. However, LIF was recently demonstrated to reduce the vertical transmission of HIV-1 virus though the placenta. Finally, LIF enhances the proliferation of normal primordial germ cells and spermatocyte differentiation.

LIF is playing an important role in the development of hippocampal and olfactory receptor neurons. It stimulates the proliferation of skeletal muscle satellite cells and is a hypertrophic factor for myocardium. Therefore, LIF may play a role in regeneration of neural tissue, myocardium, and skeletal muscle. In support of this LIF is up regulated in damaged brain, heart, and skeletal muscle. It is possible that LIF in cooperation with other factors such as stromal derived factor-1 (SDF-1) and hepatocyte growth factor (HGF)/ ▶ scatter factor recruits circulating stem cells and macrophages to damaged organs.

In the hematopoietic system, LIF shows positive costimulatory effects on megakaryocyte maturation and platelet formation. LIF also stimulates proliferation of the human factor-dependent hematopoietic cell lines TF-1 and DA as well as proliferation of the murine factor-dependent GB2 cell line. LIF together with Flk ligand has been shown to enhance blast colony formation by murine bone marrow cells. These blast colonies are enriched in macrophages and ▶ dendritic cells. LIF receptors on murine hematopoietic populations are mainly restricted to cells of the monocyte lineage. Thus, the potential influence of LIF on the compartment of hematopoietic stem cells requires further study.

LIF also exerts several metabolical effects such as inhibits lipid transport to adipocytes, enhances production of acute-phase proteins by the liver and was also identified as a factor capable of switching autonomic nerve signaling from an adrenergic to a cholinergic mode. LIF also has several endocrine effects being a major regulator of ACTH production in the pituitary and inhibits production of prolactin and growth hormone.

LIF and Oncogenesis

LIF appears to be an important developmental mottomorphogene that displays several pleiotropic biological effects in vitro and in vivo. Recent evidence has accumulated that this interesting cytokine affects survival, proliferation, and differentiation of many cell types not only normal but malignant cells as well. For example while LIF stimulates proliferation or differentiation of several established malignant hematopoietic stem cell lines (TF-1, DA, GB2, or M1), it inhibits in vitro proliferation of ▶ breast cancer cells. LIF also may play some role in tumor vascularization and ▶ angiogenesis.

Recently, based on the observation that LIF stimulates proliferation of skeletal muscle satellite cells and myocytes, as well as cardiomyocytes, we hypothesized that the LIF–LIF-R axis could be involved in progression of human ▶ rhabdomyosarcomas (RMS). We found that in RMS cells LIF stimulates (1) phosphorylation of MAPKp42/44, AKT, and STAT3, (2) ▶ adhesion and chemotaxis, and (3) increased resistance to cytostatics. Thus, we presented evidence for the first time that the LIF–LIF-R axis exerts chemotactic activity and may direct RMS metastasis. The role of this axis in progression of other non-hematopoietic malignancies requires further study.

Clinical Aspects

The LIF–LIF-R axis has emerged as an important axis regulating both the development and homeostasis of an adult organism. The novel recognized role of LIF in trafficking of normal and ▶ cancer stem cells may lead to the development of better therapeutic strategies aimed at directing ▶ migration of normal stem cells to regenerate damaged organs (e.g., heart or brain) but on the other hand inhibiting metastasis of LIF-responsive cancer stem cells. Thus, a new area of investigation on the role of gp130 signaling molecules in cancer metastasis has been established.

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Leukocyte Elastase

▶ Neutrophil Elastase

Leukocyte Endothelial Cell Adhesion Molecule-2

▶ E-Selectin-Mediated Adhesion in Cancer

Leukocyte Functional Antigens

Definition

LFAs are ▶ cell-adhesion molecules initially defined with monoclonal antibodies: LFA-1 is a β_2 integrin; LFA-2 is a member of the immunoglobulin superfamily, as is LFA-3, now called CD58. LFA-1 is particularly important in T-cell adhesion to endothelial cells and antigen-presenting cells.

Leukocyte Homing

Definition

Refers to the process by which leukocytes, or white blood cells, are targeted to the sites at which they are needed. This process involves active recruitment out of the blood stream and into tissues.

▶ CXC Chemokines

Leukocyte Interferon

► Interferon-α

Leukocytes

Definition

White blood cells found in peripheral blood and tissues, in particular the bone marrow and spleen.

► CD Antigens

Leukopenia

► Neutropenia

Leukoplakia

Definition

A white patch on the mucosa that does not rub off and is not attributable to any obvious cause. It is a diagnosis of exclusion and is not a disease entity in itself. It has a relatively low frequency for malignant transformation into squamous cell carcinoma.

► Squamous Cell Carcinoma

Leukotriene Receptors

Definition

Are seven transmembrane G-protein coupled receptors (GPCR). Two receptors for LTB_4, the high affinity receptor BLT1 and the low affinity receptor BLT2 are known. Two distinct receptors for the cysteinyl leukotrienes (CysLTs), the high affinity receptor $CysLT_1$ and the low affinity receptor $CysLT_2$ have been isolated and characterized. There are several antagonists for the $CysLT_1$ receptor, which are used in clinical treatment of asthma including Singulair™ (Monteluskast) and Accolate™ (Zafirlukast), but only one known antagonist for $CysLT_2$ receptor, Bay-U9773. Several antagonists are also known for the BLT_1 and BLT_2, such as U-75302, LY293111, and CP-105696, which block the BLT_1 receptor at low concentration and BLT_2 at higher concentrations.

► Leukotriene

Leukotrienes

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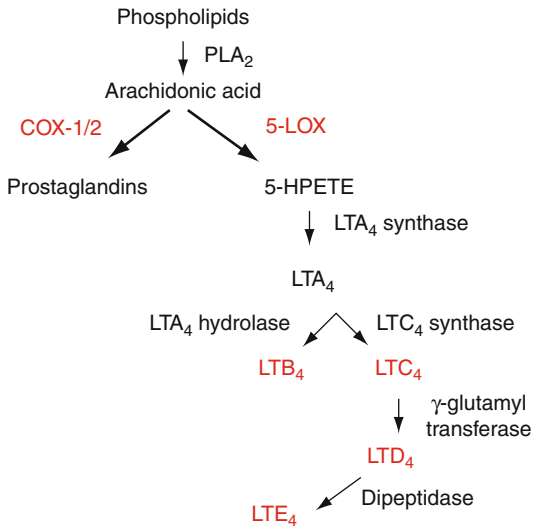
Definition

► Leukotriene, the dihydroxy leukotriene, LTB, is formed via the ► 5-LO pathway through the enzyme leukotriene A4 hydrolase. LTB4 is one of the most chemotactic molecules known. It has a preferential action on leukocytes, producing chemotaxis and chemokinesis of neutrophils and mononuclear cells, as well as aggregation, degranulation, and adherence of leukocytes to endothelial cells. The cysteinyl *leukotrienes*, including *LTC*, LTD, and *LTE*, are also known as the slow-reacting substance of anaphylaxis. CysLTs enhance contraction of smooth muscle and increase vascular permeability as well as migration and chemokine production in monocytic cells. CysLTs are produced in high amounts in activated eosinophils, basophils, macrophages, and mast cells. ► 5-Lipoxygenase (5-LO) catalyzes the biosynthesis of proinflammatory leukotrienes that have both autocrine and paracrine mechanisms and may play an important role in inflammation-induced carcinogenesis. Inflammation is an important component of tumor progression. Many tumors start from the sites of infection and inflammation.

Characteristics

Leukotrienes and ► prostaglandins are metabolites of ► arachidonic acid that play major roles in various



Leukotrienes. Fig. 1 Biosynthetic pathway of prostaglandins and leukotrienes

inflammatory conditions (Fig. 1). The release of these mediators, by cells recruited to or present at the site of inflammation, modulates or influences the magnitude of the inflammatory response. An increased understanding of eicosanoids and how their receptors trigger intracellular signaling during inflammatory conditions is helping to elucidate the well-known connection between chronic inflammatory disease and neoplastic transformation. Here, we summarize the role of leukotrienes in cancer. In addition, we delineate how continuous ▶ leukotriene receptor activation and signaling can increase cell survival and proliferation as important early steps toward oncogenicity.

Today, it is clear that the tumor microenvironment, including inflammatory cells, is an important participant in the neoplastic process, proliferation, survival, and migration. A microenvironment rich in inflammatory cells, generating growth factors, and DNA-damage agents, potentiates the risk of neoplastic formation. Several mutations of key genes also seem to be important for the transformation of the ▶ inflammation–dysplasia–carcinoma sequence.

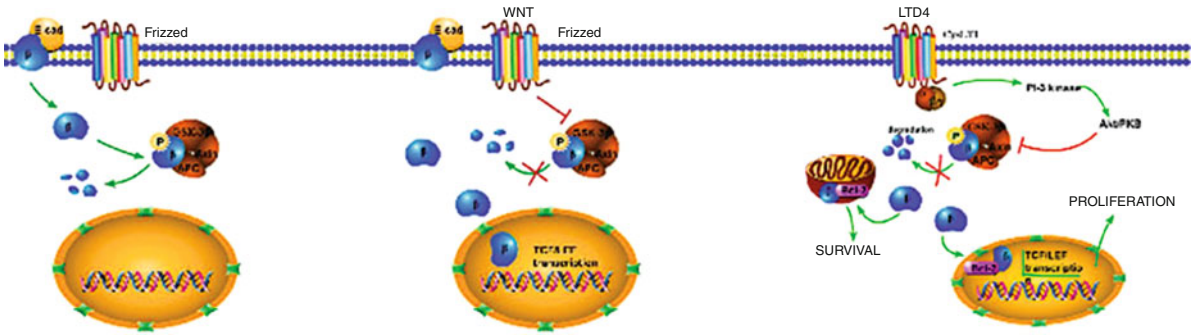
The leukotrienes potentiate biological activities in the pathogenesis of many diseases. In most chronic inflammatory conditions, such as inflammatory bowel disease, the levels of leukotrienes are increased. The strongest association of chronic inflammation with malignant disease is seen in colon carcinogenesis arising in individuals with inflammatory bowel disease. Indeed, patients suffering from inflammatory bowel disease have a 30–50% increased risk of developing colon cancer. In chronic inflammatory bowel diseases, elevated levels of leukotrienes are found, which increases the risk for development of cancer and thereby a reduced survival of these patients. As expected, it has been established that a cause-and-effect link between chronic inflammation and colon cancer occurs via activation and overexpression of the two enzymes, 5-LO and COX (▶ Cyclooxygenase, COX-1 and COX-2), responsible for regulating the production of leukotrienes and prostaglandins, respectively (Fig. 1).

The expression of ▶ COX-2 is highly upregulated both during inflammatory conditions and cancer. Elevated prostaglandin production at the site of the tumor is a good indicator of increased COX-2 activity in colon cancer tissue. There are vast amounts of data suggesting that ▶ nonsteroidal anti-inflammatory drugs (NSAIDS), ▶ COX inhibitors, reduce the risk of colon cancer. These inhibitors were shown to suppress the proliferation of intestinal cancer cell lines that express high levels of COX-2.

The production of different leukotrienes from arachidonic acid is dependent on the expression of 5-LO, an enzyme that regulates the first step in the synthesis of leukotrienes. Induction of experimental colitis in mice lacking the 5-LO protein significantly reduced the degree of infiltration of inflammatory cells and colonic injury. A number of other studies have shown that inhibition of 5-LO decreases growth and promotes cell death in several transformed cell lines.

In a recent tissue array study using colorectal cancer and control specimens, elevated levels of 5-LO and COX-2 were found in colorectal carcinomas. In accordance, similar observations were made in different colon carcinoma cell lines when these were compared with nontransformed intestinal epithelial cell lines. Interestingly, activation of CysLT receptor signaling led to an increased COX-2-mediated production of prostaglandin E_2, PGE_2. Mediating COX-2 activation and PGE_2 production has a major impact on intestinal epithelial cell survival.

5-LO metabolites may contribute to the development of several human tumors, including pancreatic, esophageal, prostate, breast, and colon cancer. COX-2 and 5-LO have been reported to be simultaneously upregulated in colorectal cancer. It is possible that blocking one of these enzymatic pathways may activate the other. Therefore, combined treatment of



Leukotrienes. Fig. 2 A simplified signaling cascade leading to accumulation of free β-catenin and increased cell proliferation

these pathways could be a better treatment option. There is an additive effect of inhibiting proliferation, inducing apoptosis, decreasing Bcl-2 levels, and increasing Bax levels in cancer cells, after combined treatment with inhibitors of COX-2 and 5-LO.

► Leukotriene receptors are upregulated in colon cancer, and their signaling facilitates the survival and proliferation of cancer cells and reduces apoptosis. In agreement with this, an increased expression level of leukotriene receptors in different colon cancer cell lines correlates well with the ability of leukotrienes to increase the survival of these cells.

Apart from Bcl-2, there are a number of other cellular proteins that are closely connected to the regulation of cell proliferation, survival, and apoptosis. One of these is ► β-catenin. This protein became of interest in relation to LTD_4-induced survival signaling when a novel LTD_4-triggered association between Bcl-2 and β-catenin in the mitochondria from intestinal epithelial cell lines was identified. It could be hypothesized that LTD4 may enhance cell survival via activation and association of β-catenin with Bcl-2 in the mitochondria. β-catenin activation and signaling execute an antiapoptotic effect through protection of cytochrome c leakage from the mitochondria.

β-catenin is a protein with many roles, one well established as an effector molecule of the ► Wnt signaling pathway. The Wnt receptors, Frizzleds, belong to the same GPCR family of receptors as the leukotrienes. The presence of a Wnt signal allows β-catenin to translocate to the nucleus where it activates the transcription factors TCF/LEF. Normally, β-catenin is regulated by the adenomatous polyposis coli (► APC) complex to control the intracellular levels of β-catenin. LTD_4 can inactivate GSK-3β, known to phosphorylate β-catenin and induce its ubiquitination and degradation, and thus has the potential to increase the amount of free β-catenin if inactivated. LTD_4 induces inactivation of GSK-3β via PI3-kinase/Akt pathway allowing free β-catenin to be able to enter the nucleus and activate the transcription factor TCF/LEF, which activates potentially oncogenic genes, such as cyclin D1, c-myc, and COX-2 (see Fig. 2).

The infection–inflammation–dysplasia–carcinoma sequence is exemplified by *Helicobacter pylori* (HP) infection, a well-known risk factor for gastric adenocarcinoma. HP infection may also be a possible risk factor for respiratory system disease, which triggers a marked local inflammatory response and a chronic system immune response. Some of the possibly mediators involved in the pathogenesis of these infections include leukotrienes that may have a role in the development of lung cancer in association with HP infection. The risk of lung cancer in patients with gastric ulcers is around three times higher than in individuals without ulcers.

Activation of leukotriene receptor signaling pathways and subsequent effects on proliferation and survival of epithelial cells indicate that the inflammatory mediator, leukotrienes, can contribute to growth of cells during pathological inflammatory conditions. This, in turn, indicates that these receptors have an important role in neoplastic transformation and development of cancer.

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Leustatin

▶ Cladribine

Lewis Antigens

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Synonyms

Histo-blood group Lewis antigens

Definition

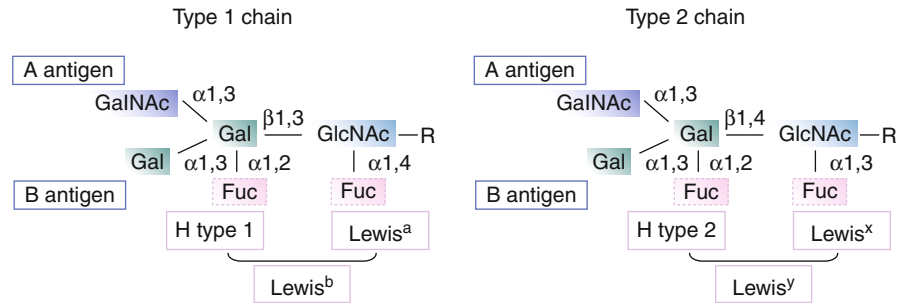
Lewis antigens are fucosylated carbohydrates linked to lipids or to proteins and thus present as glycolipids or glycoproteins.

Characteristics

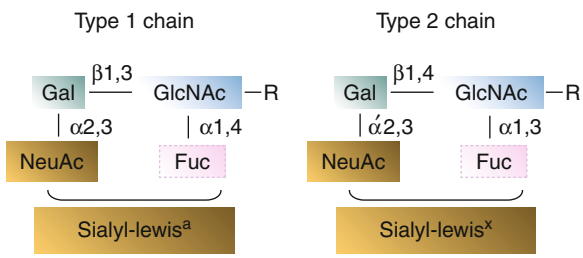
▶ G protein coupled receptors **Lewis** antigens are expressed in normal tissues on two major carbohydrate type chains, type 1 and type 2, according to the linkage type between the galactose (Gal) residue and the N-acetyl-glucosamine residue (GlcNAc), β1,3 and β1,4, respectively (Fig. 1). Type 1 Lewis structures are widely expressed in endodermally derived tissues, such as glandular epithelia, in body fluids and saliva, and are adsorbed from plasma circulating glycolipids onto the surface of erythrocytes and lymphocytes. The presence or absence of type 1 antigens in a particular individual depends upon the presence of active enzymes responsible for the addition of the fucose monosaccharide. The α1,2 fucosyltransferase, the product of the secretor gene (*Se*) (▶ Secretor (Se) enzyme), acts on the terminal galactose and produces the H type 1 structure that forms the substrate for the α1,4 fucosyltransferase, the product of the Lewis gene (*Le*) (**Lewis (Le) enzyme**), that synthesizes the difucosylated Le^b antigen. Individuals that have inactivating mutations of the *Se* gene are unable to synthesize the H type 1 structure and the Le^b antigen, and are so called nonsecretors and constitute 20% of human populations. Type 2 Lewis antigens are expressed in ecto- and mesodermally derived tissues, including skin and erythrocytes, and in a more restricted manner in endodermally derived epithelia like stomach glands. The α1,2 fucosyltransferase that acts on the terminal galactose and produces the H type 2 structure is the product of the *H* gene, the ▶ H enzyme, that forms the substrate for the α1,3 fucosyltransferase, the product of the Lewis gene (*Le*), that synthesizes the difucosylated Le^y antigen. Other ▶ fucosyltransferases may be involved, in a tissue-specific manner, in the synthesis of Lewis antigens: an α1,3 fucosyltransferase activity has been described for FUT3, FUT4, FUT5, FUT6, FUT7, and FUT9, and an α1,4 activity was described for FUT3 and FUT5. The secretor and Lewis status of individuals are implicated in susceptibility to several diseases, mostly human infections, with the most dramatic example being the virtual absence of gastrointestinal infections by Calicivirus in nonsecretors.

Lewis Antigens in Cancer

Malignant cells frequently have abnormal ▶ glycosylation with expression of modified carbohydrate antigens, among which stand sialylated forms of the Lewis antigens – sialyl-Le^a and sialyl-Le^x (Fig. 2). Sialyl-$Le^{a/x}$ cell-surface molecules, linked to lipids and proteins, when tumor cells become invasive and depolarized gain access to circulation, either linked to the cell surface or shed into the serum. The relevance of these sialylated structures in cancer was first revealed, in the 1980s, when monoclonal antibodies raised against cancer cells were shown to recognize sialyl-$Le^{a/x}$. They are widely used as tumor markers (CA19-9 for sialyl-Le^a and SLX for sialyl-Le^x) for initial serum diagnosis of cancer and for detection of cancer recurrence after surgery or treatment with radio- and chemotherapy. Later, in the 1990s,



Lewis Antigens. Fig. 1 Schematic representation of type 1 and type 2 ABH and Lewis antigens. Histo-blood group A is defined by the α1,3 terminal GalNAc, histo-blood group B is defined by the α1,3 terminal Gal, and the absence of further elongation of the H structure is characteristic of histo-blood group O individuals. Synthesis of type 1 Lewis antigens (Le^a and Le^b) and type 2 Lewis antigens (Le^x and Le^y) depends upon activity of fucosyltransferases (see text for details). *GlcNAc* N-acetyl-glucosamine, *Gal* Galactose, *GalNAc* N-acetyl-galactosamine, *Fuc* Fucose, *A antigen* Histo-blood group antigen A, *B antigen* Histo-blood group antigen B



Lewis Antigens. Fig. 2 Schematic representation of sialylated Lewis antigens – sialyl-Le^a and sialyl-Le^x. *NeuAc* sialic acid (neuraminic acid)

sialyl-$Le^{a/x}$ were identified as cancer cell-surface molecules involved in ▶ adhesion to endothelial cells, through E-▶ selectin-Mediated Adhesion (and P-▶ selectin). Binding of tumor cells to endothelial cells, in a model that mimics leukocyte extravasation, contributes to the establishment of tumor growth at distant sites by hematogenous metastization. Several studies, analyzing cancers from different organ origins, showed that expression of these sialylated Lewis antigens correlated to the prognosis of the patients, reinforcing their role on metastatic behavior. They are more frequently overexpressed in carcinomas, mainly adenocarcinomas, but also in leukemia. Mechanisms underlying overexpression of sialyl-$Le^{a/x}$ in cancer cells have been recently clarified in some cancer models and essentially result from altered expression of α2,3 ▶ sialyltransferases and/or of α 1,3/4 fucosyltransferases, responsible for their synthesis (Fig. 2). A viral gene product that induces HTLV-1 viral-associated leukemias was shown to transactivate fucosyltransferase VII, an α1,3 fucosyltransferase with rate-limiting activity for the synthesis of sialyl-Le^x in leukocytes, and therefore induces a strong constitutive expression of sialyl-Le^x in leukemic cells. Other mechanisms controlling gene expression, including methylation and identification of transcription factors, are under investigation.

The role of sialylated Lewis antigens in the metastatic process led to the development of several new candidate therapeutic approaches. Therapeutic strategies have been attempted to reduce the biosynthesis of sialylated Lewis antigens. Specifically, the synthesis of sialyl-Le^x was successfully inhibited in colon carcinoma cell lines by using a disaccharide competitive substrate as a decoy. An alternative approach is to use monoclonal antibodies directed to sialyl-$Le^{a/x}$ or analogs of the sialyl-$Le^{a/x}$ to block adhesion of tumor cells to endothelial cells and prevent metastization.

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Lewis Enzyme

Definition

A product of the Lewis gene, also named fucosyltransferase3 (FUT3), that catalyzes addition of fucose in α1,3/4 position onto type 1 and type 2 chains.

► Lewis Antigens

Lewis^x

Definition

Oligosaccharide corresponding to the histo-blood group antigen.

► CEA Gene Family

Lexatumumab

Definition

Is an anti-► DR5 humanized antibody that was generated using a phage display and screening for DR5 binding properties. In contrast to ► mapatumumab, no data on specificity, selectivity, and affinity of the antibody are publicly available. Lexatumumab exert pronounced apoptotic reactions in a wide array of malignant cell systems when used alone. The drug has proven efficacy on tumor growth in ► xenograft systems from renal cell carcinoma, non-small cell lung cancer, breast cancer, and glioma. The combination of lexatumumab with various chemotherapeutic agents (camthotecin, ► cisplatin, carboplatin, ► paclitaxel, ► doxorubicin, bortezomib) or radiation increased the efficacy in cell lines and xenografts. The underlying mechanisms of sensitization are still not completely understood. However, it seems likely that the presence of the pro-apoptotic ► Bax molecule as well as the up-regulation of the respective receptor participate in the increased efficacy of the combined approach.

► TRAIL Receptor Antibodies

Leydig Cell Tumor

Definition

Tumor derived from ► Leydig cells as normally seen in the testis. They can occur in both testis and ovaries and can be hormonally active. While testicular Leydig cell tumors may be malignant, their ovarian counterparts are usually benign.

Leydig Cells

Definition

Cells in the testes that secrete the hormone ► testosterone.

LFS

Definition

Li-Fraumeni cancer family syndrome.

► Li-Fraumeni Syndrome

LH

Definition

Abbreviation for ► Luteinizing Hormone

LHRH

Definition

Luteinizing hormone-releasing hormone: A hormone that controls sex hormones in men and women. Is released from the hypothalamus of the brain when the hypothalamus detects dropping levels of ► testosterone. LHRH binds to the receptors of the pituitary gland, which releases ► luteinizing hormone (LH) that travels to the testicles and stimulates the

production of testosterone. In ▶ prostate cancer hormonal therapy, LHRH antagonists and agonists are used to prevent the pituitary gland to release LH. The level of testosterone will drop to 5–10%, which is called ▶ castrate level. The use of an LHRH antagonist or agonist in prostate cancer hormonal therapy is referred to as chemical castration. LHRH is also referred to as gonadotropin hormone releasing hormone (GnRH). In prostate cancer hormonal therapy, the removal of testosterone from the body will temporarily slow or stop the growth and spread of the disease.

▶ Gonadotropin-Releasing Hormone

LH-RH

Definition

Luteinizing hormone-releasing hormone.

▶ Adjuvant Chemoendocrine Therapy

LHRH Agonists

Definition

Are analogues of gonadotropin releasing hormone; (LHRH = Luteinizing Hormone Releasing Hormone, also called – gonadotropin-releasing hormone (GnRH)). They act as potent inhibitors of gonadotropin secretion. When given continuously, this treatment will ultimately result in near ▶ castrate level of ▶ testosterone.

▶ Gonadotropins
▶ Prostate Cancer Hormonal Therapy

LHRH Antagonists

Definition

Are used in ▶ prostate cancer hormonal therapy to control the growth and spread of prostate cancer by ▶ testosterone ablation (reduction). An antagonist is a chemical agent that mimics the shape of a natural agent and binds to a receptor instead of the natural agent. While the antagonist is in place, the natural agent cannot bind to the receptor. LHRH antagonist mimics natural luteinizing hormone-releasing hormone (▶ LHRH) and binds to the receptor of the pituitary gland where it induces the synthesis of ▶ luteinizing hormone (LH) which in turn signals to the testicles to produce ▶ testosterone. When LHRH antagonists are bound to receptor, this signaling cascade is interrupted, and the pituitary gland does not receive the information necessary to produce LH. As a consequence, testosterone production drops to ▶ castrate level.

LI Element

Definition

▶ LINE

Lichenification

Definition

Thickening of the skin with an exaggeration of the normal skin markings resulting from chronic rubbing and scratching.

▶ Sezary Syndrome

Lichenoid Dermatitis

Definition

A form of neurodermatitis, characterized by intense pruritus with exudative, weeping patches on the skin scattered irregularly over most of the body, many of which are of the eczematous type and undergo ▶ lichenification.

▶ Rituximab

Licorice

Synonyms

Liquorice

Definition

Is a shrub native to southern Europe and Asia, the roots of which have two primary desirable qualities: first, some varieties of licorice root are 50 times sweeter than sugar and may be chewed or eaten as a sweet and making it a useful component of candies and flavorings; second, licorice has been for thousands of years sought after for its reputed medicinal qualities.

LIF

► Leukemia Inhibitory Factor

Life Table Estimates

► Kaplan-Meier Survival Analysis

Li-Fraumeni Syndrome

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Definition

Li-Fraumeni syndrome (LFS) is a rare autosomal disorder characterized by a familial clustering of tumors, with a predominance of sarcomas, breast cancers, brain tumors, and adrenocortical carcinomas, diagnosed before the age of 45 years. Other cancers, such as leukemia, lung cancer, gastric cancer, skin melanoma, pancreatic cancer, and ovarian cancer are also present in excess in some families and, in some cases, germ cell tumors, choroid plexus papilloma, and Wilms' tumor have been reported as part of the spectrum.

Characteristics

Diagnostic Criteria

The clinical criteria used to identify a classic LFS family are:

- Proband with sarcoma before the age of 45
- A first-degree relative with any tumor before age 45
- Another close relative (second or first degree) with cancer before age 45 or a sarcoma at any age

Several clinical criteria for the diagnosis of LFS-like (LFL) families have also been proposed:

- *LFL-E1* (Eeles definition 1): Two different tumors which are part of extended LFS in first- or second-degree relatives at any age (sarcoma, breast cancer, brain tumor, leukemia, adrenocortical tumor, melanoma, prostate cancer, pancreatic cancer).
- *LFL-E2* (Eeles definition 2): Sarcoma at any age in the proband with two of the following (two of the tumors may be in the same individual): breast cancer at <50 years and/or brain tumor, leukemia, adrenocortical tumor, melanoma, prostate cancer, pancreatic cancer at <60 years or sarcoma at any age.
- *LFL-B* (Birch definition): Proband with any childhood cancer or sarcoma, brain tumor or adrenocortical carcinoma at <45 years, with one first-or second-degree relative with typical LFS cancer (sarcoma, breast cancer, brain tumor, leukemia, or adrenocortical carcinoma) at any age, plus one first- or second-degree relative in the same lineage with any cancer diagnosed under age 60.
- *Chompret* (2009 version): (1) Proband with tumor belonging to LFS tumor spectrum (sarcoma, brain tumor, premenopausal breast cancer, or adrenocortical carcinoma) before 46 years and at least one first- or second-degree relative with LFS tumor (except breast cancer if the proband has breast cancer) before 56 years or multiple primary tumors; or (2) proband with multiple tumors (except multiple breast cancer), two of which belong to the LFS tumor spectrum and the first of which occurred before 46 years; or (3) proband with adrenocortical carcinoma or choroid plexus tumor, irrespective of family history.

Genetics

The genetic origin of LFS was discovered in 1990 when four LFS families had been found to carry a germ line mutation in the tumor suppressor gene ▶ TP53 (OMIM#191170). Since then, more than 400 families or individuals who are carriers of a TP53 mutation have been reported in the scientific literature. A database that compiles data from the literature and serves as a repository for data on patients and families with a TP53 germ line mutation, or with LFS/LFL syndromes is maintained at IARC (http://www-p53.iarc.fr/Germline.html). Data from the IARC TP53 database show that in approximately 81% (116/143) of LFS cases, and 67% (97/145) of LFL cases, affected family members carry a germ line mutation of one allele of the TP53 tumor suppressor gene. In some families, mutations in other genes have been reported. Two LFS and 3 LFL families had a heterozygous CHEK2 germ line mutation, while one LFL family had a ▶ CDKN2A mutation and one LFS family a ▶ PTEN mutation. These genes are all connected to the p53 pathway. CHEK2 (OMIM#604373) (▶ Checkpoint Kinases) encodes a protein kinase that is involved in checkpoint control in response to DNA damage and acts upstream of p53 in the G1 checkpoint. CDKN2A (OMIM#600160) encodes two proteins, the cyclin-dependent kinase inhibitor p16(INK4) and the tumor suppressor p14(ARF). P14(ARF) is activated by oncogenic stress and promotes cell-cycle arrest via the inhibition of the neutralization of p53 by MDM2. PTEN (OMIM#601728) is a tumor suppressor gene encoding a protein phosphatase that negatively regulates the PI3-kinase/Akt (OMIM#164730) signaling pathway. PTEN is also a transcriptional target of p53 that acts downstream of p53. Alteration of the p53 pathway is thus the main cause of LFS/LFL syndromes, mutation of the TP53 gene being the most prevalent alteration. In fact, it is now recognized that CHEK2 does not predispose to LFS/LFL, but only to the breast cancers that have occurred in the context of these families. A study on 16 LFS families with wild-type TP53 has excluded PTEN, CHEK2, and CDKN2A as candidate genes in LFS.

The clinical criteria proposed by Chompret are currently recognized as the best criteria for predicting the presence of a TP53 germ line mutation.

TP53 Germ Line Mutations

The tumor suppressor gene TP53 (OMIM:191170, synonym: P53), located on chromosome 17p13.1, has 11 exons that span 20 kb. Exon 1 is noncoding and exons 5–8 are remarkably conserved among vertebrates. The TP53 gene encodes an ubiquitous phosphoprotein involved in many overlapping cellular pathways that control cell proliferation and homeostasis, such as cell cycle, apoptosis, and DNA repair. The p53 protein is a transcription factor constitutively expressed in most cell types and is activated in response to various genotoxic and non-genotoxic stress signals. Loss of p53 function is thought to suppress a mechanism of protection against accumulation of genetic alterations.

Type and Origin of TP53 Mutations

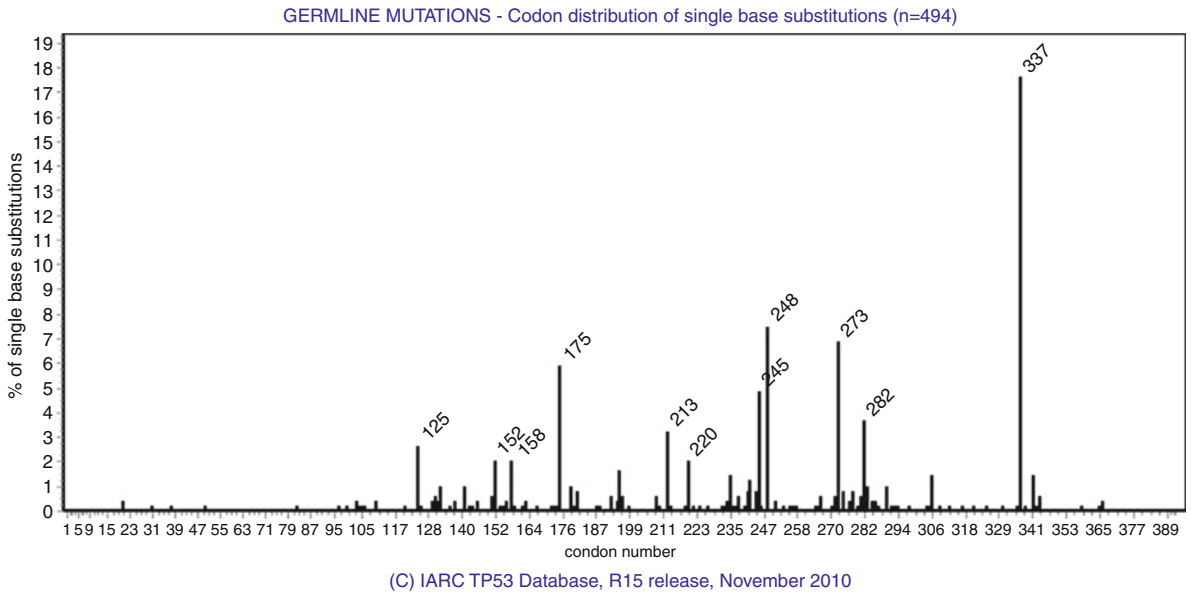
Among TP53 germ line mutations, point mutations are the most frequent (90%), followed by deletions (7%), insertions (2%), and other complex mutations.

As with somatic TP53 mutations, TP53 germ line mutations are located in highly conserved regions of the DNA-binding domain (exons 5–8), with major hotspots at codons 175, 245, 248, and 273 (Fig. 1). These mutations correspond to G:C > A:T transitions at CpG sites that are considered to be of endogenous origin, 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 transitions at CpG sites are more frequent, representing 55% of all germ line mutations compared to 25% in sporadic cancers. Overall, the spectrum of TP53 germ line mutations is consistent with a formation through endogenous mutagenesis, rather than with causation by exposure to exogenous mutagenic carcinogens.

A germ line–specific mutation hotspot has recently been described, R337H. It also results from a G:C > A:T transition at a CpG site but is located outside the DNA-binding domain, in the oligomerization domain. This mutant has been found in Brazilian children affected by adrenocortical carcinomas and in Brazilian LFL families. It is considered of low penetrance and its prevalence in Brazil has been shown to be due to a founder effect.

Tumor Spectrum in TP53 Mutation Carriers

Breast cancers, sarcomas (soft tissue sarcomas and osteosarcomas), brain tumors, and adrenocortical



Li-Fraumeni Syndrome. Fig. 1 Similar to sporadic cancers, TP53 germ line mutations preferentially occur in hotspot regions at codons 175, 245, 248, and 273. Specific to the germ line, a mutation at codon 337 (R337H, in the conserved oligomerization domain) is frequently found in the Brazilian population (IARC TP53 database, R15, November 2010)

carcinomas account for about 80% of all tumors arising in TP53 germ line mutation carriers (Table 1). Childhood adrenocortical carcinomas (ADR, 14% of tumors) have been shown to be a hallmark for the presence of a TP53 germ line mutation. Brain tumors (12% of all tumors) are mostly of astrocytic origin (65%). Pediatric brain tumors, including medulloblastomas and related primitive neuroectodermal tumors, and choroid plexus tumors are less frequent. This correlates with the occurrence of TP53 mutations in sporadic brain tumors, which prevail in astrocytomas and are considerably less frequent in medulloblastomas. In addition to the four main types of cancers strongly associated with TP53 mutation, seven types of cancers account for 14% of all cancers. They include leukemia/lymphomas, Wilms' tumor, malignant phyllodes tumor, carcinoma of the pancreas, stomach and colorectal cancers that were found to be weakly to moderately associated with TP53 germ line mutation, and lung and ovarian cancers. Although the frequent occurrence of lung and ovarian cancer might be due to the fact that they are frequent in the general population, they occur significantly earlier in TP53 germ line carriers compared to sporadic cases (Table 1).

Age Distribution in Relation to Tumor Type

While the histopathologic characteristics of tumors associated with TP53 germ line mutations are very similar to their sporadic counterparts, their age at onset show marked organ-specific differences (Fig. 2). Adrenocortical carcinomas associated with a TP53 germ line mutation develop almost exclusively in children, while sporadic adrenocortical carcinomas have a broad age distribution with a peak beyond age 40 (Table 1). Bone sarcomas occur mainly in adolescents while soft-tissue sarcomas are more frequent in childhood. Brain tumors have a biphasic age distribution, with a first pick in childhood and a second pick between 20 and 40 years. Breast carcinomas are more prevalent in the 20–40 age range.

Gender Distribution of Patients with TP53 Germ Line Mutations

The gender distribution for these tumors (Table 1) shows an excess of males for brain tumor, hematopoetic cancers, and stomach cancer, whereas an excess of females was observed for adrenocortical carcinoma and skin cancer. All of the breast cancers were in females. This gender distribution is similar to

Li-Fraumeni Syndrome. Table 1 Tumor type, age at onset, and gender distribution in TP53 germ line mutation carriers (IARC TP53 database, R12, October 2007)

		Median age at diagnosis		Male (ratio) (%)	
Tumor type	Number N (%)	TP53 carriers	Sporadic[a]	TP53 carriers	Sporadic[a]
Breast cancer	217 (25.9)	33	63.1	0 (0/217)	0.7
Soft tissue sarcoma	142 (16.9)	14	61.3	43 (56/129)	53
Adrenocortical carcinoma	117 (13.9)	2	41.9	25 (29/116)	51
Brain tumor	101 (12.0)	15.5	57.4	64 (58/91)	56
Bone sarcoma	98 (11.7)	15	43.3	49 (43/88)	56
Leukemia/lymphoma	31 (3.7)	17	65.1	59 (13/22)	55
Lung cancer	22 (2.6)	41	68.7	50 (11/22)	66
Skin	20 (2.4)	50	–	19 (3/16)	–
Colorectal cancer	18 (2.1)	39	71.6	47 (7/15)	50
Stomach cancer	15 (1.8)	38	72.6	67 (10/15)	62
Ovarian cancer	13 (1.5)	39.5	64.3	0 (0/13)	0
Other	45 (5.4)	–	–	–	–

[a]Data based on cancer registries from USA, France, and United Kingdom compiled in Cancer Incidence in Five Continents v. 7, 1997

the one of sporadic cancers observed in the general population with the exception of adrenocortical carcinoma, which occur significantly more frequently in females than in males in TP53 mutation carriers compared with sporadic cases in the general population.

Functional Consequences and Phenotype of Germ Line Mutations

The majority of TP53 germ line mutations are missense mutations (80%), followed by nonsense, frameshift deletions and insertions, mutations in splice sites, and other intronic or complex mutations. p53 mutant proteins resulting from missense mutations differ from each other in the extent to which they have lost transactivation activities, and in their capacity to inhibit wild-type p53 in a dominant-negative manner. In addition, some p53 mutants appear to exert an oncogenic activity of their own, but the molecular basis of this gain-of-function phenotype is still unclear. It is thus expected that p53 germ line mutants exert distinct biological activities that could influence tumor penetrance in term of organ sites or age at onset. Genotype/phenotype correlations have been reported in recent studies:

Brain tumors were more likely to be associated with missense mutations located within the DNA-binding surface of p53 protein that makes contacts with the minor groove of DNA.

Adrenocortical carcinomas were more likely to be associated with missense mutations located outside the DNA-binding surface.

Mutations with complete loss of transactivation activities were associated with earlier onset breast and colorectal cancers compared to mutations that retain some trans-activation capacities.

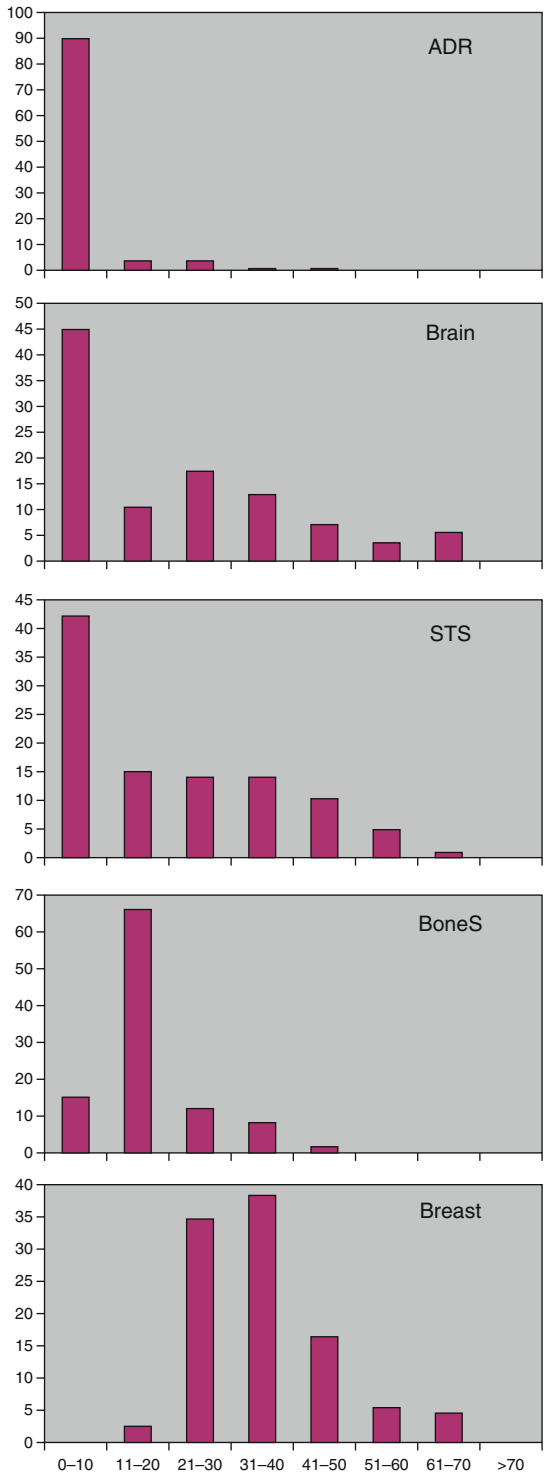
Truncating mutations were associated with earlier onset brain tumors compared to all other mutations.

Thus, the penetrance of a mutation may be related to its degree of loss of trans-activation capacities, at least in some tissues. The notion that loss of function is in itself sufficient for causing LFS is substantiated by the fact that a large deletion encompassing the whole TP53 gene has been reported in an LFS family.

These observations show that the degree of loss of trans-activation capacities may affect the type and age at onset of tumors in LFS patients. It is not yet known how other functional properties contribute to mutation phenotypes.

Mutation Detection Methods and Results Interpretation

TP53 germ line mutations are very diverse and scattered along the entire gene coding sequence, including splice sites in introns. These mutations have different biological effects that may affect their phenotype. Large deletions of the gene have also been



Li-Fraumeni Syndrome. Fig. 2 Distribution of the age at onset of the five main types of tumors associated with TP53 germ line mutations (IARC TP53 database, R12, October 2007). *ADR* adrenocortical carcinoma, *STS* soft tissue sarcoma, *BoneS* bone sarcoma

reported in some LFS families. To fully assess TP53 status in an individual, it is thus recommended to (1) use sequencing techniques to precisely identify the mutation, (2) screen all exons and splice junctions of the gene, and if no mutation is found, and (3) search for large gene deletions. A protocol for direct sequencing of TP53 is available in the IARC TP53 database (http://www-p53.iarc.fr/Download/TP53_DirectSequencing_IARC.pdf). A kit for detecting large deletions is available commercially (MLPA, http://www.mlpa.com/pages/p056pag.html).

For interpreting sequence analysis results, it is recommended to follow guidelines proposed by the ACMG (http://www.acmg.net/resources/policies/pol-027.pdf) working group and to consult the IARC TP53 Database to retrieve information on mutation prevalence and phenotype. If the mutation has already been reported as a germ line mutation, the associated tumor spectrum can be retrieved (http://www-p53.iarc.fr/TumourCriteria.ASP). If the mutation has never been described in cancer families, data on its frequency in sporadic cancers and biological impacts (assessed in functional assays or predicted by sequence conservation models) can be retrieved (http://www-p53.iarc.fr/MutationValidationCriteria.asp) to estimate its functional severity.

If no alteration is found in TP53 gene, the family history may be carefully checked since certain families with LFS/LFL resemble families with hereditary breast cancer, who are candidates for BRCA1 or BRCA2 testing (see ▶ BRCA1/BRCA2 Hereditary Breast Cancer), while other families may resemble kindreds with familial brain tumors.

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Ligament of Treitz

Definition

The suspensory ligament to the fourth part of the duodenum.

► Pseudomyxoma Peritonei

Ligands

Definition

From a chemistry viewpoint, it is an atom, ion, or molecule that generally donates one or more of its electrons through a coordinate covalent bond to, or shares its electrons through a covalent bond with, one or more central atoms or ions (these ligands act as a Lewis base). In biology, ligands are referred to small molecules (including peptides, amino acids, etc.) that bind proteins and alter their function.

► Dioxin
► Gastrointestinal Stromal Tumor
► Orphan Nuclear Receptors

Light Tomography

► Optical Mammography

Light-Induced Fluorescence Endoscopy

Definition

Is a bronchoscopy system for neoplasia detection based upon differential autofluorescence properties of early and invasive lung cancer.

► Fluorescence Diagnostics
► Laser Diagnostics

LIM Domain

Definition

Is a cysteine-rich domain composed of two tandem zinc fingers that are joined by a two-amino acid spacer. It functions as a modular protein-binding interface. The acronym "LIM" is derived from the first three proteins shown to harbor LIM domains: *Caenorhabditis elegans* LIN-11, rat Isl1, and ► *C. elegans* MEC-3. A single LIM domain consists of about 55 amino acids of which eight, mostly cysteines and histidines, are highly conserved. The latter bind two zinc ions, one for each zinc finger. The LIM consensus sequence has been defined as $CX_2CX_{16-23}HX_2CX_2CX_2CX_{16-21}CX_2$(C/H/D) (X represents any amino acid). LIM domains occur in a wide variety of eukaryotic organisms but are absent from prokaryotes. LIM proteins have a function in a wide variety of biological processes, such as cytoskeletal functions and control of gene expression.

► Lipoma Preferred Partner

LIM Domain Containing Preferred Translocation Partner in Lipoma

► Lipoma Preferred Partner

LIMD1

Definition

LIM domain-containing protein 1 is a member of the ► zyxin family of proteins.

► Lipoma Preferred Partner

LINAC

Linear Accelerator; ► radiation oncology

LINE

Synonyms

LI element

Definition

Long interspersed nuclear element; typically 6–8 kb pair long and frequent DNA sequences randomly distributed throughout the genome. In humans, LINE sequences make up approximately 20% of the genome, frequent are LINE-1, LINE-2, and LINE-3 elements. They mostly localize to AT-rich regions in the euchromatin. Because LINES move by copying themselves (instead of moving, like transposons do), they enlarge the genome. The human genome, for example, contains about 900,000 LINE sequences.

LINE sequences are transposable elements, which use RNA as an intermediary step. They encompass genes encoding two proteins: one RNA-binding, the other having activities of reverse transcriptase and endonuclease, which enable them to copy themselves. Their distribution pattern is unique for each individual genome, and their position therefore is diagnostic for the identity of individuals.

► Sticker sarcoma

LINE-1 Elements

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Definition

Long interspersed nuclear elements (LINE-1 or L1) are a family of DNA repeat sequences with ~500,000 copies comprising ~17% of the human genome.

Characteristics

Structure

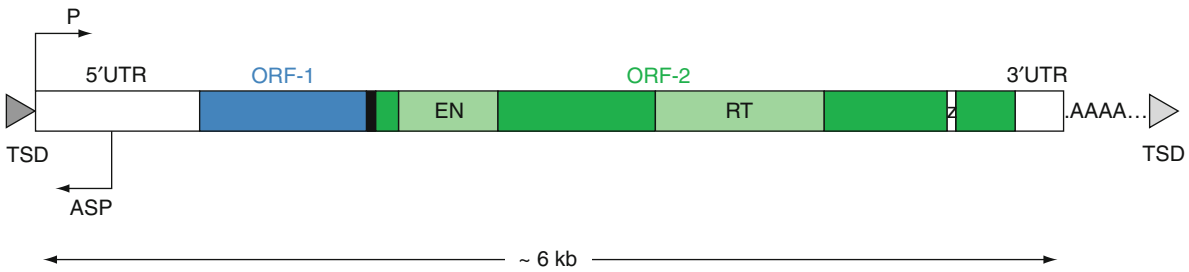
Full-length elements are ~6 kb, include a 910 bp 5′ untranslated region (UTR), two open reading frames (ORF-1 and ORF-2) that are separated by a 63 bp inter-ORF region, a 205 bp 3′UTR, a variable length poly-A tail and are typically flanked by short direct target site duplications of 2–20 bp. The 5′UTR contains an internal promoter which drives L1 expression and also has an antisense promoter permitting transcription of the 5′UTR and its upstream genomic region. ORF-1 encodes a ~40 kDa protein (p40 or ORF1p) with RNA-binding affinity and nucleic acid chaperone activity. ORF-2 encodes a ~150 kDa protein (ORF2p) with endonuclease, reverse transcriptase, and cysteine-rich domains (Fig. 1). The majority of L1 copies are either 5′ truncated, inverted, or contain point mutations. An estimated 3,000–10,000 copies are full-length with only ~1% of these elements able to encode the factors needed for autonomous movement (► retrotransposition) enabling copying of L1 sequence and insertion into a new genomic location.

Genomic Impact

Although once considered "junk DNA," it is now realized that repetitive DNA families have influenced and shaped the human genome in both detrimental and beneficial ways. L1 elements can cause constitutional and somatic cell disease through not only retrotransposal insertion, but also as facilitators of recombination giving rise to genomic deletions, translocations, and other chromosomal rearrangements. L1 elements have contributed substantially to the increase in DNA mass of human cells over evolutionary time. Over one-third of the human genome can be attributed to the activities of these elements either directly through self replication and insertion or indirectly by providing retrotransposal proteins enabling expansion of the nonautonomous ► Alu element, ► processed pseudogene, and ► SVA repeat families. L1 elements contribute to reshuffling and relocation of DNA sequence through transduction of genomic sequence either 3′ or 5′ of the parent L1 during the retrotransposition process. Further proposed roles of L1 elements include involvement in ► X chromosome inactivation, downregulating or altering mRNA expression of the host gene in some cases of intronic insertion, and repair of double strand DNA breaks by L1 insertion.

Role in Human Cancer

L1 element expression is normally restricted to developing germ cells. In somatic cells their activity is



LINE-1 Elements. Fig. 1 Structure of a full-length human L1 element. The ~6 kb retrotransposon is flanked by target site duplications (TSD) and consists of (i) a 5′UTR with internal sense (P) and antisense (ASP) promoters; (ii) two open reading frames, ORF-1 encodes a 40 kDa RNA-binding protein, ORF-2 encodes a 150 kDa protein with endonuclease (EN) and reverse transcriptase (RT) activity, and contains a conserved zinc knuckle-like domain (Z) residing within a cysteine-rich region; (iii) a 3′UTR terminating in a poly-A tail

believed to be suppressed by a number of mechanisms including DNA ▶ methylation, TP53 activity, effects leading to L1 RNA instability, and packaging into inactive chromatin. However, in some cancer cells L1 sequences become reactivated, largely through widespread ▶ hypomethylation of repetitive sequence. Cancer subtypes showing hypomethylation of L1 sequences identified so far include testicular, colon, liver, ovarian, bladder, prostate, breast, and the hematological malignancies ▶ chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML, ▶ BCR-ABL1). In some instances hypomethylation is found early in the disease, for example, in colon and bladder cancers. In other cases, including CML, CLL, prostate, ovarian, and breast cancer, L1 hypomethylation is reported to occur as the disease progresses. Limited studies also suggest that L1 hypomethylation may be associated with clinical outcome.

It would be expected that with reactivation of L1 elements, assaults to the genome associated with retrotransposition, that is, mutagenesis, illegitimate recombination, altered transcriptional activity, and gene regulation, would occur. However, whereas there is considerable evidence pointing to L1 transcriptional reactivation through hypomethylation, there are limited documented examples of L1 elements associated with cancer. Rare cases have been reported and include L1 insertions into the ▶ *APC* and upstream of the ▶ *MYC* genes resulting in deregulation of these genes causing colon and breast cancer, respectively. L1 elements are found at breakpoint sites, for example, inserted into the junction of a t(11;22) in a ▶ desmoplastic small round cell tumor or are enriched at carcinoma genomic breakpoints, for example, 3p14.1 and 9p21 deletions.

Reasons for the small number of reports in this field of interrogation could be due to either technical or biological factors. Current laboratory techniques used to investigate genetic alterations may have difficulty detecting subtle changes in L1 activity, and limited numbers of cases are examined at the detailed level required to identify L1 insertions or other L1 mediated genomic rearrangements. There also may be additional safeguards within cells that provide protection against the activities of L1 elements. Therefore, it remains to be clarified the actual extent that reactivation of L1 elements in cancer cells plays in the initiation and progression of cancer.

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Lineage

Definition

The natural progression from an immature cell type to one or more differentiated cell types.

Lineage Differentiation Tumor Antigens

Definition

Tumor antigens that are expressed in a cell type-restricted manner by malignant cells and their normal counterpart. For example, proteins of the tyrosinase family of enzymes, which are responsible for the synthesis of melanin pigment in melanocytes, are frequently recognized by the cellular as well as the humoral immune system in melanoma patients.

► Melanoma Vaccines

Lineage Restriction

Definition

The inability of one lineage to give cell types of another, that is, to cross lineage boundaries.

Linear Accelerator

Definition

LINAC is a device that accelerates electrons and other charged particles using electromagnetic waves. For radiotherapy applications, a high-energy electron beam is guided through a vacuum conduit to hit the metal foil in the path of the electron beam and to generate high energy X-rays.

► Radiation Oncology

Linearly Patterned Programmed Cell Necrosis

Definition

Is a kind of cell death. It shares morphological characteristics of traditional ► necrosis, but it is regulated by the ► apoptosis-related gene program. Under the stimuli of ► hypoxia, a cluster of tumor cells exert this death and connect with each other by lines and networks, providing the space basement for vasculogenic mimicry.

► Vasculogenic Mimicry

Lingual Cancer

► AAV

Linkage

Definition

Tendency for ► alleles at two genetic locations to be transmitted from parent to child in combination.

► Linkage Disequilibrium

Linkage Disequilibrium

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Synonyms

Allelic association; LD

Definition

Linkage disequilibrium refers to the non-random association of ► alleles at two or more loci in a general population. When alleles are in linkage disequilibrium, ► haplotypes do not occur at the expected frequencies. Linkage disequilibrium between two alleles is related to the time of the mutation events, genetic distance, and population history. It can be used to improve the power of cancer ► genetic association studies.

Characteristics

Introduction to Linkage Disequilibrium

Two or more alleles are said to be in linkage equilibrium when they occur randomly in a population. Conversely, alleles are in linkage disequilibrium when they do not occur randomly with respect to each other.

Consider two neighboring polymorphic loci A and B with two alleles each (A_1 and A_2, and B_1 and B_2, respectively) having the allele frequencies shown in Table 1.

Under linkage equilibrium, the four haplotypes formed by these loci have the frequencies shown in Table 2. These are equal to the product of the component allele frequencies. The alleles are independent, for example, whether an individual carries allele A_2 at locus A is not dependent on whether this individual carries allele B_2 at locus B on this chromosome.

Under linkage disequilibrium, haplotypes do not occur at the frequencies expected when the alleles were independent. Positive linkage disequilibrium exists when two alleles occur together on the same haplotype *more* often than expected, and negative LD exists when alleles occur together on the same haplotype *less* often than expected (Table 3). For example, suppose linkage disequilibrium existed such that the alleles A_2 and B_2 tended to occur together, that is, alleles A_2 and B_2 were in positive linkage disequilibrium. The probability that an individual carries allele A_2 at locus A is, to some extent, predictable based on whether this individual carries allele B_2 at locus B on this chromosome. The observed haplotype frequencies will differ from that expected as shown in Table 3.

Measures of Linkage Disequilibrium

Deviation of the observed from expected haplotype frequencies can be quantified by several linkage disequilibrium measures. The basic linkage disequilibrium parameter, D, was introduced in 1918 and is defined as $D = x_{11} - p_1 \times q_1$. This difference between observed and expected haplotype frequencies is presented in Table 4. Although D is simple to calculate, its disadvantage is that it is sensitive to allele frequencies at the extreme values of 0 to 1. D maximizes when allele frequencies are both 0.5 and is not calculated if an allele frequency equals 0 or 1.

In 1964, Lewontin suggested D', a normalized D calculated by dividing D by its theoretical maximum for the observed allele frequencies ($D' = D/D_{\max}$; Table 5). D' thus ranges from -1 to 1 and reflects both positive and negative linkage disequilibrium. D' is commonly used to characterize linkage disequilibrium, particularly, in the definition of haplotype blocks (see below).

A particularly useful metric of linkage disequilibrium is r^2 which is equivalent to the Pearson correlation coefficient. r^2 is calculated as $D^2/(p_1 \times p_2 \times q_1 \times q_2)$ and ranges from 0 to 1. Because it is less sensitive to extreme allele frequencies than D or D', r^2 is commonly used to describe linkage disequilibrium in genetic epidemiologic studies of

Linkage Disequilibrium. Table 1 Allele frequencies

Locus	Allele	Observed frequency
A	A_1	p_1
A	A_2	p_2
B	B_1	q_1
B	B_2	q_2

Linkage Disequilibrium. Table 2 Haplotype frequencies under linkage equilibrium

Haplotype	Expected frequency
$A_1\,B_1$	$p_1 \times q_1$
$A_1\,B_2$	$p_1 \times q_2$
$A_2\,B_1$	$p_2 \times q_1$
$A_2\,B_2$	$p_2 \times q_2$

Linkage Disequilibrium. Table 3 Haplotype frequencies under linkage disequilibrium

Haplotype	Observed frequency	A_2 and B_2 in positive LD	A_2 and B_2 in negative LD
$A_1\,B_1$	x_{11}	$x_{11} > p_1 \times q_1$	$x_{11} < p_1 \times q_1$
$A_1\,B_2$	x_{12}	$x_{12} < p_1 \times q_2$	$x_{12} > p_1 \times q_2$
$A_2\,B_1$	x_{21}	$x_{21} < p_2 \times q_1$	$x_{21} > p_2 \times q_1$
$A_2\,B_2$	x_{22}	$x_{22} > p_2 \times q_2$	$x_{22} < p_2 \times q_2$

Linkage Disequilibrium. Table 4 The linkage disequilibrium parameter D

Haplotype	Observed frequency
$A_1\,B_1$	$x_{11} = p_1 \times q_1 + D$
$A_1\,B_2$	$x_{12} = p_1 \times q_2 - D$
$A_2\,B_1$	$x_{21} = p_2 \times q_1 - D$
$A_2\,B_2$	$x_{22} = p_2 \times q_2 + D$

Linkage Disequilibrium. Table 5 The linkage disequilibrium parameter D'

D	D′
$D > 0$	$D' = (x_{11} - p_1 \times q_1)/\min(x_{12}, x_{21})$
$D < 0$	$D' = (x_{11} - p_1 \times q_1)/\min(x_{11}, x_{22})$
$D = 0$	$D' = 0$

cancer. In addition, r^2 has utility in adjustment of estimates of statistical power in genetic association studies (see below) in which an assayed ▶ polymorphism may be in linkage disequilibrium with the truly causal polymorphism. Sample size must be increased by a factor of $1/r^2$. For example, if a sample size of 2,000 were required to detect a particular association between phenotype and a causal polymorphism ($r^2 = 1.0$), a sample size of 2,500 ($= 2{,}000 \times 1.25$) would be required to detect the association if the causal polymorphism was in linkage disequilibrium with an assayed polymorphism at $r^2 = 0.8$ but was itself not assayed.

Estimation of Linkage Disequilibrium

Estimation of linkage disequilibrium between alleles at two loci requires observations of haplotype frequencies which is not currently technologically feasible in diploid organisms. Haplotype frequencies are, therefore, often estimated using statistical tools such as the expectation maximization (EM) algorithm. These methods take as input the observed combined ▶ genotype frequencies at the two loci (for example, the distribution of the nine possible combinations of A_1 A_1, A_1 A_2, and A_2 A_2, with B_1 B_1, B_1 B_2, and B_2 B_2).

Estimates of the observed haplotype frequencies are used for calculation of linkage disequilibrium measures. Numerous software packages facilitate these calculations ranging from EH (which provides estimates of observed haplotype frequencies) to Haploview (which calculates and graphically displays multiple linkage disequilibrium measures, Fig. 1).

Haplotype Blocks

Because of linkage disequilibrium in the human genome, there are regions in which only a reduced set of haplotypes occur relative to all possible haplotypes. These are referred to as haplotype blocks, and they can be defined in a variety of ways. A common definition for haplotype blocks is a region without "substantial" ancestral recombination; pairs of polymorphisms are considered to be in "strong LD" if the one-sided upper 95% confidence bound on D' is $\geq$0.98 and the lower bound is $\geq$0.70. Pairs are deemed to have "strong evidence of historical recombination" if the upper confidence bound on D' is $\leq$0.90. Based on this, haplotype blocks can be defined as regions over which $\leq$5% of comparisons among informative polymorphism pairs show strong evidence of historical recombination (Fig. 2).

Linkage Disequilibrium Versus Linkage

Genetic ▶ linkage exists when two alleles are co-inherited within a pedigree and this phenomenon is observed across multiple pedigrees. These loci are in linkage because they occur near enough to each other on the same chromosome such that the frequency of recombination (measured as θ) is relatively low. Linkage disequilibrium differs from linkage, in that the former describes alleles while the latter describes loci.

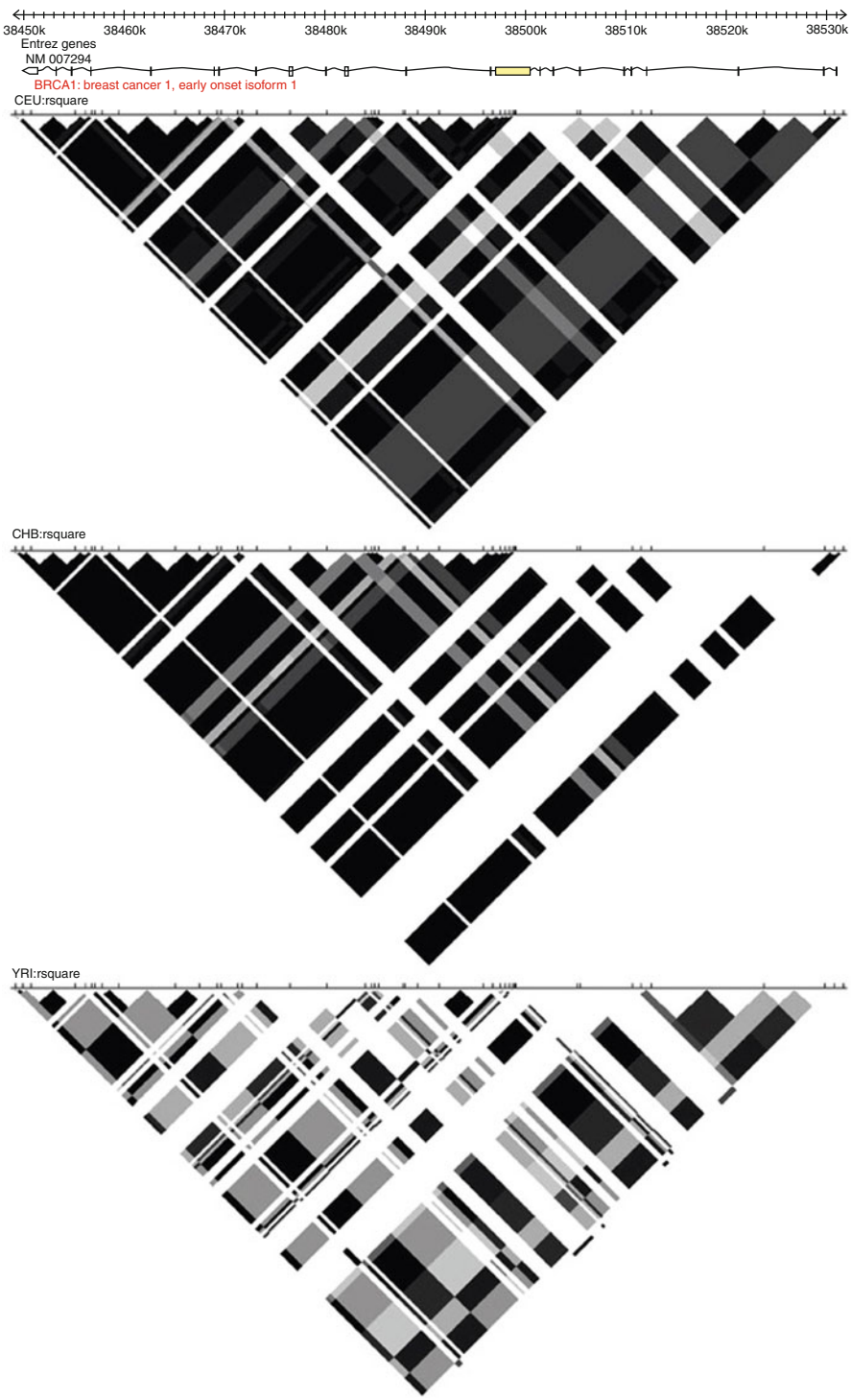
Origins of Linkage Disequilibrium

Linkage disequilibrium arises when a mutation event gives rise to a new allele on a particular chromosome in an individual. The new allele will be associated with the alleles already present on that individual's chromosome for all other loci. In time, as this person reproduces and the population grows, recombination between the new mutation and surrounding loci will return alleles in this region to equilibrium, and the new mutation will occur on chromosomes regardless of the background of surrounding loci.

In stable populations, two factors inhibit this return to linkage equilibrium: time and genetic distance. The closer two loci are, the more time (number of recombination events) is required for linkage disequilibrium to break down, and the more recent the mutational event occurred, the larger the region of linkage disequilibrium.

Linkage Disequilibrium in Human Populations

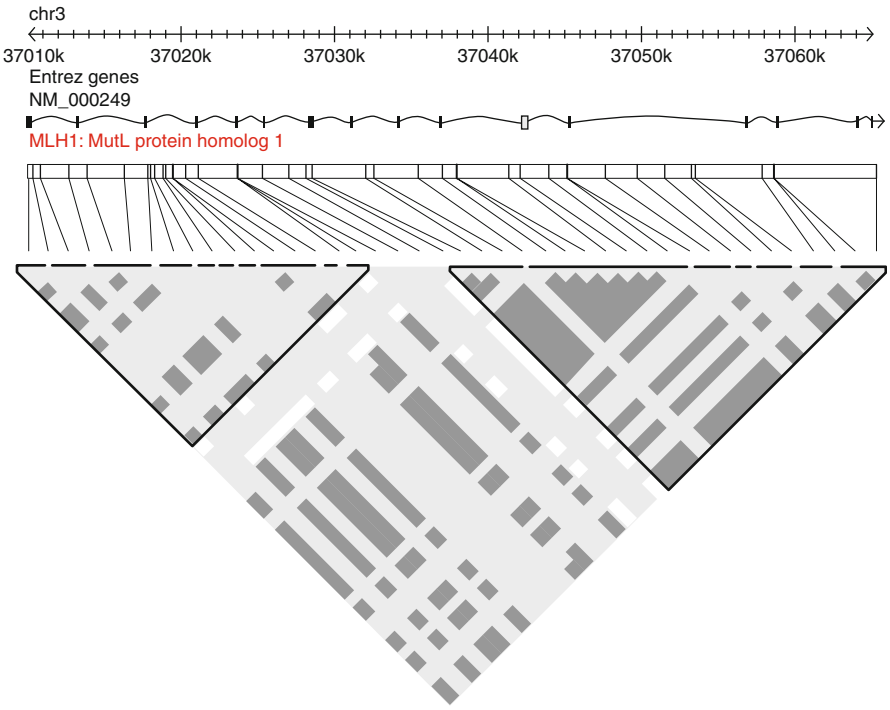
Linkage disequilibrium can also reflect instability or changes in populations. Because modern human chromosomes represent the bottom generation of a very large *Homo sapiens* pedigree, patterns of linkage disequilibrium track with human migration patterns. The migration out of Africa into Europe and Asia is seen, for example, in the observation that modern-day Africans tend to have less linkage disequilibrium,



Linkage Disequilibrium. Fig. 1 Plots of pairwise linkage disequilibrium (r^2) for polymorphisms in the ▶ *BRCA1* region genotyped in three populations by the International HapMap Project. CEU, Utah residents with ancestry from northern and western Europe; CHB, Han Chinese in Beijing, China; YRI, Yoruba in Ibadan, Nigeria; white, $r^2 = 0$; black, $r^2 = 1.0$; HapMap Release 22; chromosome 17 NCBI Build 36

generally, than Europeans or Asians. The relatively small number of "founding" chromosomes which migrated to new continents limited the variation induced by recombination. Therefore, more linkage disequilibrium is generally observed in populations which arose relatively recently.

The International HapMap Project is a large consortium formed in 2002 which seeks to characterize



Linkage Disequilibrium. Fig. 2 Plot of haplotype blocks among polymorphisms in the *MLH1* region (► colon cancer) genotyped in Utah residents with ancestry from northern and western Europe by the International HapMap Project. *Black*, strong linkage disequilibrium; *gray*, uninformative; *white*, recombination; HapMap Release 22; chromosome 3 NCBI Build 35

haplotypes and linkage disequilibrium in a variety of human populations. Researchers have performed genotyping on millions of genetic markers in individuals from four populations including Yoruba in Ibadan, Nigeria; Japanese in Tokyo, Japan; Han Chinese in Beijing, China; and Utah residents with ancestry from northern and western Europe. These data are publicly available to researchers who aim to analyze these genetic markers in relation to health conditions, including cancer.

An alternative way to characterize linkage disequilibrium in human populations involves resequencing genes of interest in a variety of DNA samples. Resequencing data has the advantage that a variety of polymorphism types may be detected (including insertions and deletions) and that rare alleles may be characterized. In 1998, the US National Institute of Environmental Health Sciences embarked on the Environmental Genome Project including resequencing of key environmentally responsive cancer candidate genes in the populations also assessed by the International HapMap Project. These dense polymorphism data complement the genome-wide HapMap approach and allow researchers access to finer-scale linkage disequilibrium information in key genes involved in ► DNA repair, ► inflammation, ► apoptosis, ► cell cycle, and other pathways.

Using Linkage Disequilibrium to Select Informative Markers

Genetic association studies in ► cancer epidemiology aim to identify common inherited variants which are related to risk of cancer (► case–control studies), treatment response, survival, and other endpoints. These studies often focus on particular candidate genes (suspected because of known biological function related to cancer). Candidate gene studies may utilize linkage disequilibrium in that, rather than genotyping every genetic marker in a suspected gene or genomic region, only those markers thought to be independent are assessed. This "tagging SNP" approach can allow for greater gene coverage and cost efficiency. For example, in a study of 260 candidate genes related to the ► NF-κB pathway, 17,360 common polymorphisms were estimated to be captured by 2,181 tagging polymorphisms.

Numerous methods have been developed to identify subsets of tagging polymorphisms based on analysis of multiple polymorphisms in a genomic region. For example, pairwise-binning methods aim to identify the subset of polymorphisms which are linkage disequilibrium at a specific r^2 threshold with all available polymorphisms, and haplotype-tagging methods aim to identify the subset of polymorphisms which tag all

estimated haplotypes over a certain haplotype frequency threshold. ldSelect and Tagger are two commonly used software tools for identifying tagging polymorphisms.

Data from the HapMap Project, the Environmental Genome Project, other public sources, as well as study participant data, can be used for selection of the optimal set of tagging polymorphisms. It is important to note that population differences in linkage disequilibrium (for example, due to ethnicity or sampling variation) between the more densely genotyped samples (for example, HapMap populations) and the target population (for example, a cancer genetic association study population) must be accounted for when using tagging polymorphisms.

Utility of Linkage Disequilibrium to Genome-wide Association Studies

Genetic association studies can also search the entire genome allowing for identification of cancer-related loci in unpredicted regions. Genome-wide association studies would not be feasible without capitalizing on linkage disequilibrium. This allelic association allows for a reduced set of markers to represent over 12 million common polymorphisms thought to exist. Numerous commercial panels are available for high-throughput assessment of genome-wide polymorphisms, including from 300,000 polymorphisms to 1,000,000 polymorphisms. For example, a panel available in 2007 from Illumina Corporation (San Diego, CA) assessed approximately 550,000 polymorphisms but was estimated to offer as much genetic information (at $r^2 = 0.85$) as assaying 57%, 89%, and 90% of the 3.7 million HapMap polymorphisms among the Yoruban, Asian, and Utah populations described above.

Other Uses of Linkage Disequilibrium in Cancer Research

Linkage disequilibrium also informs hypothesis testing in genetic association studies. The causal allele, if it exists, may be included in the set of assayed markers or it may lie on a haplotype defined by the set of assayed markers. Analysis of haplotypes may, therefore, provide additional information on association. Numerous methods exist for testing haplotype associations with disease (including score tests, logistic regression, and Bayesian methods). All methods first estimate haplotype frequencies (or probabilities) because haplotypes are usually not directly observed.

If an association signal is detected, linkage disequilibrium can be used in refinement of the signal where an association is detected (fine-mapping). A particular polymorphism found to be associated with disease may be in linkage disequilibrium with the truly causal allele. Thus, additional analysis of nearby polymorphisms may increase the association signal and provide a targeted region for functional genomics analysis. In summary, incorporation of linkage disequilibrium has the potential to improve the power and efficiency of genetic association studies leading to increased understanding of the genetic causes of cancer.

▶ Case-Control Association Study

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Linoleic Acid

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Definition

Linoleic acid (LA) is an 18-carbon, polyunsaturated fatty acid (PUFA), which contains two *cis* double bonds. Because mammals cannot introduce a double bond at carbon atoms beyond *C-9* in the fatty acid chain, linoleic acid (18:2 *cis*-Δ^9,Δ^{12}) and linolenic acid (18:3 *cis*-Δ^9, Δ^{12},Δ^{15}) are two essential fatty acids. LA possesses low melting temperature and provides fluidity to cell membranes. LA is mainly contained in plant oils, such as safflower oil and corn oil.

Characteristics

The effect of linoleic acid (LA) on health is still controversial. LA is one of the two essential fatty acids, which means dietary supplementation of LA is necessary for maintaining cell activity. Saturated fatty acids have been implicated in obesity, heart disease, diabetes, and cancer while PUFAs generally have a positive effect on health; however, a high ω-6/ω-3 ratio, which is associated with today's Western diets, promotes the pathogenesis of many diseases, including cardiovascular disease, inflammatory diseases, and cancer. Several in vivo studies suggest that a high amount of ω-6 PUFA such as LA might enhance the incidence of some types of cancers via stimulation of epithelial cell proliferation. Experiments in animal models of mammary and colorectal carcinogenesis suggest that fatty acids promote tumor development; ω-6 PUFA generally stimulate tumor growth, while ω-3 fatty acids oppose this effect.

Other studies lead to opposite conclusions. The associations between monounsaturated fatty acids, trans fatty acids, PUFAs such as LA, alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), or ω-3/ω-6 ratio, and colorectal cancer are not convincing. Contrary to data from animal experiments, human studies do not show an association of breast cancer risk with ω-6 PUFA intake. High LA and arachidonic acid (AA) concentrations have been observed in insulin resistance–associated diabetic complications and in some tumors, but these are multifactorial processes that include many lifestyle determinants. It is therefore questionable to involve only ω-6 fatty acids in their etiology.

The controversial roles of LA might be due to the possibility that different metabolic pathways result in opposite effects. ω-6 PUFAs play a significant role in inflammatory and/or immune responses by bioactive molecules including PGE2.

LA is integrated into cytoplasmic membrane and is metabolized to AA through γ-linolenic acid, and dihomo-γ-linolenic acid (▶ Arachidonic Acid-Pathway and Cancer). AA is stored as phospholipids in cytoplasmic membrane and serum, and is released by cleavage with phospholipase A2. AA is metabolized by cyclooxygenase-1 and cyclooxygenase-2 (COX-2) (▶ Cyclooxygenase-2 in Colorectal Cancer) to ▶ prostaglandins (PGs), thromboxanes (TXs), and ▶ Leukotrienes (LTs). COX-2 is an inducible enzyme of COXs, which is absent in normal cells. In contrast, COX-1 is a constitutive enzyme expressed ubiquitously. COX-2 expression is induced in carcinogenic processes in many malignancies, involving colorectal, esophageal, breast, lung, pancreatic, and bladder cancers. Induced COX-2 expression in epithelial cells and also in stromal cells provides PGE2 in the local tissues. PGE2 possesses immunosuppressive and pro-inflammatory effects. COX-2-dependent overproduction of PGE2 is hypothesized to be an important part of sustained proliferative and chronic inflammatory conditions in colorectal epithelium, which are closely associated with carcinogenesis (▶ Inflammation in Cancer). PGE2 also has a strong association with colorectal cancer progression by promoting cell survival, cell growth, migration, invasion, and angiogenesis (▶ Colon Cancer). The various biological effects exerted by PGE2 are through the G-protein coupled cytoplasmic membrane E-prostanoid receptors termed EP1 to EP4.

15-Lipoxygenase-1 (15-LOX-1) is known for its anti-inflammatory properties and has a profound influence on the development and progression of cancers. 15-LOXs belong to the structurally and functionally related nonheme iron dioxygenases family. 15-LOXs are responsible for oxidative metabolism of ω-6 PUFAs, such as LA and AA to eicosanoids. Two isoforms are known in 15-LOXs; 15-LOX-1 (leukocyte type) and 15-LOX-2 (epidermis type). Both isoforms are expressed in normal and tumor tissues in various combinations. Different from other LOXs, such as 5-LOX and 12-LOX, 15-LOXs, 15-LOX-1 is revealed also as an ▶ apoptosis inducer in human cancers and inhibits cancer progression in several types of cancers, including colorectal, and breast cancers. By the contrary, reduction of 15-LOX-1 is correlated with the disease progression of breast and colon cancers.

For antitumor effects, 15-LOX-1 is closely associated with peroxisome proliferator-activated receptor γ (PPARγ) (▶ Peroxisome Proliferator-Activated Receptor and Cancer) activation. The oxidative metabolites of LA by 15-LOX-1 can function as endogenous activators and ligands of PPARγ. In particular, 9-hydroxyoctadecadienoic acid (9-HODE), 13-hydroxyoctadecadienoic acid (13-HODE), and 13-oxooctadecadienoic acid (13-OXO) have biological effects as a PPARγ ligand. PPARγ is originally identified to induce adipocyte differentiation. PPARγ

is a nuclear hormone receptor superfamily of ligand-activated transcription factors. PPARγ is dimerized with retinoic X receptor (▶ Retinoid Receptor Crosstalk in Cancer), and binds specific responsive element within promoter DNA sequence to regulate gene expression. PPARγ initiates transcription of genes associated with energy homeostasis, cell growth, and anti-/pro-inflammatory effect. PPARγ is activated by endogenous secreted prostaglandins and fatty acids. 15-Deoxy-δ(12,14)-prostaglandin J2 is a strong endogenous ligand of PPARγ. Decrease in PPARγ expression is associated with cancer metastasis. PPARγ plays a role in transcriptional regulation of cancer-related genes. Ligand activation of PPARγ in colorectal cancer cells attenuates colonic inflammation and causes a reduction growth via the induction of apoptosis. Conjugated LA (CLA), a strong ligand for PPARγ, has a substantial anticarcinogenic effect. Synthesized PPARγ ligands including troglitazone have been shown to be effective chemopreventive agents in a rat model of carcinogenesis and in AOM-induced colon cancer in mice. In in vitro transformation model, LA inhibits intestinal cell transformation. Inhibitory effect of PPARγ to cancer metastasis is also reported in several cancers, such as non-small cell lung cancer, colon cancer, thyroid cancer, and breast cancer. Downregulation of EGFR, TGF-α (▶ Epidermal Growth Factor Receptor Ligands), and upregulation of Bax, p21Waf-1 (▶ p21(WAF1/CIP1/SDI1)), ▶ E-cadherin by PPARγ activation induce antiproliferative, proapoptotic, and prodifferentiation effects. These alterations of gene expressions provide LA-induced anticarcinogenic, antitumor, and antimetastatic effects on cancer cells.

The sequential alteration of concurrence of COX-2 upregulation and 15-LOX-1 downregulation is found in the adenoma–carcinoma transition in colorectal neoplasia (▶ Colorectal premalignant lesions). Low-grade adenomas express 15-LOX-1 but not COX-2; high-grade adenomas and early carcinomas show decreased 15-LOX-1 expression and induction of COX-2 expression; and advanced carcinomas express COX-2 but not 15-LOX-1. It possibly shows close association of the switching of LA-metabolizing pathways with colon cancer development and progression. In expression of 15-LOX-1, several conditions play an important role. Cytokines, such as IL-4 (▶ Interleukin-4) and IL-13, high ratio of ω-3/ω-6 fatty acids, and ▶ nonsteroidal anti-inflammatory drugs (NSAIDs), such as sulindac sulfone induce 15-LOX-1 expression and activation of PPARγ. Reciprocally, activated PPARγ represses COX-2 by inhibition of NFκB (▶ Nuclear Factor κB) and ▶ AP-1. This negative regulation of COX-2 expression by PPARγ activation might be one of mechanisms of reverse expression between 15-LOX-1 and COX-2. Furthermore, promoter DNA methylation is responsible for silencing of 15-LOX-1 expression (▶ Epigenetic Gene Silencing). The epigenetic alteration might be a trigger to switch 15-LOX-1 repression and COX-2 upregulation along with malignant transformation and cancer progression in colon cancer.

Excess uptake of LA increases cancer metastasis by enhancing cell embedding into the target organs. LA-derived TXA2 accelerates platelets aggregation involving cancer cells. LA also affects cancer cell activity. Short-term treatment with LA induces apoptosis in cancer cells. In the nude mice peritoneal dissemination model, LA treatment inhibits formation of peritoneal metastasis. In contrast, cancer cells exposed to LA for a long term show quiescent condition in vitro and dormancy in transplanted animals. In these cells, decrease of EGFR, VEGF (▶ Vascular Endothelial Growth Factor), and increase of ▶ BCL-2 are observed. Thus, LA might play a role in formation of cancer cell dormancy and delayed metastasis.

In summary, the effects of LA on human health are still not clearly figured out. As LA is an essential fatty acid, we cannot cease LA uptake. In further studies, it is important to make use of the beneficial side of the double-edged sword of LA for prevention and treatment of cancer.

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Lipid Mediators

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Synonyms

Bioactive lipids; Lipid second messengers

Definition

Lipid mediators are bioactive molecules rapidly produced upon cell activation either by enzymatic process or by oxidative fragmentation of polyunsaturated fatty acids attached to their glycerol backbone. The majority of lipid mediators are products of degradation and/or phosphorylation/dephosphorylation of glycerophospholipids by defined enzymes acting in concert and commonly designated as ▶ phospholipases (PLs), phosphokinases, and phosphatases. Hydrolysis of ▶ phospholipids also generates free fatty acids, including arachidonate, a direct precursor of ▶ eicosanoids.

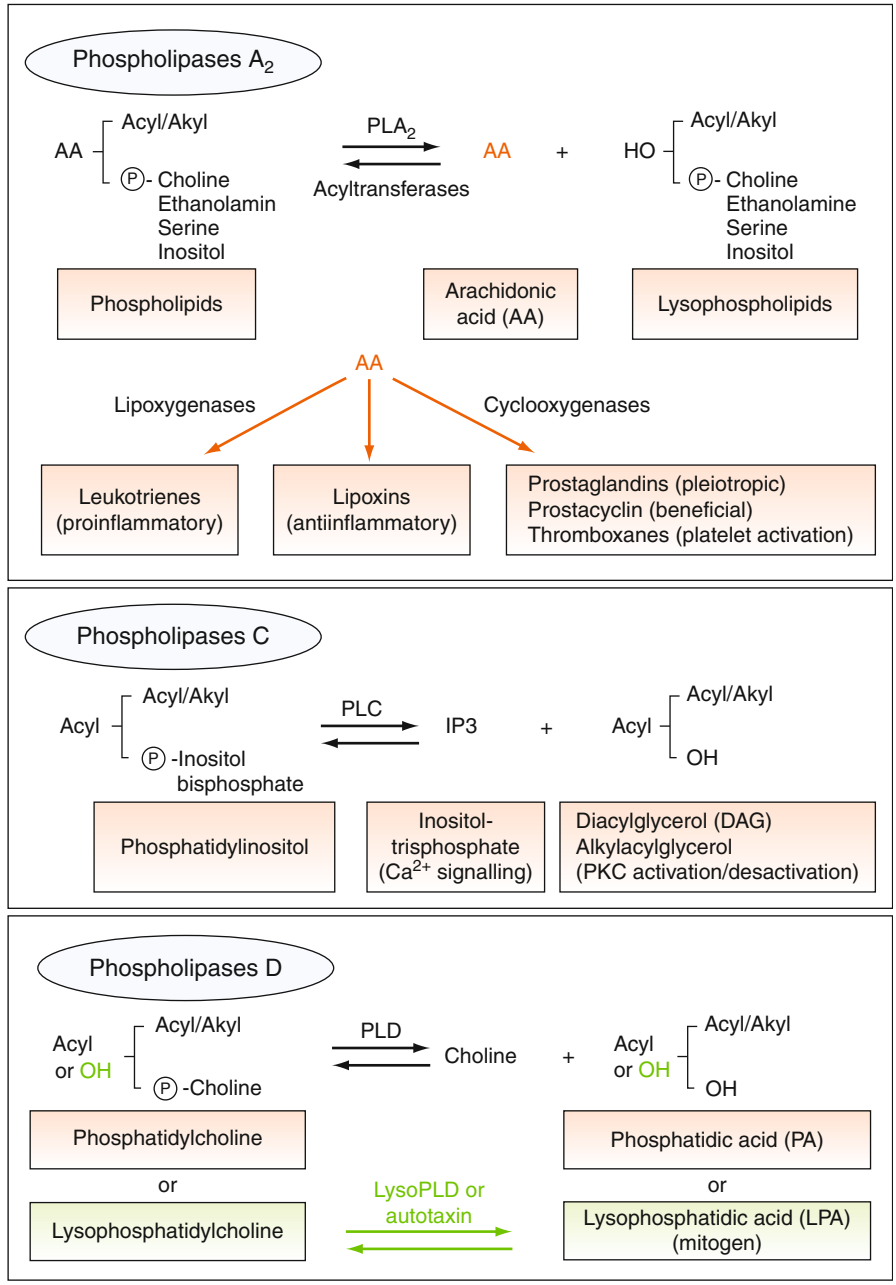
Characteristics

Within seconds after cell activation, certain phospholipids are either selectively hydrolyzed by defined phospholipases leading to highly bioactive molecules (lipids and/or fatty acids) or phosphorylated by specific kinases to bioactive ▶ phosphoinositides Inositol lipids. Essentially three phospholipase (PL) families, namely, ▶ Phospholipase A2 (PLA2), ▶ Phospholipase C (PLC), and ▶ Phospholipase D (PLD) (Fig. 1) are involved in the degradation of structural phospholipids, leading either to direct formation of lipid mediators (▶ lysophosphatidylcholine in Cancer, ▶ inositoltrisphosphate (IP3), and ▶ diacylglycerol (DAG)) or to freeing of their immediate precursors (polyunsaturated fatty acids, including arachidonic (▶ Arachidonic Acid-Pathway And Cancer) and ▶ Linoleic acid). Among PLA2s, the secretory group II PLA2 can produce (Lysophospatidic Acid (LPA) directly from phosphatidic acid. Recently described ▶ autotaxin, which is widely implicated in tumor progression, possesses lysoPLD activity and generates LPA from lysophosphatidylcholine (Fig. 1).

Free arachidonate, produced by degradation of phospholipids by PLA2, is a substrate for three major classes of lipid mediators (Fig. 1) (▶ Arachidonic acid-pathway and cancer): prostanoids/thromboxanes (▶ Prostaglandins), (▶ Leukotriene), and lipoxins, the latter being beneficial as they induce the resolution of the inflammatory response (▶ Inflammation). Upon (▶ Oxidative stress) arachidonate esterified to phospholipids at the sn-2 position of glycerol is oxidized either to ▶ isoprostanes, very potent proinflammatory mediators (▶ Bioactive lipid signaling) or to several ▶ oxidized phospholipids with short carbon chains resembling ▶ Platelet-activating-Factor (PAF) and endowed with potent capacity to modulate the immune response and (▶ Angiogenesis). Interestingly, an analogue of PAF containing a non-hydrolysable methyl group at the sn-2 position of glycerol, ▶ ET-18-OCH3 or edelfosine, is a selective antitumor phospholipid targeting (▶ Apoptosis) via endogenous activation of Fas/CD95 death receptor.

▶ Sphingolipids are a subclass of phosholipids which enter many metabolic pathways and constitute an interconnected network of signaling molecules with crucial roles in both cancer development and progression (▶ Cancer). ▶ Sphingomyelin, a structural phospholipid, is hydrolyzed by sphingomyelinases to a tumor suppressor lipid, ▶ ceramide, with proapoptotic and antiproliferative properties (Fig. 2) (▶ Ceramide). Ceramide may be either phosphorylated to ▶ ceramide-1-phosphate (C1P) with proinflammatory and antiapoptotic properties or may be degraded to ▶ sphingosine, a substrate for phosphorylation leading to S1P, a potent tumor-promoting lipid endowed with angiogenic and immunosuppressive properties. Additionally ceramide is glycosylated into ▶ gangliosides, bearing various sugars and endowed with highly tumorigenic properties (Fig. 2) (▶ Ganglioside). It is admitted today that the balance between the tumor suppressor molecule – ▶ ceramide – and its tumor-promoting metabolites is of prime importance in (▶ Carcinogenesis) and anticancer therapy.

▶ Conjugated linoleic acid (CLA), which is a mixture of positional and stereoisomers of octadecadienoate (18:2) found in foods derived from ruminants, may reduce carcinogenesis; however, the data are still controversial. Equally, prostaglandin J2, which derives from oxidation of arachidonate by cyclooxygenases

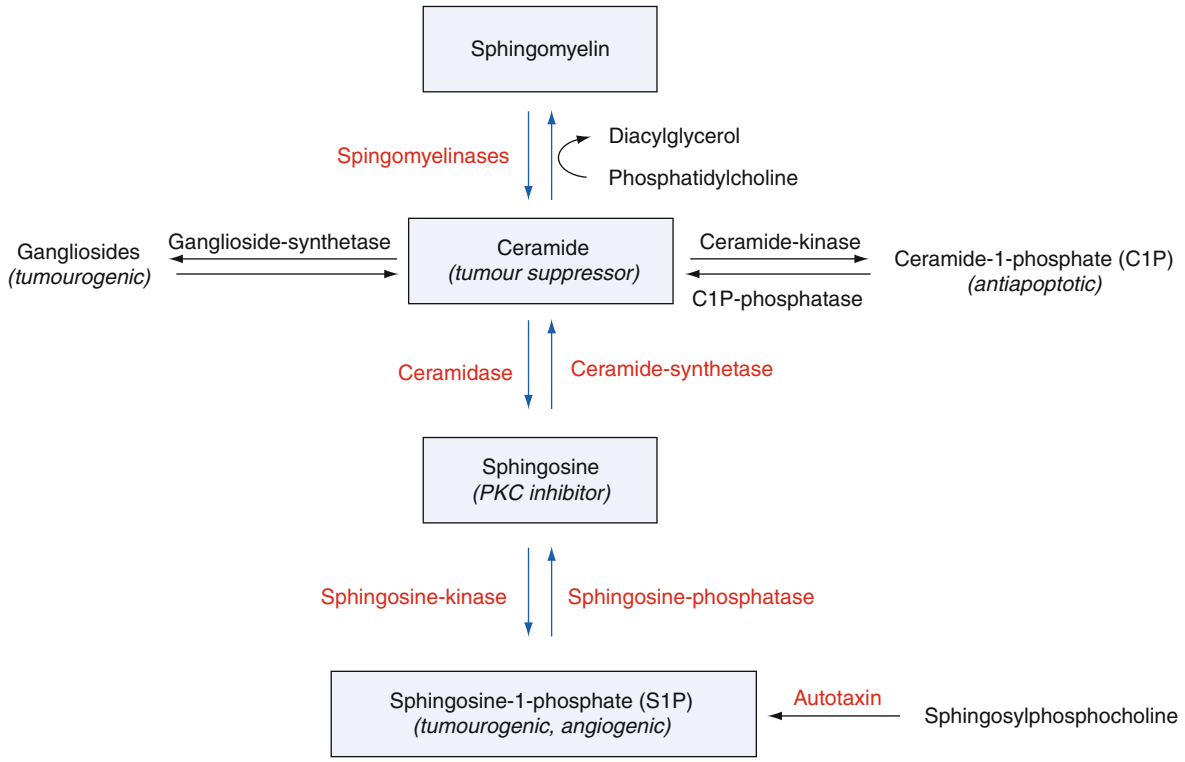


Lipid Mediators.
Fig. 1 Schematic representation of production of various lipid mediators by phospholipases

(COXs) is an emerging antineoplastic agent as it induces death receptor 5 (DR5), a specific ▶ receptor for TNF-related apoptosis-inducing ligand (TRAIL).

The majority of lipid mediators target distinct families of G-protein coupled receptors and activate vital cellular defenses usually defined as inflammatory reactions; however, some of these mediators, including LPA, modulate cell proliferation, growth, survival, and (▶ Migration) enabling the expansion of defined cell subsets. LPA levels are elevated in situ and in circulation in ovarian tumors and in multiple myeloma, supporting its causal effect in proliferation and thus eventual use as a target and/or a diagnostic marker.

▶ Protein Kinase C (PKC) isoforms are a family of serine/threonine protein kinases commonly divided into three subfamilies: classical, novel, and atypical. PKC isoformes, whose expression is cell-type specific and developmentally regulated, are key transducers in



Lipid Mediators. Fig. 2 Schematic representation of bioactive sphingolipid formation from sphingomyelin

many agonist-induced signaling pathways (▶ Protein kinase C family). At least ten different PKC isoforms have been identified and are believed to play distinct regulatory roles. PKC isoforms are catalytically activated by several lipid cofactors, including DAG generated by receptor-mediated hydrolysis of membrane phospholipids. IP3 triggers Ca^{2+} release from internal stores, and the elevation of cytosolic Ca^{2+} acts synergistically with DAG to activate the relevant forms of PKC. PKC isoforms reside in the cytoplasm in an inactive conformation and undergo translocation to the plasma membrane or cytoplasmic organelles upon cell activation; however, PKC isoenzymes are also capable of translocating to the nucleus and certain isoforms can even reside within the nucleus. Certain PKC isoforms are often overexpressed in cancer. Tumor-promoting phorbol esters and DAG activate classical and novel PKC isoforms. Naturally occurring retinoids (▶ Retinoic Acid), antisense oligonucleotides against specific PKC isoforms and specific PKC inhibitors can block this activation. Beta-carotene and retinoid derivatives act as anticarcinogenic agents and can antagonize some of the biological actions of phorbol esters and oxidants.

One of the major inositol-containing lipid second messengers, the ▶ phosphoinositide-3,4,5-trisphosphate (PIP3) (▶ Phosphoinositide-3,4,5-trisphosphate-kinases (PI3-kinases)) is generated by the action of PI3-kinases activated in response to a variety of extracellular signals (▶ PI3K Signaling). Phosphatase ▶ PTEN, one of the most frequently mutated genes in human cancer, acts as a tumor suppressor by dephosphorylating PIP3, thus preventing both elevated levels of PIP3 and tumorigenesis (▶ PTEN). Loss or inactivation of PTEN leads to the formation of a variety of tumors, probably by deregulating ▶ the mammalian target of rapamycin (mTOR) signaling pathway. As mTOR activity can be suppressed by various compounds, including (▶ Rapamycin), it may be useful to treat tumors carrying inactivated PTEN with such drugs.

Inflammation has been associated with cancer recently. Besides cytokines and chemiokines incriminated already, lipid mediators are also implicated since the discovery that cyclooxygenase-2 (COX-2) inhibitors (▶ coxibs) (▶ Celecoxib) slow the progression of colorectal cancer (▶ Cyclooxygenase-2 in colorectal cancer) and that the COX-2 levels are increased in several tumors (colon, breast, ovarian, and melanomas); for

this reason, the gene encoding for COX-2 is now considered an (► Oncogene). Equally, the products of 5-lipoxygenase may play a role in human tumors (brain, skin). Finally, the relatively recently discovered ► oxidized phospholipids, the analogues of the mediator PAF, are involved in the induction of immune suppression and might be instrumental in inducing cancer.

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Lipid Peroxidation

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Definition

Lipid peroxidation is the metabolic process in which ► reactive oxygen species (ROS) result in the oxidative deterioration of lipids. This may significantly affect cell membrane structure and function.

Characteristics

Lipid peroxidation most often affects ► polyunsaturated fatty acids, because they contain methylene –CH2– groups which contain hydrogen that is especially reactive with ROS. Increased ROS production occurs in inflammation, during radiation, or during metabolism of hormones, drugs, and environmental toxins. This can overwhelm endogenous protective ► antioxidant mechanisms and increase ROS-mediated damage to membrane structure and function. Such ROS reactions can also lead to protein damage, including DNA repair enzymes and polymerases, impairment, and production of aldehyde by-products such as malondialdehyde (MDA; β-hydroxy-acrolein) and 4-hydroxy-2-nonenal (HNE). MDA is formed during homolytic decomposition of lipid hydroperoxides that contain more than two double bonds. MDA reacts with DNA to form primarily a propane adduct with 2′-deoxyguanosine (M1G-dR). Although they have important physiological roles in cell proliferation, transformation, differentiation, and ► apoptosis these aldehydes are also strongly carcinogenic. Mutagenicity of MDA and HNE, the major aldehyde products, has been clearly demonstrated. These can promote the formation of DNA-adducts which are required to be repaired in order to maintain the fidelity of the DNA. If not, DNA ► mutations can occur. For example, the reaction between the epoxide of HNE with DNA leads to the formation of unsubstituted etheno-dAdo adducts. Etheno adducts are mutagenic and have been detected in human tissue samples providing an important link between lipid peroxidation and in vivo DNA-adduct formation. Alternatively, lipid peroxidation and ROS are triggers and essential mediators of apoptosis, which eliminates precancerous and cancerous, virus-infected and otherwise damaged cells. This suppression of cell cancer growth is enhanced by pro-oxidants and eliminated by antioxidants, and this elimination is proportional to the inhibition of lipid peroxidation products by antioxidants. Lipid peroxidation may also play an important role in the potential anticarcinogenic effects of other dietary factors including soy, marine n-3 fatty acids, isothiocyanates, green tea, and vitamin D and calcium.

Mechanisms

As with any radical reaction, the reaction consists of three major steps: initiation, propagation, and termination. Initiation is the step whereby a fatty acid radical is produced. The initiators in living cells are most notably ROS such as hydroxyl radical, which combines with a hydrogen atom to make water and a fatty acid radical. The fatty acid radical is not a stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This too is an unstable species that reacts with another free fatty acid producing a different fatty acid radical and a ► hydrogen peroxide molecule or a cyclic peroxide molecule if it had reacted with itself. This cycle propagates itself as the new fatty acid radical reacts in the same way. This results in a chain reaction and the only way to stop a radical reaction is for two radicals to react and produce a non-radical species. This occurs when the concentration

of radical species is high enough for there to be a high probability of two radicals actually colliding. However, in organisms there are a number of different molecules which bind and quench free radicals and so protect lipids from oxidation. These are usually lipid-soluble vitamins such as alpha-tocopherol or vitamin E.

ROS-mediated formation of lipid hydroperoxides involves the initial abstraction of a bis-allylic methylene hydrogen atom. Lipid hydroperoxides can also be formed by the action of cyclooxygenases and lipoxygenases on polyunsaturated fatty acids (PUFAs). LOX- and COX-mediated pathways of PUFA metabolism can potentially provide a rich source of lipid hydroperoxides.

Clinical Aspects

Chronic inflammation, part of the host immune response, has long been recognized to be associated with the development and progression of cancer. The combination of excess oxidant production and antioxidant depletion, and therefore, oxidative stress, may play a role in the development and progression of cancers. High ROS generation and persistent oxidative stress have been recognized as characteristic features of carcinoma cells both in vivo and in vitro. Also, it is widely accepted that patients with advanced cancer have reduced circulating antioxidant concentrations. Therefore, in the cancer patient the risk for structural and functional damage of cell membranes is likely to be increased. Higher levels of circulating plasma MDA have been observed in different malignancies, including lung, gastrointestinal, and hormone-dependent cancers. However, whether such increased MDA concentrations are primarily due to the tumor, the ► inflammatory response or some other factors remain to be determined.

► Reactive Oxygen Species

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Lipid Phosphatases

Definition

A family of signaling molecules in the cells that includes MAPK and PI3K.

► MAP Kinases
► Tight Junction

Lipid Raft

Definition

Cholesterol- and sphingolipid-enriched microdomains in plasma membranes; they function as signaling platforms.

► Natural Killer Cell Activation

Lipid Second Messengers

► Lipid Mediators

Lipid Therapy

► Membrane-Lipid Therapy

Lipogenesis

Definition

The process that converts nonfat food materials into body fat.

► Cachexia

Lipoma

Definition

Is a benign neoplasm of adipose tissue. One of the most common soft tissue tumors in men and form part of the daily practice of every surgical pathologist. Like fat, lipomas are mainly composed of mature fat cells, but the cells vary slightly in size and shape and are somewhat larger. The tumors are usually thinly encapsulated and have a distinct lobular pattern. A minority of all lipoma patients has multiple lesions, but most patients only have one tumor. Most of these solitary lipomas cause few problems other than those of a localized mass.

- ▶ Adipose Tumors
- ▶ Cowden Syndrome
- ▶ Lipoma Preferred Partner

Lipoma Preferred Partner

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Synonyms

LIM domain containing preferred translocation partner in lipoma; LPP

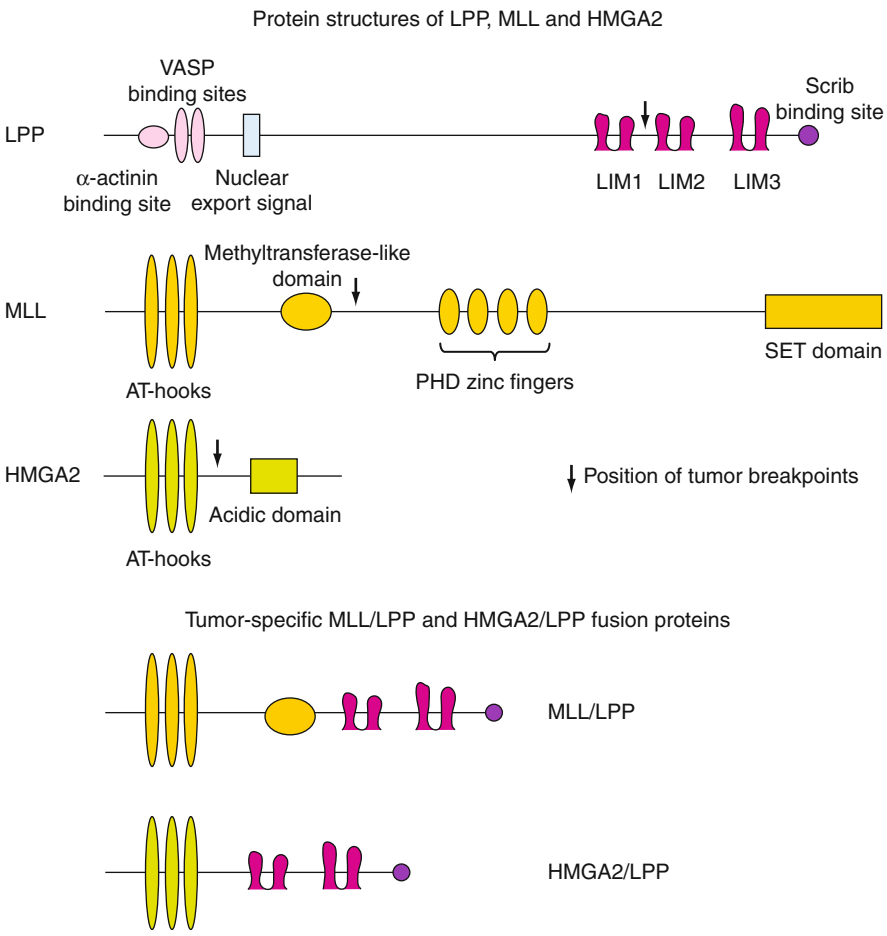
Definition

Lipoma-preferred partner (LPP) is a ▶ LIM domain protein that belongs to the ▶ zyxin family of cytoskeletal adaptor proteins. LPP is localized in cellular adhesion sites and is in certain conditions also detected in the nucleus where it acts as a transcriptional co-activator. It is highly expressed in smooth muscle cells and plays a role in cell migration. In particular tumors, aberrant LPP proteins are expressed in which the LIM domains are fused to DNA-binding domains. These tumor-specific LPP fusion proteins function as transcription factors.

Characteristics

LPP and Tumors

▶ Lipomas are benign tumors of adipose tissue and one of the most commonly occurring soft tissue tumors in humans. A minority of patients have multiple tumors, however most patients have only one tumor. More than 60% of human solitary lipomas have an abnormal ▶ karyotype. Two-thirds of the latter carry chromosomal aberrations, mostly translocations, involving chromosomal region 12q15. The gene on 12q15 that is affected by these translocations is ▶ HMGA2. The HMGA2 protein consists of three AT-hooks (DNA-binding domains) followed by an acidic carboxyterminal tail (see Fig. 1). By means of the translocation, the DNA-binding domains are separated from the acidic tail. Multiple chromosomes have been identified as translocation partner of 12q15 in lipomas by cytogenetic analysis, indicating that many different genes are able to act as translocation partner of HMGA2 in these tumors. However, in a quarter of the cases, chromosome 3 at bands q27-q28 is found as the translocation partner of chromosome region 12q15. This means that the most consistent chromosomal aberration in lipomas is represented by t(3;12)(q27-q28;q15), being present in about 10% of all solitary lipomas. In 1996, the gene on 3q27-28 that is affected by the t(3;12) was discovered in the lab of Wim Van de Ven from the University of Leuven, Belgium and named "LPP" for "lipoma-preferred partner." After publication, the HUGO gene nomenclature commission advised to change the name of LPP in "LIM domain containing preferred translocation partner in lipoma." Indeed, the LPP protein contains three LIM domains in its carboxyterminus that are preceded by a proline-rich pre-LIM region (Fig. 1). Through the t(3;12), an *HMGA2/LPP* fusion gene is created encoding an HMGA2/LPP fusion protein consisting of the three DNA-binding domains of HMGA2 followed by the two most carboxyterminal LIM domains of LPP (see Fig. 1). Fusion transcripts encoding identical HMGA2/LPP fusion proteins have also been detected in other tumors including pulmonary chondroid hamartomas, a ▶ parosteal lipoma, and a soft tissue chondroma. The HMGA2/LPP fusion protein localizes in the nucleus of cells and is a tumor-specific transcription factor: It binds DNA in the promoter of its target genes with the DNA-binding domains of HMGA2 and activates transcription



Lipoma Preferred Partner.
Fig. 1 *Upper part*: Schematic representation of the LPP, MLL, and HMGA2 proteins. *Lower part*: Schematic representation of the tumor-specific MLL/LPP and HMGA2/LPP fusion proteins

through the LIM domains of LPP. In addition to benign tumors, rearrangements of the *LPP* gene have also been found in a case of malignant secondary acute monoblastic leukemia with a t(3;11)(q28;q23). In the latter, the two most carboxyterminal LIM domains of LPP are fused to the AT-hooks (DNA-binding domains) of the mixed lineage leukemia (MLL) protein (see Fig. 1). As such, as well in benign as in malignant tumors, tumor-specific fusion proteins are composed of AT-hooks, either from HMGA2 or from MLL, and LIM domains from LPP.

Cell Biological Characteristics of the LPP Protein

LPP is a member of the zyxin family of LIM domain proteins, which consists of seven members: zyxin, TRIP6/►ZRP1, LPP, ►ajuba, ►LIMD1, ►WTIP, and ►migfilin/Cal. LPP is most closely related to zyxin and ►thyroid receptor interacting protein 6 (TRIP6). All members of the zyxin family contain a proline-rich region (in LPP this region covers the amino-terminal 2/3 of the protein), which is followed by three LIM domains. LPP contains multiple protein–protein interaction domains. Known binding partners include the cytoskeletal proteins α-actinin, VASP (vasodilator-stimulated phosphoprotein) and palladin, the ETS domain transcription factor PEA3, and the tumor suppressor Scrib. In addition, LPP probably also interacts with a number of yet unidentified proteins that bind its three carboxyterminal LIM domains, which are modular protein-binding interfaces.

In cultured fibroblasts and aortic SMCs, LPP colocalizes with vinculin at focal adhesions, which are attachment sites to the extracellular matrix. The three LIM domains of LPP cooperate to target the protein to these adhesion sites. LPP is also found in cell–cell contacts, and in transverse sections of bladder smooth muscle, an association of LPP with peripheral dense bodies is suggested. In addition to these

cytoskeletal localizations, LPP also shuttles to the nucleus. Its nuclear-cytoplasmic localization is regulated in part by a nuclear export signal (NES) which is sensitive to the drug leptomycin B.

LPP as a Transcriptional Coactivator

In 2006, the lab of Andy Sharrocks from the University of Manchester, UK, discovered a nuclear function for LPP as a coactivator for ▶ PEA3. PEA3 is an ETS domain transcription factor whose expression is regulated by a number of signaling cascades, including the mitogen-activated protein (MAP) kinase pathways. PEA3 is a transcriptional activator and regulates transcription of multiple genes, including the matrix metalloproteases (MMPs) MMP-1 and MMP-9. MMPs are enzymes that degrade the extracellular matrix during normal remodeling events and cancer metastasis. PEA3 is expressed during normal mammary gland development and is an important player in breast tumor metastasis. Also LPP is expressed in normal and cancerous breast tissue, and is recruited to the MMP-1 promoter in a PEA3-dependent manner.

LPP as a Smooth Muscle Marker

In 2003, two independent laboratories (the Somlyo lab from the University of Virginia, USA and the Lindahl lab from the University of Göteborg, Sweden) identified LPP as a novel smooth muscle cell marker. Although, in adults, LPP is ubiquitously expressed, it is highly expressed in vascular as well as visceral smooth muscle-containing tissues such as uterus, stomach, bladder, aorta, and portal vein. Concerning the function of the LPP protein in smooth muscle cells, LPP is involved in the regulation of their migration, as shown in in vitro experiments.

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Lipomatous Tumors

▶ Adipose Tumors

Lipophilic

Definition

Literally means fat loving or greasy. These are drugs that much prefer to dissolve in fats than in water.

▶ ADMET Screen

Lipophilicity

Definition

Defines the molecular characteristic of being attracted to nonpolar environments – literally the property of being "oil loving." This term is often used to describe a particular drug's preference for organic (nonpolar), compared to water-based (polar) environments.

▶ Chelators as Anticancer Drugs

Lipoprotein

Definition

Biochemical assembly that contains both proteins and lipids.

▶ Wnt Signaling

Liposarcomas

▶ Adipose Tumors

Liposomal

Referring to ▶ liposomes

Liposomal Chemotherapy

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Synonyms

Drug carriers; Drug delivery vesicles

Definition

The central problem in cancer chemotherapy (▶ Chemotherapy of Cancer, Progress and Perspectives) is the severe toxic side effects of anticancer drugs on healthy tissues. The use of ▶ liposomes as drug delivery vesicles (▶ Drug Delivery Systems in Cancer) for antitumor therapeutics has great potential to revolutionize the future of cancer therapy. As tumor architecture causes liposomes to preferentially accumulate at the tumor site, their use as drug carriers results in the localization of a greater amount of the drug load at the tumor site, thus improving cancer therapy and reducing the harmful nonspecific side effects of chemotherapeutics. In addition, targeting of liposomal anticancer drugs to antigens expressed or overexpressed on tumor cells provides a very efficient system for increasing the therapeutic indices of the drugs.

Characteristics

The Magic Bullet Concept

Within medical practice, there has long been the desire to achieve selective delivery of drugs to specific areas in the body in order to maximize drug action and minimize side effects. It is well known that many drugs, while having a beneficial action, can also exhibit deleterious effects that may limit their clinical utility. Drugs used in cancer chemotherapy represent a clear example of this problem. Cytotoxic compounds can kill target cells, but also normal cells in the body.

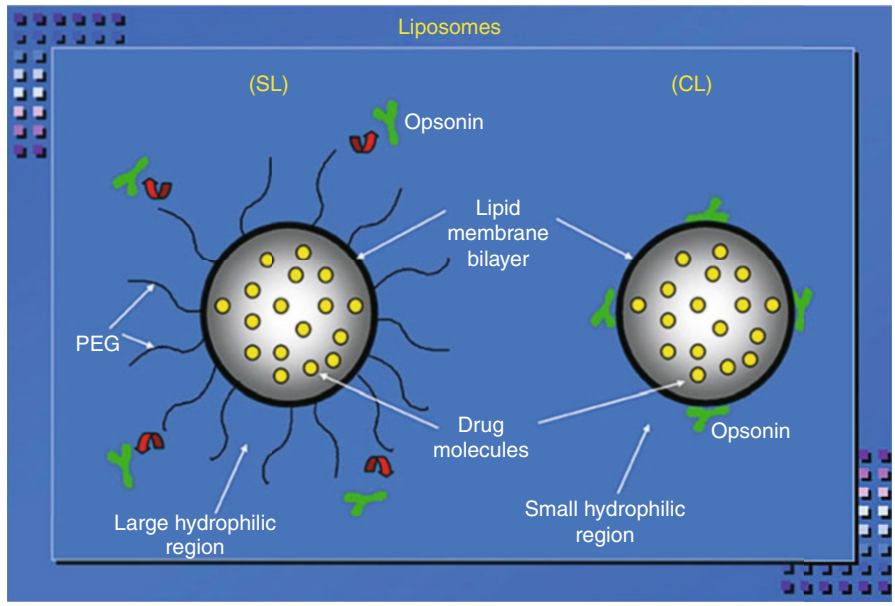
The ▶ magic bullet concept, first expounded by the German physician Paul Ehrlich, represents an early description of the drug-targeting paradigm. Indeed, about one century ago Ehrlich coined the word "chemotherapy" to indicate the possibility to design and produce, by chemical synthesis, drugs, the so-called "magic bullets" as they were able to kill infectious agents without affecting the human body. However, at that time, the recipe for the synthesis was based on four "G": Geld (money), Geduld (patience), Geschick (skill), and Glùck (luck). Indeed, until the last decade of the twentieth century, the most impressive progression in discovery of new chemotherapeutics has been due to "serendipity."

Drug Delivery Technology

The medical community has recently sought alternative therapies that improve selective toxicities against cancer cells. Nanobiotechnology, defined as biomedical applications of nano-sized systems, is a rapidly developing area within ▶ nanotechnology. ▶ Nanoparticles, such as liposomes, allow unique interaction with biological systems at the molecular level. They can also facilitate important advances in detection, diagnosis, and treatment of human cancers and have led to a new discipline of nano-oncology. Nanoparticles are being actively developed for tumor imaging in vivo, biomolecular profiling of cancer biomarkers, and targeted drug delivery.

Sterically Stabilized (Stealth) Liposomes

Several nanotechnological approaches have been used to improve delivery of chemotherapeutic agents to cancer cells with the goal of minimizing toxic effects on healthy tissues while maintaining antitumor efficacy. Among the most popular and well-investigated drug carriers are liposomes. Conventional liposomes (CL), first described by professor Alec D. Bangham in 1965, are made up of amphiphilic phospholipids and cholesterol, which, upon hydration, self-associate to form bilayers surrounding an aqueous interior (Fig. 1). Liposomes are artificial phospholipid vesicles with sizes varying from 50 to 1,000 nm, which can be



Liposomal Chemotherapy. Fig.1 Comparison of the chemical structure of second generation liposomes (sterically stabilized SL)

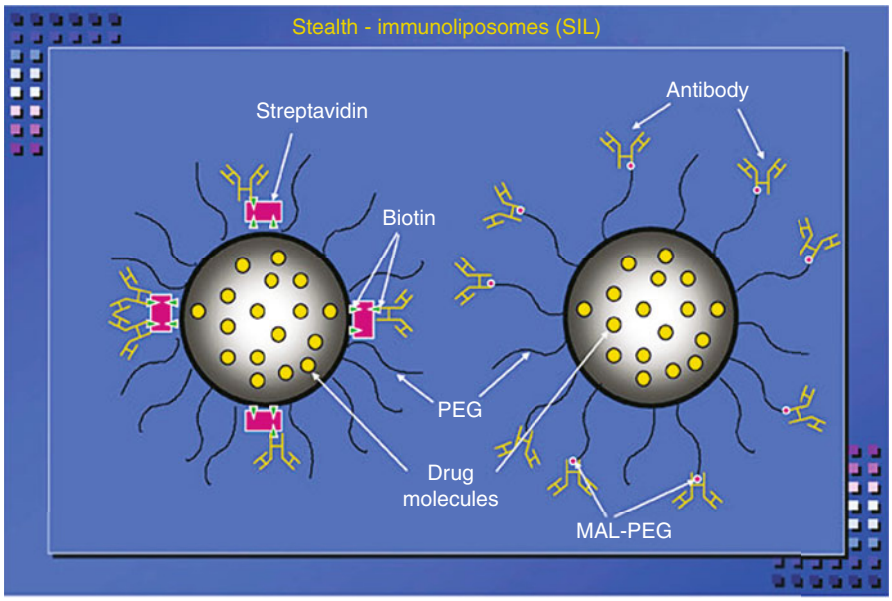
loaded with a variety of water-soluble drugs (into their inner aqueous compartment) and sometimes even with water-insoluble drugs (into the hydrophobic compartment of the phospholipid bilayer).

One of the major drawbacks of conventional liposomes has been their rapid clearance from blood, due to adsorption of plasma proteins (opsonins) to the "naked" phospholipid membrane, triggering recognition and uptake of the liposomes by the mononuclear phagocytic system (MPS), also referred to as the reticuloendothelial system (RES). A major advance in the field of liposomes came with the development of sterically stabilized (Stealth®) liposomes (SL), which utilize a surface coating of a hydrophilic carbohydrate or polymer, usually a lipid derivative of polyethyleneglycol (PEG), to help evade MPS recognition (Fig. 1). The inclusion of PEG or other hydrophilic polymers extends the half-life of liposomes from less than a few minutes (classical liposomes) to several hours (Stealth liposomes).

Both conventional and Stealth liposomes rely on "passive" targeting to increase the localization of anticancer drugs to solid tumors. Growing solid tumors, as well as areas of infection and inflammation, have capillaries with increased permeability as a result of the disease process (e.g., tumor ► angiogenesis). Pore diameters in these capillaries can range from 100 to 800 nm. Drug-containing liposomes that have diameters in the range of approximately 50–200 nm are small enough to extravasate from the blood into the tumor interstitial space through these pores (► extravasation). Normal tissues contain capillaries with tight junctions that are impermeable to liposomes and other particles of this diameter. This differential accumulation of liposomal drugs in tumor tissues relative to normal cells is the basis for the increased tumor specificity of liposomal drugs relative to free drugs. In addition, tumors lack lymphatic drainage and therefore, there is low clearance of the extravasated liposomes from tumors.

Passive targeting can result in increases in drug concentrations in solid tumors of several-fold relative to those obtained with free drugs. The mechanism of action of the liposomal drugs is thought to be due to sustained release of drug from the liposomes and diffusion of the released drug throughout the tumor interstitial fluid, with subsequent uptake of the released drug by tumor cells. This phenomenon has been termed the enhanced permeability and retention effect (► EPR).

Liposomal formulations of few ► anthracycline anticancer drugs have received clinical approval. Three liposomal chemotherapeutic agents, all of which are nanoparticles measuring about 100 nm, are indeed being assessed in human cancer: liposomal daunorubicin, (Daunosome®), approved in the USA



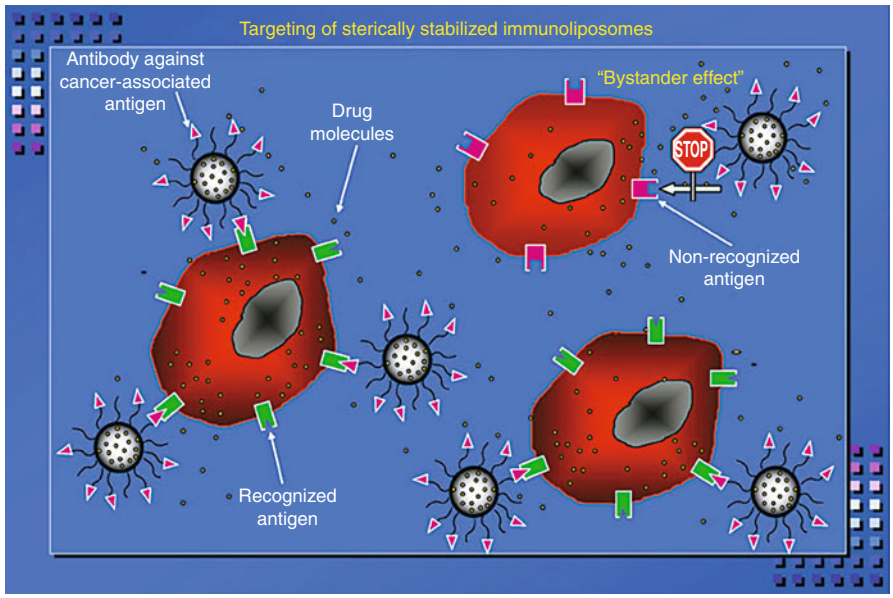
Liposomal Chemotherapy. Fig.2 Immunoliposomes: Liposomes chemically

and Europe to treat AIDS-related Kaposi's sarcoma; liposomal doxorubicin, Myocet®, which, in combination with cyclophosphamide, is approved for the treatment of metastatic breast cancer in Europe; and pegylated liposomal doxorubicin, Doxil®/Caelyx®, approved for both Kaposi's sarcoma, refractory ovarian cancer, and metastatic breast cancer in Europe and USA. In addition, many other liposomal anticancer drugs are in clinical trials (i.e., liposomal cytosine arabinoside, Depocyt®, for the treatment of lymphomatous and neoplastic meningitis, liposomal cisplatin for patients with malignant pleural mesothelioma, sphingosomal vincristine for treatment of recurrent or refractory adult acute lymphocytic leukemia, liposomal muramyltripeptide phosphatidylethanolamine for patients with osteosarcoma).

Ligand-Targeted Stealth Liposomes

In the attempt of increasing the specificity of interaction of liposomal drugs with target cells and the amount of drug delivered to latter, recent efforts in the liposome field have been addressed to the development of ligand-targeted liposomes. These liposomes utilize targeting moieties coupled to the liposome surface to selectively deliver the drug–liposome package to the desired site of action (active targeting). Targeting moieties may include antibody molecules, or fragments thereof, small molecular weight, naturally occurring or synthetic ligands like peptides, carbohydrates, glycoproteins, or receptor ligands, that is, essentially any molecule that selectively recognizes and binds to target antigens or receptors overexpressed or selectively expressed on cancer cells.

To date, liposomes chemically coupled to antibodies or antibody fragments have been the most extensively used ligand-targeted Stealth liposomes (stabilized ▶ immunoliposomes or SIL) (Fig. 2). This chemical complexation can be either non-covalent or covalent in nature. In the former case a biotin–streptavidin–biotin bridge between the liposome and the antibody is involved, while covalent binding can be achieved through the use of heterobifunctional cross-linkers or maleimide-derivatized PEG–lipid complexes (PEG–MAL). The great advantages of SIL encapsulating cytotoxic drugs over free drugs have been unquestionably demonstrated in a number of experimental models of cancer. The mechanism whereby SIL appears to act is related to localized release of the encapsulated drug at the targeted cell surface following binding of the drug carrying liposomes to the cell. With some particular antibodies, moreover, internalization of the liposomal drug package may occur and also contribute to the mechanism of cytotoxicity. Interestingly, it has been shown that approximately 400-fold more monoclonal antibody was required to achieve similar results with antibody–drug conjugates. Hence, high drug:



Liposomal Chemotherapy. Fig. 3 Mechanisms of drug delivery of immunoliposomes: specific targeting to tumor cells

antibody ratios can be achieved with SIL, thus decreasing the need for expensive and potentially immunogenic antibodies. Since most tumors are heterogeneous with regard to tumor-associated antigen expression, another advantage may be the "► bystander effect": specific binding of a SIL to a tumor cell, with release and diffusion of the drug and uptake by surrounding tumor cells may result in cytotoxicity of bystander cells lacking the specific epitope (Fig. 3).

In conclusion, liposomal formulation of chemotherapeutic drugs is considered a very promising modality of drug delivery because liposomes are biologically inert and completely biocompatible, they do not cause toxic or antigenic reactions, and the drugs included into liposomes are protected from the destructive action of the external environment. Association of drugs with carriers, such as liposomes has pronounced effects on the ► pharmacokinetic profile of the drug resulting in delayed drug absorption, restricted drug biodistribution, decreased volume of drug biodistribution, delayed drug clearance, and slower drug metabolism.

Future Perspectives for Liposomal Chemotherapy

Monotherapy is not common in chemotherapy. A combination of drugs generally produces better therapeutic results. The rationale for use of multiple agents takes into consideration the heterogeneity of the tumor cells and differences in tumor cell sensitivity to individual drug classes. Treatment strategies generally include drugs with different mechanisms of action and nonoverlapping side effects in order to attain maximum therapeutic benefit. The evaluation of targeted liposomes using drug or ligand combinations is only beginning to be explored, for example, targeting either the same liposomal drug against different epitopes, targeting different drugs against the same epitope, or targeting different drugs against different epitopes. Combinations of liposomal drugs might also help to lower drug dosages and increase responses, which could help reduce drug toxicities. Given the exhaustive possibilities available to liposome chemistry, research will be quickly directed at multifunctional liposomes, combining tumor-targeting and tumor therapy in an all-in-one system, providing a useful multimodal approach in the battle against cancer.

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Liposomes

Definition

Artificial lipid bilayer vesicles formed from an aqueous suspension of phospholipids and cholesterol. These vesicles can be used to incorporate water-soluble materials, including drugs, proteins, and nucleic acids, enabling the delivery of such molecules to various cells. Synthetic cationic lipids can be used to form electrically charged (cationic) liposomes, which show robust association with DNA and enhance the efficiency of its delivery to target cells.

- ▶ Drug Delivery Systems
- ▶ Immunoliposomes
- ▶ Liposomal Chemotherapy
- ▶ Non-Viral Vector for Cancer Therapy

5-Lipoxygenase

Synonyms

5-LO

Definition

Key enzymes in the arachidonic acid metabolizing pathway into leukotrienes.

- ▶ Leukotrienes

Lipoxygenase

- ▶ Arachidonic Acid Pathway

Lisch Nodules

Definition

Lisch nodules are pigmented ▶ hamartomatous nevi (singular ▶ Nevus), a type of benign tumor affecting the iris, named after Austrian ophthalmologist Karl Lisch, who first recognized them in 1937. They are clear, yellow to brown, oval to round, dome-shaped papules that project from the surface of the iris. These nodules typically do not affect vision, but are very useful in diagnosis. They are detected by slit lamp examination. They are a typical feature of ▶ neurofibromatosis Type 1.

Liver

Definition

Large, glandular organ located in the upper right side of the abdominal cavity, divided by fissures into lobes and functioning in the secretion of bile and various metabolic processes.

- ▶ Hepatic Ethanol Metabolism

Liver Cancer

Definition

Primary liver cancer is a cancer that forms in the tissues of the liver. Secondary liver cancer is a cancer that spreads to the liver from another part of the body.

Estimated new cases and deaths from liver and intrahepatic bile duct cancer in the United States in 2009:

- New cases: 22,620
- Deaths: 18,160

- ▶ Hepatitis B Virus x Antigen Associated Hepatocellular Carcinoma
- ▶ Hepatocellular Carcinoma
- ▶ Hepatocellular Carcinoma – Etiology, Risk Factors, and Prevention

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 2 years of age. Cholangiocarcinoma (CC) develops from the epithelium of intrahepatic biliary ducts, and angiosarcoma is a rare malignant mesenchymal (vascular) tumor. This entry presents only the molecular biology of HCC.

Characteristics

Epidemiology

Liver cancer is one of the most common human cancers worldwide, with an estimate of 626,000 new cases diagnosed each year, and incidence is continuously rising in America and Europe. 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 and 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 few human neoplasms seroepidemiologically related to viral infections. More than 80% of HCC cases worldwide are associated with chronic infection with hepatitis B virus (HBV) (▶ hepatitis viruses) or ▶ hepatitis C virus (HCV). Other major risk factors include alcoholic cirrhosis, dietary intake of aflatoxin B1, a fungal metabolite which contaminates crops in some tropical areas, and inherited metabolic disorders such as tyrosinemia, hemochromatosis, and α1-antitrypsin deficiency. Recently, diabetes and obesity have also been recognized as significant etiological factors of HCC. The risk of developing HCC is greatly increased in chronic viral carriers exposed to other recognized risk factors, including aflatoxin B1, alcoholic cirrhosis, and diabetes.

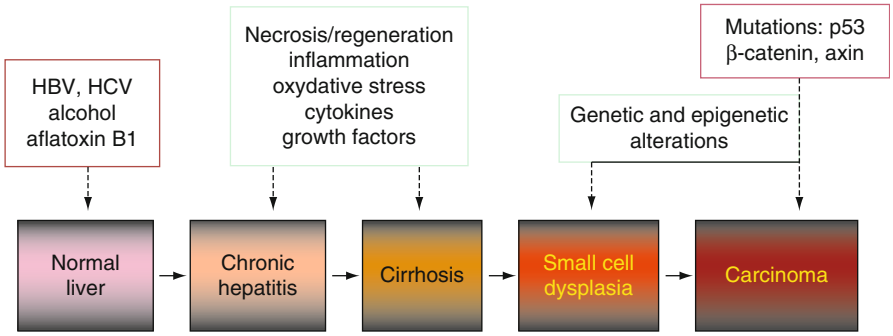
Role of Viral Factors

In HCC as in other human tumors, multiple steps involving independent lesions are required to reach the fully malignant phenotype. Chronic HBV infection plays a complex role in liver carcinogenesis, involving various cooperative mechanisms (Fig.1).

Following liver injury by viral infections (HBV or HCV) or by excessive intake of alcohol or aflatoxin B1, a long period of necroinflammatory liver disease is generally followed by liver cirrhosis. Small cell dysplastic nodules are considered as direct precursor lesions of HCC. Accumulation of genetic and epigenetic changes, including mutations of the p53 and ▶ axin 1 tumor suppressors or the β-catenin oncogene lead to the fully malignant phenotype.

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 with gross genetic alterations such as chromosomal translocations, deletions, or amplifications of large chromosomal regions. Therefore, it may promote genomic destabilization, in turn favoring the accumulation of genetic mutations at early steps of HCC development. Insertion of HBV DNA into cellular genes and subsequent modification of target gene expression or function (a process called ▶ insertional mutagenesis) is another potential oncogenic mechanism. Initial studies of four independent HCC cases identified viral DNA integration sites within cellular genes that play important roles in the control of cell 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 has allowed to better evaluate the prevalence of such oncogenic insertional events. Large-scale analysis of HBV integration sites provided evidence for viral integration into transcribed regions in 62% of cases and for recurrent insertion sites



Liver Cancer Molecular Biology. Fig. 1 The multistep process of liver carcinogenesis

in the human telomerase (hTERT) and mixed lineage leukemia (MLL) genes.

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), a hepadnavirus closely related to HBV, develop frequent and early onset HCC. 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. The critical role of WHV DNA integration in *MYC* oncogenes 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 particular, HBx has been reported to modulate calcium homeostasis, mitochondrial functions, proteasome activity, and signal transduction pathways. Described as a weak transcriptional activator, HBx exerts its functions by interacting with a variety of cellular partners such as DDB1 (a subunit of the Cul4A ubiquitin ligase complex), the histone acetyltransferases CBP/p300, and p53. In transgenic mouse strains, liver expression of HBx can either induce liver cancer or act as a cofactor 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 viral replication itself. Transgenic mouse models have provided evidence for an indirect role of HBV in cancer formation. 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 which ultimately leads to malignant transformation. Moreover, stimulation of cellular immune responses in mice transgenic for the HBV surface antigen (HBsAg) leads to frequent emergence of liver tumors. Thus, an important factor in tumorigenesis is the accelerated turnover of infected hepatocytes resulting from continuous cell death triggered by the host immune response and subsequent cell proliferation. Hepatocyte DNA lesions during persistent HBV infection may be induced by exposure to mutagenic products secreted by inflammatory cells, endogenous production of mutagens such as oxygen-free radicals and nitrosamines, and impaired detoxification pathways or DNA repair mechanisms.

Chromosomal Aberrations

In the recent years, global insights into chromosomal alterations profiles have been provided by genetic studies of large sets of tumors. These studies have shown that a large variety of genetic and epigenetic alterations are present in different combinations among individual HCC cases. Therefore, HCC might rank among the most complex and heterogeneous types of human solid tumors, which is consistent with the multiple etiologies of HCC and the long period of chronic inflammatory disease that fosters accumulation of genetic and epigenetic defects.

Studies using PCR-based microsatellite marker analysis (MSA) and Comparative Genomic Hybridization (CGH) have identified the major genetic changes in HCC 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. The relative frequency of allelic losses may vary with the associated risk factors, implying that viral and chemical agents implicated in liver cancer may preferentially select some pathways rather than others. Importantly, genomic instability has been found at higher rates in HBV-related tumors than in tumors associated with HCV or other risk factors. CGH studies have confirmed prevalent chromosomal losses and demonstrated frequent DNA copy gains at 8q, 1q, 6p, and 17q.

Moreover, specific chromosomal gains and losses were found to occur at different stages of tumor progression, suggesting nonrandom chromosomal gains and losses occurring in an orderly fashion in liver cancer. The recent development of array-based CGH might allow more accurate evaluation of chromosomal changes and identification of cancer-related genes located on affected chromosomal arms.

In addition to genetic mutations, epigenetic mechanisms such as hypermethylation of promoters containing CpG island have been shown to modify gene expression patterns in HCC. A number of tumor suppressor genes including $p16^{INK4A}$, SOCS-1, APC, RASSF1A, GSTP1, and E-cadherin are silenced by DNA methylation in a large proportion of liver tumors, and this process often starts at preneoplastic (cirrhotic) stages.

Tumor Suppressor Genes and Oncogenes

▶ *P53 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 HCC, LOH at chromosome 17p13 has been observed in 25–60% 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 seen at lower rate, and they are distributed over the coding exons.

The retinoblastoma gene. Allelic deletions at chromosome 13q14 have been associated with the inactivation of the RB tumor suppressor gene (▶ RB1). 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 downregulated in 30–50% of tumors. While no mutation of the RB gene itself has been documented, inactivation of the RB pathway is achieved mainly by methylation-dependent silencing of $p16^{INK4}$, an inhibitor of cyclin-dependent kinases which blocks the cell cycle. In addition, overexpression of gankyrin, a new oncogene homologous to a subunit of the 26 S proteasome, promotes RB degradation by the ubiquitin–proteasome pathway.

β-Catenin. Activation of the Wnt pathway has been implicated in liver oncogenesis by the finding of frequent mutations in the β-catenin gene. 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 about 22% of liver tumors. All mutant forms of β-catenin harbor missense mutations or short deletions in the amino-terminal domain (so-called destruction box) and they are resistant to degradation. Thus, wild type β-catenin is expressed at the cell membrane in normal epithelial cells, whereas the mutants accumulate in the cytoplasm and nucleus of tumor cells. In about 7% of HCC cases, activation of β-catenin is also achieved in HCC by loss-of-function mutations of axin 1, a tumor suppressor protein known to bind β-catenin and promote its degradation. In contrast with colorectal cancer, another β-catenin partner, the adenomatous polyposis coli (▶ APC) tumor suppressor gene, is not mutated in HCC. Interestingly, β-catenin mutations are less frequent in HBV-related HCCs than in HCV-related or nonviral HCCs. No mutation of β-catenin could be evidenced in intrahepatic cholangiocarcinoma.

TCF1. The finding of frequent LOH at chromosome 12q24.2 in hepatocellular adenomas has led to identify mutations and bi-allelic inactivation of the TCF1 gene encoding the liver-enriched HNF1-α transcription factor. This finding links MODY 3 diabetes to benign liver tumors, and also occasionally to HCC.

► *Ras family oncogenes*. None of the genes of the ras family has been found to be mutated at significant rate in HCC, whereas K-ras mutations are frequent in intrahepatic cholangiocarcinoma.

Oncogenic Pathways

Deregulation of various signaling pathways has been reported in different HCC subtypes, comprising Wnt/β-catenin signaling and the $p14^{ARF}$/p53, $p16^{INK4A}$/RB, TGF-β, and PTEN/Akt pathways [3]. Evidence for activation of the Ras and Jak/Stat pathways in HCC by epigenetic silencing mechanisms has also been reported. Additionally, altered expression of growth factors such as HGF, IGFs, amphyregulin, and their receptors, as well as genes involved in angiogenesis have been involved in the development and progression of HCC.

Molecular characterization of HCC by gene expression profiling using DNA microarray technology has allowed to distinguish several tumor subtypes harboring significant biological differences. Importantly, these newly discovered subtypes may differ also by a number of clinicopathological features, such as tumor stage, etiological factor, association with recurrence or metastasis, and survival of the patients. New progress will come from integrating gene expression profiles and comprehensive genetic surveys of HCC.

Conclusions

Our understanding of the molecular pathogenesis of HCC has been obscured by the striking clinical and biological heterogeneity of this tumor. It is surmised that new tools provided by sequencing of the human genome and high-throughput screening of DNA, RNA, and protein alterations in HCC would permit a comprehensive view of the signaling networks operating in liver cell transformation. Great insights will come from integrating the signals from different pathways at pretumoral and tumoral stages. Because HCCs carry a severe prognosis and remain refractory to current chemotherapy regimens, it is urgent to accurately evaluate new molecular targets for therapeutic treatments.

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Liver Cell Carcinoma

► Hepatocellular Carcinoma

Liver Cirrhosis

Definition

Cirrhosis is a chronic disease of the liver caused by damage from alcohol, chronic viral hepatitis, biliary disease, iron accumulation, etc. Normal parenchyma is replaced by delicate bands or broad scars of fibrous tissue, active parenchymal nodules created by hepatocyte regeneration, and passive nodules created by constrictive scarring, with disruption of liver architecture. These changes are irreversible and vascular intrahepatic system is reorganized with the creation of interconnections between vascular inflow and hepatic vein outflow channels. The end stage of cirrhosis is the complete loss of liver function. In some instances, cirrhosis predisposes to liver cancer.

► Liver Cancer, Molecular Biology
► Preneoplastic Llesions

Liver Flukes

Definition

Parasites that are ingested and form cysts within the liver, causing chronic ► inflammation, predisposing to cholangiocarcinoma. Fluke eggs are eaten by snails and develop into cercaria which bore out of the snails and bore into the muscle of fish, where they

form metacercaria. The metacercaria are then ingested by humans from raw or undercooked fish and adult parasites form liver cysts, feeding off bile.

► Cholangiocarcinoma

Liver Resection

Definition

Surgical removal of a part of the liver.

► Hepatic Epithelioid Hemangioendothelioma

Liver Transglutaminase and G_h

► Transglutaminase-2

Liver Transplantation

Definition

An operation harvesting the liver from a donor and grafting to a recipient.

► Hepatic Epithelioid Hemangioendothelioma

LMP2

Definition

Low molecular mass protein 2; the generation of antigenic protein fragments for (low molecular mass protein 2) loading onto ► MHC class I molecules and subsequent display on the surface of the cell uses the cellular machinery for protein degradation, the proteasome. This is a large multi-subunit complex consisting of seven subunits and three proteolytic active sites. Although constitutively expressed in the cell, under the influence of IFN-γ, subunits are exchanged to create the immunoproteasome, which is more competent to produce peptides of appropriate size for MHC I binding. Two of the subunits, LMP2 and LMP7 are encoded by genes within the MHC locus and are components of the immunoproteasome.

► Immunoediting

5-LO

► 5-Lipoxygenase

Lob 1

Definition

Lobule 1; is defined as the terminal ductal lobular unit of the human breast and the site of origin of breast cancer.

► Lobular Carcinoma of the Breast

Lobectomy

Definition

An operation done to remove a lobe of an organ such as the lobe of a lung or a lobe of the thyroid gland. The lung has five lobes, three on the right and two on the left. A lobectomy may be performed for a disease such as ► lung cancer.

Lobular Carcinoma of the Breast

Definition

Is one of the main types of ► breast cancer. Lobular carcinoma is the second most common type of breast cancer, making up about 10% of all breast cancers.

There are various types of breast cancer categorized by the way the cells appear under the microscope. The main types are:

- Ductal carcinoma (70–80%): cancer cells that resemble the ducts of the breast
- Lobular carcinoma (5–10%): cancer cells that resemble the lobules or glands of the breast

- Medullary carcinoma (1–5%)
- Mucinous carcinoma (1–6%)
- Tubular carcinoma (2%)

Lobular carcinoma (in situ); "in situ" refers to pre-invasive breast cancer. This is breast cancer which has not yet penetrated ("invaded") through the basement membrane (the membrane at the base of the epithelial lining of ducts or glands). In situ carcinoma has the potential to become invasive carcinoma, and so is treated as an early form of breast cancer.

Lobular carcinoma in situ (LCIS) is thought to be a "pre-cancerous" condition. This means that it is not true cancer, because the cells, while abnormal, have not developed the ability to spread beyond the lobules of the breast. Lobular carcinoma in situ is still an important finding, however, because a woman with LCIS is at higher risk of developing true invasive breast cancer than unaffected women. This risk is thought to be about 7–9 times higher. Early breast cancer has a good (greater than 80%) 5-year survival. Important prognostic factors which can help predict survival include involvement of lymph nodes, the size of the tumor, and how aggressive the tumor cells are. If the cancer has metastasized (spread) to lung, liver, or bone at diagnosis, 5-year survival rates are significantly lower. Women with LCIS are at higher risk of developing invasive breast cancer than the general population.

Lobular Involution

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Synonyms

Senile involution

Definition

A descriptive term, involution refers to a progressive decrease in the size of an organ that is usually associated with a decline in its function. Organs that undergo involution include the post-partum uterus, the ovaries after menopause, the thymus gland, and breast. Except for the post-partum uterus, involutional changes are regarded as a manifestation of aging and are often irreversible.

Characteristics

Anatomy of the Human Breast

The adult human female breast consists of 15–20 irregular lobes that are separated by layers of connective tissue as they fan out from the nipple into the mammary gland proper. These lobes empty independently into the nipple through the lactiferous ducts, which conduct milk to the nipple. The ducts subdivide and terminate in multiple small lobules, which subdivide into multiple glandular acini or alveoli that produce milk during lactation. These lobules are the functional units of the mammary gland. If the terminal duct is also included, they are often referred to as terminal ductal-lobular units. A system of branching intralobular ducts, which eventually converge with the larger lactiferous ducts, connects the individual lobules within each lobe. The highest concentration of lobules is usually found in the upper outer quadrant of the breast. The development of the breast begins at puberty. Its maximum development is reached sometime after the twentieth year with atrophic changes setting in by the age of 35. Lobules are not present in the male breast.

Involution

The breast continually undergoes structural changes depending on the menstrual cycle, pregnancy, and age. Thus, for normal development and function, a balance must exist between cell proliferation, differentiation, and death. A major change entails involution, which refers to two markedly different physiological processes that affect the lobules. The first relates to pregnancy and the production of milk. During pregnancy, in preparation for lactation, these lobules increase in size as a result of cellular proliferation. There is rapid epithelial proliferation with additional branching of the ducts and lobulo-alveoli growth. Following the cessation of lactation, the lobules shrink and return to their pre-pregnancy size, a process described as post-lactational involution. In

animals, this process is very rapid occurring in a matter of days through a process of ► apoptosis. In humans, the time in which this occurs is somewhat uncertain because of the limitations on the opportunity for tissue sampling. This process repeats after each pregnancy. The second process, which greatly differs from the first, is not associated with pregnancy. It is referred to as age-related lobular involution and usually begins near the end of child bearing.

Age-Related Lobular Involution

In humans, information on age-related lobular involution is limited primarily to morphological descriptions. Involution is characterized by a gradual reduction in the number of the terminal lobular structures of the breast. The lobules progressively regress in size, that is, atrophy, until they disappear ultimately being replaced by fat and fibrous tissue. Nearly all of the lobular structures are eventually lost in the process of age-related involution. A few partially involuted lobules or glands persist in the breast years after menopause, usually surrounded by a dense fibrous tissue stroma.

Although it is a normal physiological process, the age of onset, rate, and extent of age-related lobular involution varies greatly among women. In most women, involution begins around age 35, at least 10–15 years before the time of menopause. In some women, however, it may start in the early 30s or even later. It is a relatively slow process often continuing for many years, even into menopause. By age 55, involution is practically complete and the breast has in a general way returned to its prepubertal condition. Involution is not uniform in the breast, but occurs in a patchy or focal distribution. Some areas of the breast may show nearly complete involution while others show only minimal change. Age 35 years appears to be a crucial year. A full-term pregnancy before age 35 confers some degree of protection against future ► breast cancer. Pregnancy after age 35 has been associated with an increased risk of subsequent breast cancer.

The mechanism of lobular involution has not been extensively studied. Post-lactational involution involves apoptosis. Therefore, it can be presumed that age-related lobular involution also involves apoptosis, which in contrast occurs at a much slower rate than post-lactational involution.

Factors Affecting Involution

Very little is known about the factors that govern involution. The process does not follow the regular pattern of aging seen in other organs or tissues. Most likely, the physiological aging process in the breast is under the influence of various hormones. Although it is unknown what factors control the onset and rate of involution, the following associations exist. Oopherectomy at a young age leads to atrophy of the lobules and involution of the lobular epithelium, which resembles the atrophy normally seen in older women. This is of some interest, because oopherectomy also reduces the risk of breast cancer. Since involution predates menopause by many years, it is possible that early changes in ovarian function that precede menopause trigger the onset of involution.

There have been no international comparisons on the extent of physiologic involution in different populations. Surveys of the incidence of different histological tumor types of breast cancer in different geographic areas have suggested international variations exist. This possibility depends on the assumption that the incidence of different tumor types reflects lobular involution. Variation in lobular involution would offer one explanation for the substantial variability of breast cancer observed between populations.

More than 55 years ago, Clemmensen showed that the age-specific incident rate of breast cancer increased until age 50, then decreased for a few years, then increased again, but at a much slower rate than previously. Although many reasons were advanced for the decrease, it has been proposed that Clemmensen's hook reflects involution with its loss of glandular epithelium. The second increase has been attributed to persisting ductal-lobular structures that are susceptible to malignant transformation.

Measurement of Involution

Currently, there is no way to assess quantitatively the extent of involution. Radiologically, dense mammographic parenchymal patterns have been associated with mammary gland mass, which apparently reflects the amount of epithelial tissue present. In general, there is an inverse association between the prevalence of dense parenchymal patterns and age. However, parenchymal patterns are variable; they often vary in women who are the same age. There is no explanation as to why some women have dense breasts and others

do not even at the same age. A decrease in the prevalence of dense parenchymal patterns with age most likely reflects the loss of epithelial lobular structures as a result of involution. Dense parenchymal patterns are also a predictor of breast cancer risk, the greater the density the greater the risk for breast cancer, which suggests a relationship between the amount of epithelial tissue and cancer.

Failure to Involute

It is tempting to speculate on the consequences of a delay or failure of involution to occur at the appropriate time. It is known that lobular epithelium can persist beyond the time of normal involution, which has lead to speculation about the association between abnormal lobular involution and the increased risk of breast cancer. Abnormal involution could involve incomplete involution, genetically abnormal involution, or normal but delayed involution.

In the only systematic examination of age-related lobular involution in the context of breast cancer, a strong inverse relationship was shown between the extent of lobular involution and breast cancer risk, independent of all known breast cancer risk factors investigated. Among women with benign breast disease, lobular involution was significantly associated with reduced breast cancer risk.

There are several possible biologic mechanisms by which aberrant involution could modify a woman's breast cancer risk. It has been suggested that the progressively smaller number of epithelial cells present upon gradual lobular involution could lead to a reduction in breast cancer simply because a lesser amount of tissue is available for malignant transformation. It has also been suggested that abnormal involution may merely be a surrogate for an underlying susceptibility to breast cancer. Failure of timely involution allows for prolonged exposure of epithelial cells to mutagenic stresses thereby increasing breast cancer risk.

Certain known breast cancer risk factors may interfere with the process of involution. Women whose first full-term pregnancy occurs after 35 years of age are at increased risk of breast cancer compared to both nulliparous women and women with earlier first full-term pregnancies. It is likely that the ductal-alveolar epithelium proliferation that accompanies the late pregnancy may interrupt the process of involution. The persistent estrogen activity that results from a late age at menopause possibly explains the increased risk of breast cancer.

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Local Ablation Therapy

► Locoregional Therapy

Local Therapy

Definition

Treatment that affects cells in the tumor and in the surrounding area.

Local Treatment

Definition

Therapy which targets the tumor and surrounding regional area. Surgery and radiotherapy are forms of local treatment. The purpose of local treatment can be curative or ► palliative. Local treatment is distinguished from ► systemic treatment.

► Induction Chemotherapy

Locoregional Therapy

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Synonyms

Local ablation therapy

Definition

There has been no clear definition for locoregional therapy. Most often, locoregional therapy refers to various minimally invasive therapeutic procedures. With the aid of ultrasound guidance, an antitumoral device, drug, or chemical is introduced directly into the tumor and causes tumor death.

Characteristics

For most human cancers, surgical resection remains the standard treatment modality. However, resection is sometimes possible in only a minority of patients. When surgical treatment is less likely to perform, physicians may choose other therapeutic procedures. Among the available methods, locoregional therapy has been frequently used to treat cancer. It is so named because the tumors can often be treated locally. The best example to demonstrate the role and usefulness of locoregional therapy is applying this kind of therapy in patients with ▶ hepatocellular carcinoma, or ▶ HCC, which is primary liver cancer originated from transformed liver cells. HCC is one of the common malignant tumors in the world, and most cases are found in Asia and Africa. Among the various locoregional interventions, ▶ transarterial chemoembolization, percutaneous ultrasound-guided therapy including injection of ▶ ethanol or acetic acid, and thermal ablation using radiofrequency and microwave energy, are the most commonly used methods (Table 1). These locoregional therapies possess the advantages of preserving the uninvolved liver parenchyma and avoiding potential morbidity and mortality of major hepatic surgery.

Transarterial Chemoembolization (TACE)

Most liver cancers, when formed, have a feeding artery that delivers nutrition and oxygen to tumor cells to enable their growth. TACE, using iodized oil and chemotherapeutic agents, combines the effect of targeted chemotherapy with that of ischemic necrosis induced by arterial embolization. Although TACE was originally used for ▶ metastatic liver tumors from colorectal cancers or other malignancies, it was later used to treat primary HCC because HCC nodule frequently has a high arterial blood supply (hypervascular lesion). In addition, TACE can target single- or multi-nodular HCCs in one treatment session and could be repeatedly administered. TACE is generally a palliative treatment for unresectable HCC, and a better outcome can only be expected in properly selected patients.

Percutaneous Ethanol Injection (PEI)

PEI was originally developed in the 1980s when the real time ultrasound-guided aiming became possible. The injected chemical, pure ethanol, induces local tumor necrosis as a result of direct protein denature, cellular dehydration, and thrombosis of blood vessels. HCC usually is hypervascular and well-encapsulated by a tumor capsule that can limit the spread of ethanol. These characteristics have made PEI one of the most commonly used methods of local ablation therapy. Usually PEI is limited to patients with small-sized tumor nodules. Compared with the transarterial approach, PEI has the advantages of being safer, less expensive, and easy to perform. In addition, PEI allows selective treatment of HCC without significant damage to the adjacent liver parenchyma, and can be used for patients with moderately advanced cirrhosis. Side effects are mostly minimal.

Percutaneous Acetic Acid Injection (PAI)

Acetic acid induces profound tumor necrosis at a concentration of 15–50% through a similar mechanism as

Locoregional Therapy. Table 1 Comparison of various locoregional therapies for HCC

Treatment	Advantages	Disadvantages
TACE	Target multiple tumors in one treatment session	No effect for hypovascular tumors, may induce liver or renal failure
PEI	Minimally invasive	Limited to small lesions, need multiple treatment
PAI	Minimally invasive, probably more effective than PEI	Limited to small lesions, need multiple treatment
RFA	Effective for small and medium sized HCCs	Probably higher complication rate, more expensive
MCT	Effective for small to medium sized HCC	Probably higher complication rate, more expensive

TACE transarterial chemoembolization, *PEI* percutaneous ethanol injection, *PAI* percutaneous acetic acid injection, *RFA* radiofrequency ablation, *MCT* microwave coagulation

ethanol. It has a strong ability to penetrate cancer cells, and can dissolve lipids and extract collagen from intratumoral septa and capsule that frequently contain viable cancer cells. Therefore, acetic acid is at least equally effective compared with ethanol in treating HCC.

Radiofrequency Ablation (RFA)

RFA for human HCC was first reported in the mid-1990s. The puncture needle (or probe) has an insulated shaft and a noninsulated tip, which is inserted into the lesion under ultrasound or computed tomography guidance. The patient is part of the electric circuit with grounding pads on the thighs or the back. The radiofrequency energy emitted from the needle tip induces ionic agitation and frictional heat. The surrounding tissue, rather than the electrode itself, is the source of heat that destroys the cancer cells. RFA has now become one of the very efficient local ablative therapies for HCC due to its excellent necrotizing effect. While many investigators consider RFA a safe procedure, a few reports found that the complication rate of RFA could be quite high. When the location of tumor nodule is close to the major blood vessels, the radiofrequency energy will be carried away by the blood flow (the "heat sink" phenomenon) and result in a suboptimal treatment response.

Microwave Coagulation Therapy (MCT)

As a thermal ablation method for HCC, MCT utilizes a microwave coagulator that generates and transmits microwave energy to a needle electrode which is inserted into the lesion. MCT has been studied in patients with HCC as well as in liver ▶ metastasis. It can be applied with percutaneous or laparoscopic approach to ablate unresectable HCC, and is useful to control tumor bleeding from ruptured HCC or prevent massive blood loss in liver surgery.

Comments

Locoregional therapy has also been applied to other cancers other than HCC, but the overall experience is relatively limited. The choice and application of various locoregional therapies for HCC and other cancers may vary from center to center and is likely to depend on the experience, preference, and facility of the referral center. Long-term survival in patients with unresectable HCC may be achievable in selected patients provided various locoregional therapies can be appropriately performed.

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Locus

Definition

Plural, *loci*; a fixed position or location in a DNA sequence.

► Linkage Disequilibrium

Log-Kill Hypothesis

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Synonyms

Skipper–Schabel model; Skipper–Schabel–Wilcox model

Definition

The *log-kill hypothesis* proposes a model for the effect of cytotoxic chemotherapy on tumor size. It states that a given dose of chemotherapy kills the same fraction of tumor cells regardless of the size of the tumor at the time of treatment.

Characteristics

Experimental and mathematical models have attempted to describe the fundamentals of tumor cell growth and kinetics. From these systems arose an improved understanding of tumor growth characteristics, a foundation for the key principles of chemotherapy and, eventually, recognition of the importance of dose scheduling. The Skipper–Schabel–Wilcox model is one of the most influential and pioneering experiments in the history of oncology.

Investigators at the Southern Research Institute developed a simple model of tumor growth using L1210 murine leukemia cells. This system had two notable features: a seemingly exponential growth pattern and the ability to spontaneously generate drug-resistant cells. These factors made it a reasonable reflection of the growth of rapidly fatal human malignancies and of the heterogeneity in drug sensitivity seen clinically in many cancers.

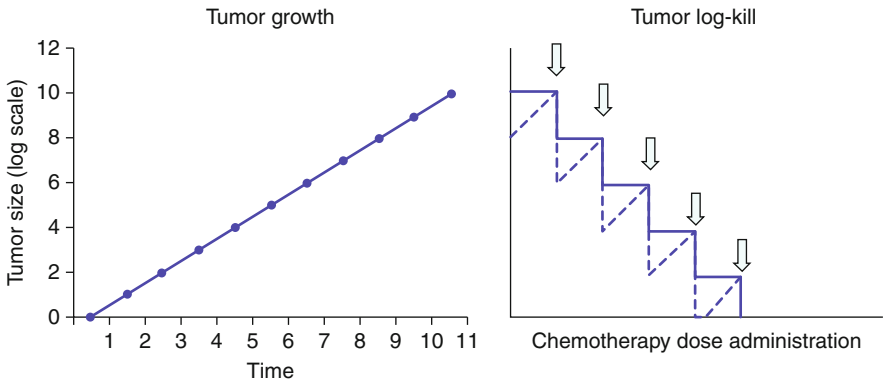
Leukemia in this murine model grows exponentially until it reaches a lethal volume of $\sim 10^9$ cells. A significant observation was that the doubling time remained constant regardless of the size of the tumor. This pattern of growth could be generalized to any fractional increase so that if it takes a certain amount of time (x) to grow from 10^3 cells to 10^4 cells, it will similarly take x amount of time for 10^7 cells to grow into 10^8 cells.

Building on the observations and assumptions above, the investigators hypothesized that when such a tumor is treated with chemotherapy, the fraction of cells killed is similarly constant. This fixed, relative cytoreduction suggests that a dose of drug active against a homogeneously sensitive population should always kill a constant proportion of the cells, regardless of the size of the tumor at the start of treatment. According to this model, if a dose of drug could improve survival by some period of time, then enough additional doses could delay death and lead to cure.

Graphically, when this fractional cell kill is expressed on a logarithmic scale, cytotoxicity occurs in the reverse of an exponential growth pattern (Fig. 1). Therefore, a drug dose that reduces 10^7 cells to 10^5 cells (a log-kill of 2) will also reduce the same tumor from 10^5 cells to 10^3 cells. Exponential cell growth is matched by exponential cell kill. Accordingly, enough cycles of enough drugs at high enough doses should be able to kill a high percentage of cells, if not all of them.

The log-kill model led to enthusiasm regarding the application of chemotherapy to the postoperative setting. ► Adjuvant therapy would theoretically cure patients of micrometastases as small volume tumors should be more easily eradicated as predicted by this model.

However, the Skipper–Schabel model does not accurately represent the entire clinical reality. The log-kill model presumes that growth is exponential between treatment doses and does not vary as a function of tumor size. Additionally, clinical experience with several solid tumors does not support this model. The observations that cure is rare for patients with advanced cancer, and that many patients with early stage cancer recur despite treatment suggest that this model does not completely predict or explain tumor cytokinetics.



Log-Kill Hypothesis.
Fig. 1 The Skipper–Schabel–Wilcox "log-kill" model. Tumor growth appears exponential when plotted on a logarithmic scale (*left*). With each dose of chemotherapy, a constant fraction of cells are killed (*right*)

Subsequent mathematical models based upon Gompertzian growth (▶ Gompertzian growth curve; ▶ Norton-Simon hypothesis) improved upon the concept established by the Skipper–Schabel–Wilcox model.

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LOH

Definition

▶ Loss of heterozygosity

LOI

Definition

Loss of ▶ Imprinting.

▶ Genomic Imprinting

Long Dispersed Nuclear Element

Definition

▶ LINE Element

Long Terminal Repeat

Synonyms

LTR

Definition

Regulatory sequence of the genome of a ▶ retrovirus.

▶ Retroviral Insertional Mutagenesis

Loss of Heterozygosity

Definition

Abbreviation LOH; also ▶ 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; ▶ Knudson Hypothesis). LOH can be identified in cancer cells by a number of technical approaches that all make use of the fact that the two alleles on the two homologous parental chromosomes differ in their DNA sequence. Consequently, normal cells can be shown to be ▶ heterozygous for a given chromosomal region. If cancer cells of the same person lack this heterozygosity, one of the two alleles must have been lost. In essence, determining LOH in a cancer cell is a technical approach to show the deletion of chromosomal material from one of the homologous parental chromosomes.

- ▶ CCCTC-Binding Factor
- ▶ Circulating Nucleic Acids
- ▶ Tumor Suppression
- ▶ Tumor Suppressor Genes
- ▶ WWOX

Loss-of-Function Mutation

Definition

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.

Lovastatin

Definition

Member of the statins family.

- ▶ Statins

Low Density Lipoprotein

Definition

LDL as a lipoprotein of the blood plasma transports triglycerides, cholesterol, and antioxidative vitamins from the liver and small intestine to peripheral cells. High LDL levels can lead to atherosclerosis.

- ▶ Photodynamic Therapy

Low Grade/Well Differentiated MEC

- ▶ Mucoepidermoid Cancer

Low Molecular Weight G-Proteins

- ▶ Rho Family Proteins

Low-Grade

Definition

Referring to a tumor that has progressed minimally and is still relatively benign.

LOX

- ▶ Arachidonic Acid Pathway

L-PAM

Definition

- ▶ Melphalan

LPP

► Lipoma Preferred Partner

LRF

Definition

Luteinizing hormone (LH)-releasing factor.

► Gonadotropin-Releasing Hormone

LRP

► Major Vault Protein

LRP5/6

Definition

Members of the ► low-density lipoprotein (LDL) receptor superfamily; single-pass transmembrane receptors for Wnt and DKK proteins; recruit Axin to the membrane.

► Wnt Signaling

LS-80558

► Temozolomide

L-Sarcolysin

Definition

► Melphalan

LTBP

Definition

Latent TGF-β binding protein.

► Transforming Growth Factor Beta

LTR

Definition

Long terminal repeat, the promoter of HIV genome transcription.

► TAT Protein of HIV

Lucifer Yellow

Definition

Lucifer yellow is a low molecular weight (457 Da) hydrophilic fluorescent compound able to diffuse through gap junctions.

► Gap Junctions

Luciferase Reporter Gene Assays

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Synonyms

Bioluminescent reporter gene assays

Definition

Luciferase ► reporter gene assays monitor the transcription of specific genes in cells and in vivo through

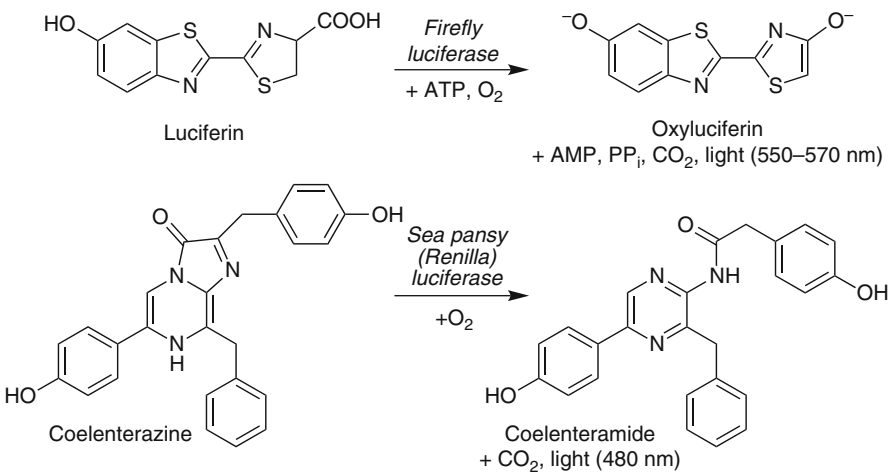
the detection of light generated by the enzyme luciferase. Their primary uses are for mapping the control regions of genes, for measuring ▶ signal transduction pathways modulated by hormones or disease, as a tool in ▶ gene therapy and drug discovery, and for noninvasive whole-animal imaging.

Characteristics

Luciferase reporter gene assays are a type of reporter gene assay that utilizes the light generated by one of a family of enzymes known as luciferases. Reporter genes are essential tools in the study of gene expression and regulation, as they are designed to function as surrogates for genes involved in key cellular processes and disease. To achieve this role, they are engineered so that their expression is directly correlated with the expression of the gene to be studied, but they have the advantage that they are easier to measure. The characteristics of a good reporter gene include an easily measurable phenotype, low background activity in the cell under investigation, a large dynamic range, and high reliability and sensitivity. A number of such genes have been identified over the past 20 years, including members of the family of luciferases, β-galactosidase (β-Gal), chloramphenicol acetyltransferase (CAT), β-lactamase (β-lac), alkaline phosphatase (SEAP or SPAP), and green fluorescent protein (GFP) and derivatives. Each of these reporter genes has distinct advantages and disadvantages depending on the particular system being studied, but all are engineered in a similar way. The reporter gene is first cloned into an expression vector downstream of the regulatory elements of the cellular gene to be studied. The regulatory elements include the promoter, where specific transcription factors bind to modulate the gene's activity, and enhancers that can affect the promoter depending on the input from other signaling pathways in the cell. When this reporter gene expression vector is transfected into a cell, it becomes controlled by the same signals as the gene under study, and will function as a "reporter" of its transcriptional status. The proteins expressed by reporter genes can be measured directly by their intrinsic properties such as fluorescence, by enzymatic activities that generate fluorescent or luminescent products, or indirectly with antibodies. The primary advantages of reporter genes, as compared to direct measurements of gene transcription, are that they are easy to measure and allow for continuous monitoring of gene expression.

Luciferase is a general term for a family of enzymes that catalyze the oxidation of a number of substrates and in the process produce photons of visible light with emission spectra between 400 (purple) and 620 nm (orange). Luciferase genes have been cloned from a variety of bacteria, insects, and marine organisms, but the luciferases from firefly (*Photinus pyralis*), and sea pansy (*Renilla reniformis*) are most widely used as reporter genes in mammalian cells. The chemical reactions of the firefly and sea pansy luciferases are shown in Fig. 1.

Firefly luciferase is a monomeric protein of 61 kDa that produces an initial burst of light (seconds) followed by a sustained low level of luminescence (hours) when incubated with substrates. In the original assay, cell lysis was required prior to the addition of luciferin, but the development of membrane-permeable substrates has eliminated this step and also allowed imaging in vivo. Other improvements that have been introduced include modification of the reaction conditions to produce a stable luminescent output (important for applications that require consistent light output such as drug screening), and genetic changes that optimize expression and increase enzyme turnover. Light output is typically measured through the use of plate-based luminometers or charged coupled device (CCD) cameras. Sea pansy luciferase (more commonly referenced as *Renilla*) is a monomeric protein of 36 kDa that uses the membrane-permeable substrate coelenterazine as a substrate and does not require ATP. While its structure is unrelated to firefly luciferase, it catalyzes a similar reaction and generates light of a shorter wavelength. The differences in substrate requirement and emitted wavelength have allowed the development of dual reporter systems that utilize both luciferase genes in a single assay. For example, cells have been transfected with firefly and *Renilla* reporter genes under the control of two separate signal transduction pathways, allowing the effects of compounds on each individual pathway to be monitored by the sequential addition of substrates. Similar dual reporter assays have been developed in living mice by taking advantage of the different emission wavelengths and kinetics of the two luciferases. Luciferases can also be utilized in ▶ protein-fragment

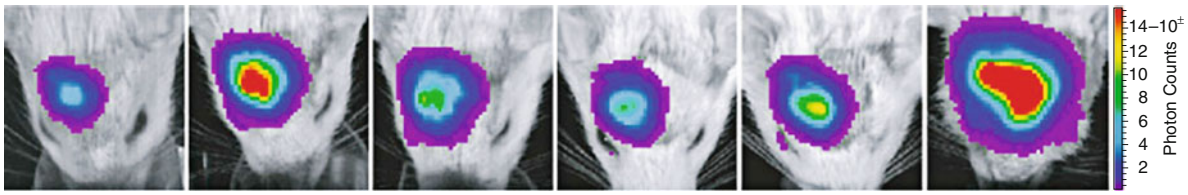


Luciferase Reporter Gene Assays. Fig. 1 Chemical reactions catalyzed by (**a**) firefly (*Photinus pyralis*) and (**b**) sea pansy (*Renilla reniformis*) luciferases. The firefly luciferase reaction requires both ATP and O_2, while the sea pansy luciferase only requires O_2. The products of both luciferases include CO_2 and visible light

complementation assays (PCAs) to measure the interaction between two proteins. In these assays, the luciferase gene is split into two parts, one of which is fused with the gene of the target protein and the other with its potential partner. The interaction of the target protein with its partner brings together the two fragments of luciferase and thus reconstitutes enzymatic activity. Such PCAs have been developed for a number of luciferases and function as highly sensitive detectors of protein–protein interactions in cells and in vivo. The primary advantages of either luciferase over the other reporter genes described above are their combination of high specific activity and low background (mammalian cells do not express light-generating enzymes), large and linear dynamic range (7–8 orders of magnitude), and the relatively rapid turnover of the enzyme. Highly stable reporter proteins can lead to a higher signal through accumulation, but their concentrations consequently become poorly linked to changes in transcription. The luciferase assay is also high-throughput, nonradioactive, nontoxic, relatively low cost, and easily adaptable to automation. The primary disadvantages of luciferase are the requirement for exogenous substrates and the 4–6 h delay from stimulus to response to allow transcription to occur.

Luciferase reporter gene assays have a number of important applications in biomedical science and cancer biology. Their initial uses were to map and characterize transcriptional control elements such as promoters, enhancers, silencers, and transcription factors, and these are still valuable applications today. In these experiments, an expression vector is developed with the control region of the gene to be studied inserted upstream of luciferase. Mutations or deletions are then made in the control region at different locations, and the effect of these changes on luciferase output is measured. Once the different regions are mapped in this way, they can be characterized further by removing them from the control region and inserting them immediately upstream of a constitutively active viral promoter driving luciferase. For example, tissue-specific enhancer elements inserted in either orientation should cause an increase in transcription only in the relevant cell types, and silencer elements should decrease expression. The contribution of individual transcription factors to the overall response can be characterized by monitoring the effect of the overexpression or ▶ siRNA-mediated inhibition of specific factors on the output of luciferase.

Another use of luciferase reporter gene assays is in the study of components of signaling pathways upstream of transcription. A wide variety of assays have been developed that can quantitate modifications of cell-surface proteins such as G-protein coupled receptors (GPCRs), signal transduction proteins such as protein kinases, and protein–protein signaling cascades. In the majority of cases, the particular protein to be studied is known and the assay is designed to respond to changes in its activation status. Specific examples include the site-directed mutagenesis of ▶ receptor tyrosine kinases, the identification of ligands for ▶ orphan GPCRs, and the involvement of particular proteins in different transcriptional



Luciferase Reporter Gene Assays. Fig. 2 The effect of chemotherapy on luciferase-expressing tumor cells implanted into mice. Tumor cells were implanted into the mice 16 days before the initiation of chemotherapy on day 0 (*left panel*), and luciferase activity was measured every 4 days thereafter (*panels from left to right*) [5]

activation pathways. One particularly important use for this type of assay is in the discovery of ▶ small molecule drugs. The sensitivity, convenience, and broad dynamic range of luciferase reporter gene assays make them especially suitable for cell-based high-throughput screening for inhibitors and activators of drug targets. Assays have been developed for nearly all classes of drug targets, including GPCRs, protein kinases, nuclear hormone receptors, and protein–protein interactions, and a number of successful drug discovery programs have been launched as a result. The assays are easily miniaturized to 384-well microtiter plate formats, and can be used to screen over 100,000 compounds a day with automated systems. Luciferase reporter gene assays also have applications in pathway identification and characterization when a specific target is not under study. These "black box" assays monitor the effect of a particular treatment on a cell which expresses a luciferase reporter gene linked to a general signaling cascade. Examples include their use in the identification of new drug targets through a ▶ chemical tool approach, and in genetic or chemical cytotoxicity screens for drug candidates or environmental pollutants.

An exciting and more recent use of luciferase reporter gene assays is in the visualization of gene expression in vivo, known as ▶ bioluminescent imaging. These assays allow the noninvasive, real-time monitoring of biological processes in living animals, and have already contributed to the development of better ways to diagnose and treat disease. The key observation that enabled these assays was that the light generated by luciferase could be transmitted through an animal's tissues and visualized by highly sensitive CCD cameras. Two general types of assays have been described: those where a normal animal is injected with cells or a virus engineered to contain a luciferase reporter gene, and those that utilize a luciferase reporter gene transgenic animal. The initial application for bioluminescent imaging was in animal models of bacterial infection. Pathogenic bacteria such as *Salmonella* and *Pseudomonas* were engineered to express bacterial luciferase without the need for exogenous substrate (other luciferases require the injection of luciferin or coelenterazine before imaging), and the process and distribution of infection was followed upon injection of the cells into an animal. This model has been used to identify those host cells that are important in defense against pathogens, and test possible treatments. A similar approach has been used to study the trafficking and fate of transplanted immune cells, carcinoma cells, or tumor xenografts that have been transfected with luciferase reporter genes. In these cases, the fate of the cells and the effects of different chemotherapeutic and immunotherapeutic regimens can be measured after injection (see Fig. 2). Finally, the effectiveness of viruses used for gene therapy can be monitored in vivo by engineering them to express luciferase reporter genes and measuring the degree and location of light output. This approach has been used to optimize the construction, delivery method, safety, and long-term efficacy of such viruses.

The use of transgenic animals in combination with luciferase reporter genes is another area of current development. For example, a number of groups have generated animals that express luciferase under the control of specific promoters (e.g., NFkB), or in particular tissues (e.g., pituitary). These animals are useful for determining the function of particular genes and the role of signal transduction pathways, for characterizing the effects of drug or other treatments on physiology and disease, and for measuring the tissue distribution and ▶ pharmacokinetics/pharmacodynamics of therapeutic drugs. One limitation of luciferase reporter gene assays in vivo is that they provide only a 2-dimensional view of expression patterns, but better detection technologies and the combination of bioluminescent

imaging with other noninvasive technologies such as MRI or CT promise to improve resolution in the coming years.

Luciferase reporter gene assays are an essential in vitro and in vivo tool in the study of biology and medicine due to their simplicity, sensitivity, and ease of use. They have contributed greatly to the study of gene transcription and the elucidation of gene function, and are a valuable technology for drug discovery. Their application in vivo promises to facilitate the development of new treatments for cancer and other diseases.

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Lugol Unstained Lesion

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Definition

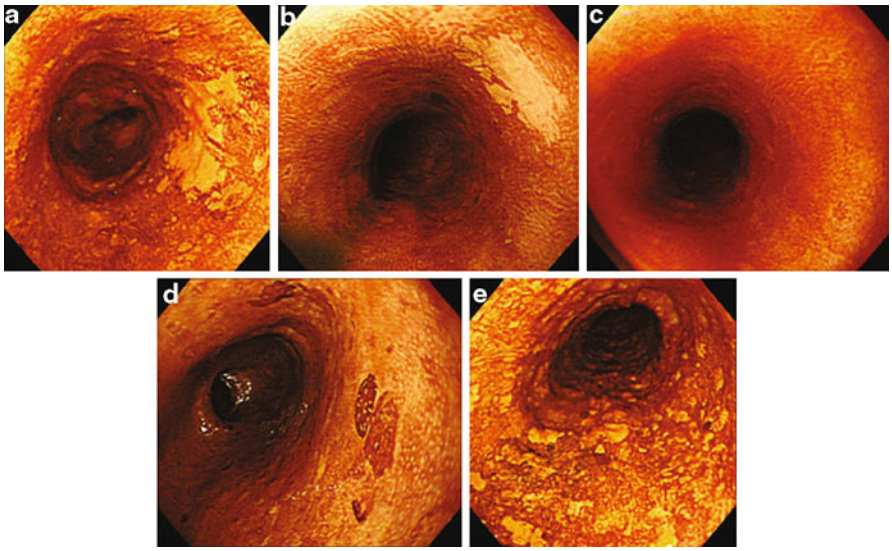
In 1933, the Lugol spraying method was introduced for the diagnosis of uterine cervical neoplasia using gynecologic colposcopy.

Since the 1980s, this technique has been used for screening of esophageal squamous cell carcinoma (ESCC) with Lugol chromoendoscopy. The Lugol spraying method has developed for early detection of ESCC from asymptomatic patients.

Characteristics

After ordinary endoscopic observation, 5–10 mL of 2.0% glycerin-free Lugol iodine solution, which is a brown liquid consisting of 2.0 g potassium iodine and 4.0 g iodine to 100 mL distilled water, is sprayed from the gastroesophageal junction to the upper esophagus using a plastic spray catheter passed through the biopsy channel of the endoscope. The whole esophagus is reobserved and epithelial areas are categorized as unstained (Fig. 1a, b), normally stained (Fig. 1c), or overstained (Fig. 1d). Lugol unstained lesions (LULs) are defined as those areas either staining less intensely than normally stained epithelium, or completely unstained; this group of lesions includes carcinoma, dysplasia, and esophagitis. According to Lugol staining patterns, completely "unstained" areas are found in approximately 90% of high-grade dysplasia and carcinoma, while approximately 90% of areas, which stain less intensely than normally stained epithelium, represent non-dysplastic lesions and the remaining 10% are dysplastic. Therefore, LULs are detectable not only in dysplasias and carcinomas but also in non-dysplastic areas, for example with esophagitis, or in the setting of Barrett esophagus. Overstained areas represent glycogenic acanthosis (GA), which has been referred to as leukoplakia and hyperkeratosis of the esophagus. GA is a common benign lesion easily observed during ordinary endoscopic observations, usually varying in number and consisting of oval, slightly elevated, firm, gray-white plaques, measuring from 0.2 to a maximum of 1.5 cm in length. In addition, GAs stain a deep brown color when treated with Lugol solution. An iodine starch reaction occurs momentarily on esophageal epithelium when sprayed with Lugol solution. Therefore, since normal squamous epithelium contains glycogen that interacts with the iodine in the Lugol solution, normal esophageal epithelium becomes uniformly greenish-brown when stained [1]. Dysplasia and esophagitis are not stained, since these regions have a reduced content or no glycogen. Therefore, these minute lesions that were not identifiable by conventional endoscopic observation become visible when Lugol solution is used. Most individuals with LULs have only single or a few LULs, while multiple LULs (Fig. 1d) were found in some ESCC patients.

Carcinoma of the esophagus is considered to be a widespread global disease estimated in 2000 to



Lugol Unstained Lesion. Fig. 1 (**a**) Endoscopic findings of a Lugol unstained lesion, which was completely unstained. The lesion was 12 mm in diameter, and histologic finding was intraepithelial squamous cell carcinoma. (**b**) Endoscopic findings of a Lugol unstained lesion, which was staining less intensely than normally stained epithelium. The lesion was 4 mm in diameter, and histologic finding was esophagitis. (**c**) Endoscopic findings of normally Lugol staining epithelium without a Lugol unstained lesion. (**d**) Endoscopic findings of an overstained lesion. (**e**) Endoscopic findings of multiple Lugol unstained lesions. Many irregular lesions which were stained less intensely than normally Lugol staining epithelium were located in one endoscopic view

be responsible for 338,000 deaths worldwide (▶ Squamous Cell Carcinoma). In Asia, some parts of Africa, South America, and South Europe, squamous cell carcinomas are the dominant form of esophageal cancer. Despite recent advances in the surgical treatment for ESCC, surgical outcome is not satisfactory, with an overall 5-year survival rate of 17–39% worldwide. In contrast, standard ▶ chemoradiotherapy has a curative potential for locally advanced ESCC, with inoperable cases having a 3-year survival rate of approximately 20%. The survival rate for advanced stage squamous cell carcinomas of the esophagus is poor, mainly due to their late detection and the poor efficacy of therapy containing surgery and chemoradiation. Conversely, the prognosis of patients treated endoscopically for carcinomas confined within the intraepithelium or proper mucosal layer is excellent (▶ Tumor Staging), with 5-year survival rates of 77–100% in Japan and Europe. These results indicate that endoscopically mucosal resection (EMR) for superficial ESCC can improve the prognosis of ESCC, highlighting the need to detect this disease at an early stage. Thus, the Lugol spraying method is a clinically important adjunct to endoscopic screening for ESCC, since two thirds of esophageal intraepithelial carcinomas are overlooked by conventional endoscopy alone. This simple technique of spraying Lugol solution in the esophagus is a highly sensitive tool for delineating areas of squamous dysplasia and carcinoma [1]. In recent screening studies for ESCC, Lugol chromoendoscopy has been used worldwide.

Carcinogenesis (Precursor of ESCC)

Characterization of human esophageal precancerous lesions at the molecular level is of critical importance in our understanding of the etiology of ESCC and for the identification of useful ▶ biomarkers for prevention studies of this disease. The identification of ESCC precursors will ultimately lead to the prevention of poor-prognostic ESCC. Mutation analyses have demonstrated that *p53* gene mutations occur at an early stage of esophageal carcinogenesis, both in the setting of non-dysplastic lesions (i.e., basal cell hyperplasia (BCH), Lugol unstained lesions with non-dysplastic epithelium (LULs-NDE), chronic esophagitis) and in

dysplastic lesions (i.e., low-grade and high-grade dysplasia, cancer in situ). Since *p53* mutations are frequently found in invasive ESCC, most of these lesions, both dysplastic and non-dysplastic, should be considered to be precancerous.

Mutations of the *p53* tumor suppressor gene (▶ p53 Protein, Biological and Clinical Aspects) are the most common genetic abnormality in solid human cancers. Missense mutations are found in 78% of the 6,177 somatic *p53* mutations in exons 5–8, suggesting a correlation between the degree of evolutionary diversity and the structural or functional importance of individual amino acid residues. In contrast, *p53* gene mutations have been proposed to be concentrated in six hotspots [2]. Based on the updated *p53* gene mutation database containing 5,961 mutations, codons 175, 245, 248, 249, 273, and 282 have been identified as mutation hotspots in human cancers, and the incidence of the hotspot mutation is specific to the molecular alterations in solid human cancers [2]. A hotspot can identify a relationship between the mutation, protein structure and function, and carcinogenesis. Furthermore, hotspot mutations in carcinomas represent protein alterations that provide a selective growth advantage to the cell, and missense mutations at six hotspots account for 25–30% of the mutations [2]. In contrast, malignant transformation of human esophageal epithelium is a multistage progressive process from non-dysplastic lesions through dysplasia to carcinoma [3]. The *p53* hotspot mutation was found in not only dysplasia or carcinoma but also Lugol unstained lesion with non-dysplastic epithelium (LUL-NDE) [4]. Therefore, protein alterations that provide a selective growth advantage to the cell would have already occurred in cells of LULs-NDE before histologic transformation into dysplastic cells. Mutations at codon 175 and 273 have been shown to have transforming frequencies that are 22- and 8-fold, respectively, of the basal level for wild-type p53 protein. The LUL-NDE or low-grade dysplasia containing mutations with high transforming activities, such as codon 175 and codon 273 mutations, might have growth advantages favoring progression to invasive ESCC with the acquisition of other genetic changes, and may acquire malignant potential before morphologically manifested cell proliferation at an early molecular level of carcinogenesis. In endoscopic biopsy samples, the *p53* missense mutation containing a hotspot mutation was found in NDE, which was identified as a LUL. No hotspot mutation was found in normal Lugol staining area or BCH. These findings suggest that some LULs-NDE may represent the earliest state of esophageal squamous cell carcinoma.

Multiple LULs

A single or few LULs were detected in one fifth of patients attending for routine endoscpy. Although subjects with multiple LULs (ten and more than ten LULs in one endoscopic view, Fig. 1d) were found in only 1% of the general population, dysplasia occurred frequently (60%) in these subjects. In contrast, multiple LULs were found in 27% of head and neck cancer patients, and secondary ESCCs were found in 72% of such cancer patients with multiple LULs [5]. These results indicate essential information about field cancerization and malignant potential in respect of multiple LULs. The field cancerization phenomenon proposes that multiple squamous cell carcinomas occur either simultaneously with the primary lesion (synchronous) or after a period of time (metachronous) in the esophagus and the head and neck region. There is the possibility that widespread epithelial oncogenic alterations are found in patients with multiple LULs, indicating that esophageal epithelium with multiple LULs has unique characteristics in carcinogenesis.

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Luminal Epithelial Cells

Definition

One of the two major epithelial cell types in the breast that are located in an inner cell layer surrounding the lumen of the gland and produce milk.

▶ Basal-Like Breast Cancer

Lunasin

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Definition

Lunasin is a novel seed peptide with ▶ cancer-preventive properties against chemical carcinogens and ▶ oncogenes.

Characteristics

Lunasin is a unique 43-amino acid peptide that contains eight Asp (D) residues in its carboxyl end (bold) preceded by a cell adhesion motif Arg-Gly-Asp (RGD) (italics) and a predicted helix (underlined) with structural homology to a conserved region of chromatin-binding proteins: SKWQHQQDSCRKQ KQGVNLTPC-EKHIMEKIQG-*RGD*-**DDDDDDDD**.

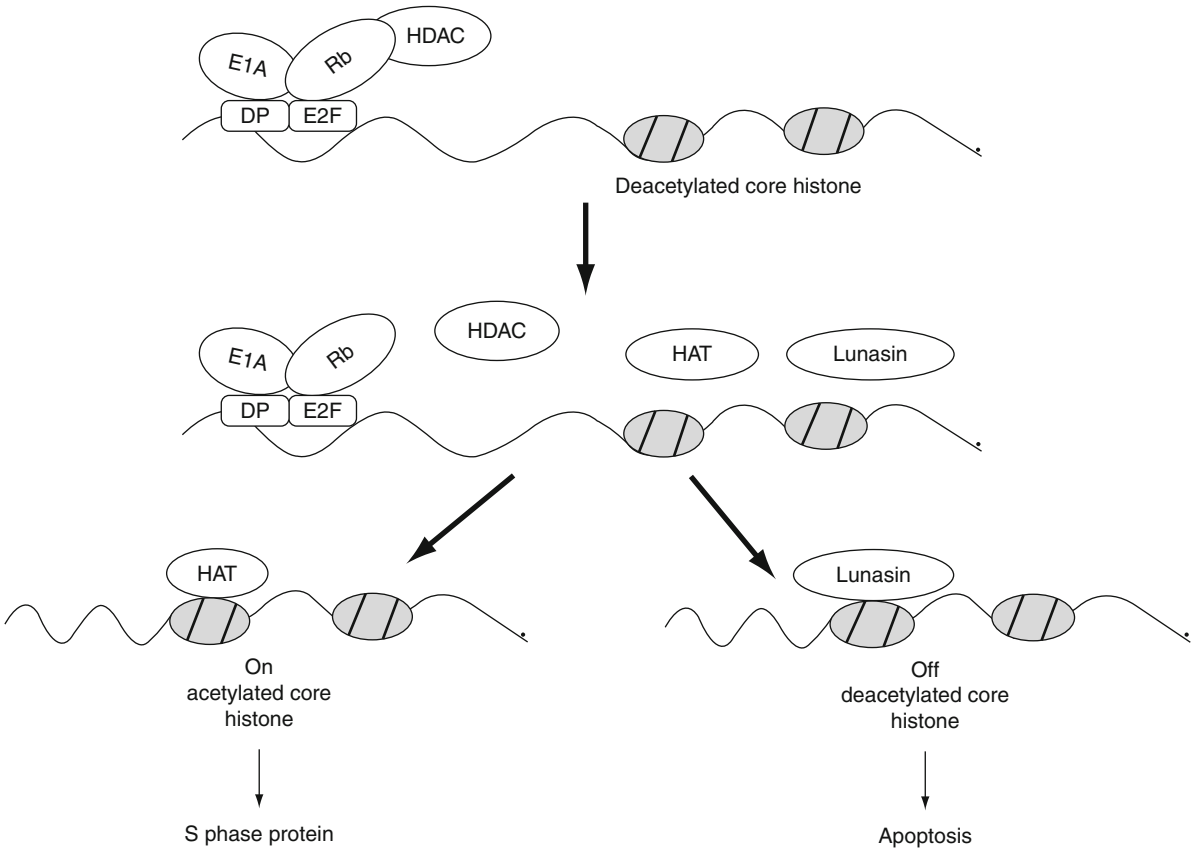
Initially identified in the soybean cotyledon, Lunasin has been recently found in barley, wheat, pepper, amaranth, and the *Solanaceae* family. It is likely that additional screening would reveal its presence in many other seeds. Lunasin has ▶ chemoprevention properties both in vitro and in vivo. In the absence of carcinogens, Lunasin does not seem to affect cell morphology and proliferation but prevents cell ▶ transformation in the presence of carcinogens. At nanomolar concentrations, Lunasin added exogenously significantly suppresses chemical-carcinogen-induced (▶ DMBA and ▶ MCA) foci formation in mouse fibroblasts cells.

Lunasin also prevents transformation of mammalian cells by viral oncogenes. It inhibits, in a dose-dependent manner, foci formation in experimental mouse cells transfected with the ▶ adenovirus early gene E1A, that induces cell ▶ proliferation by inactivating the ▶ tumor suppressor protein ▶ Rb.

▶ Chemical carcinogenesis and viral oncogenesis share common mechanism (s) involving changes in chromatin status that Lunasin disrupts to suppress cancer formation. Lunasin peptide added exogenously to mammalian cells treated with the ▶ histone deacetylases (HDAC) inhibitor sodium butyrate, internalizes into the cell and crosses the nuclear membrane, localizing in the nucleus. There, it binds specifically to deacetylated core ▶ histones H3 and H4, inhibiting their acetylation. The affinity of Lunasin for hypoacetylated chromatin and its inhibitory effect on histone acetylation is relevant to the proposed ▶ epigenetic mechanism of action (E1A-Rb-HDAC model; Fig. 1). This model stipulates that Lunasin selectively kills cells that are being transformed by disrupting the dynamics of ▶ histone acetylation–deacetylation when a transforming event occurs. The tumor suppressor protein ▶ Rb functions by interacting with ▶ E2F ▶ promoter and recruiting ▶ HDAC to keep the core histones in the deacetylated (repressed) state. The inactivation of Rb by the ▶ adenovirus E1A ▶ oncogene dissociates the Rb-HDAC complex, exposing the deacetylated core histones for acetylation by ▶ histone acetyltransferases (HATs). \When this event occurs, Lunasin is triggered into action and binds to the deacetylated core histones, turning off ▶ transcription, which is perceived as abnormal by the cell and commits ▶ apoptosis. This ▶ epigenetic mechanism shows that Lunasin can influence regulatory pathways involving chromatin modifications that may be fundamental to carcinogenic pathways in general.

In the first animal model, Lunasin applied topically suppresses skin ▶ papilloma formation in ▶ SENCAR mice treated with ▶ DMBA and ▶ TPA. Tumor multiplicity is also reduced, and the appearance of ▶ papillomas is delayed by this peptide.

Oral administration is an important feature of an ideal cancer-preventive agent. To exert this effect, once orally ingested, the peptides have to survive



Lunasin. Fig. 1 E1A-Rb-HDAC model to explain the ability of Lunasin to suppress ▶ Adenovirus early gene (E1A)-induced ▶ transformation. *Top diagram*: ▶ Rb controls ▶ G1/S ▶ transcription by interacting with the ▶ E2F ▶ promoter and recruiting ▶ HDAC to keep the core ▶ histones in the deacetylated (repressed) state. In a cell being transformed, E1A inactivates Rb and dissociates the Rb-HDAC complex, exposing the deacetylated core histones in the ▶ E2F ▶ promoter (*medium diagram*). Lunasin competes with ▶ histone acetyltransferase (HAT) in binding to the deacetylated core ▶ histones. *Bottom diagram*: HAT binds and acetylates core histones, turning on E2F ▶ cell cycle ▶ transcription factors, leads to cell proliferation. Lunasin binds, turns off the transcription, perceived as abnormal by cell, results in ▶ apoptosis

degradation by gastrointestinal and serum proteinases and peptidases, reaching the target tissue or organ in an active form. Bioavailability studies have demonstrated that Lunasin, orally administered to rats, is protected from the gastrointestinal digestion by soy-naturally occurring protease inhibitors, such as ▶ Kunitz Trypsin Inhibitor (KTI). Lunasin is absorbed and ends up in various tissues as an intact and bioactive peptide. This capacity and its in vitro demonstrated properties that make Lunasin the perfect candidate to exert an in vivo cancer-preventive activity. Animal studies demonstrating this preventive activity against different kinds of cancer, such as ▶ prostate cancer, ▶ colorectal cancer and ▶ breast cancer, are currently being carried out.

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Lung Cancer

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Synonyms

Bronchogenic carcinoma; Lung carcinoma

Definition

The term lung cancer is used for malignant epithelial tumors arising from the respiratory mucosa (bronchi, bronchioles, and alveoli). Mesotheliomas, lymphomas, and stromal tumors (sarcomas) are distinct from epithelial lung cancers.

Characteristics

Lung cancer is the leading cause of cancer death worldwide with over one million deaths each year. The disease is largely preventable as the majority of cases are attributable to smoking. However, global statistics estimate that 15% of lung cancer cases in men and 53% in women occur in never smokers.

The 5-year overall lung cancer survival rate (15%) has nearly doubled in the past 30 years as a result of advances in combined-modality treatment with surgery, radiotherapy, and chemotherapy. Thus, primary carcinoma of the lung is a major health problem with a generally poor prognosis.

Pathology

Four major cell types make up 88% of all primary lung neoplasms according to the World Health Organization classification. These are ▶ squamous cell carcinoma, ▶ small cell carcinoma, ▶ adenocarcinoma (including bronchioloalveolar), and ▶ large cell carcinoma. The remainder includes rarer tumor types including carcinoids. The various cell types have different natural histories and responses to therapy, and thus a correct histologic diagnosis is essential. In the past 25 years, adenocarcinoma has replaced squamous cell carcinoma as the most frequent histologic subtype, and the incidence of small cell carcinoma is on the decline. These dramatic histologic shifts may be due to changes in the composition of cigarettes.

Etiology

Most lung cancers are caused by ▶ carcinogens and tumor promoters inhaled via ▶ cigarette smoking. The relative risk of developing lung cancer is increased about 13-fold by active smoking and about 1.5-fold by long-term passive exposure to cigarette smoke. Exposure to industrial pollutants and chemicals, including ▶ asbestos, ionizing radiation, ▶ radon etc., contribute to ▶ pathogenesis, often exacerbating the effects of ▶ tobacco carcinogensis.

Biology and Molecular Pathogenesis

Molecular genetic studies have shown the acquisition by lung cancer cells of a number of genetic lesions, including activation of dominant ▶ oncogenes and inactivation of ▶ tumor suppressor genes. Lung cells may have to accumulate a large number of such lesions before developing a fully invasive/metastatic phenotype.

Activation of Dominant Oncogenes

Changes in dominant oncogenes include point mutations in the coding regions of the RAS family of oncogenes (especially ▶ KRAS gene in adenocarcinoma of the lung); mutations in the tyrosine kinase domain of the ▶ Epidermal Growth Factor Receptor (EGFR) found in nonsmoking adenocarcinomas (~10% in Caucasioans, with much higher rates in East Asians) with rates >50% in nonsmoking East Asian patients); occasional mutations in ▶ BRAF and ▶ PIK3CA or activation of the PIK3CA/AKT/▶ mTor pathway; amplification, rearrangement, and/or loss of transcriptional control of myc family oncogenes are found in non-small cell cancers, while changes in all myc family members are found in small cell lung cancer); overexpression of bcl-2 and other anti-apoptotic proteins; and overexpression of other EGFR family members such as ▶ Her-2/neu and ERBB3; and activated expression of the ▶ telomerase gene in >90% of lung

cancers. Genome-wide approaches are identifying other ▶ amplified or mutated dominant ▶ oncogenes (such as ▶ MET) which could be important new therapeutic targets.

Inactivation of Tumor Suppressor Genes

Selected study and genome-wide approaches have identified a large number of tumor suppressor genes (recessive oncogenes) that are inactivated during the pathogenesis of lung cancer. This usually occurs by a tumor-acquired inactivating mutation of one allele (seen, e.g., in the ▶ *p53* and ▶ retinoblastoma, ▶ *RB1*, tumor suppressor gene) or tumor-acquired inactivation of expression by tumor-acquired promoter DNA ▶ methylation (seen for example in the case of the *p16* and *RASSF1A* tumor suppressor genes) which are then coupled with physical loss of the other parental allele ("▶ loss of heterozygosity"). This leaves the tumor cell with only the functionally inactive ▶ Allele and thus loss of function of the growth regulatory tumor suppressor gene. Genome-wide approaches have identified many such genes involved in lung cancer pathogenesis including ▶ *TP53*, ▶ *RB1*, *RASSF1A*, *SEMA3B*, *SEMA3F*, *FUS1*, *p16*, *LKB1*, *RARβ*, and ▶ *FHIT*. Several tumor suppressor genes on chromosome 3p appear to be involved in nearly all lung cancers. Allelic loss for this region occurs very early in lung cancer pathogenesis, including in histologically normal smoking damaged lung epithelium.

Autocrine Growth Factors

The large number of genetic and epigenetic lesions indicates that lung cancer, like other common epithelial malignancies, is a multistep process that is likely to involve both carcinogens causing mutation ("initiation") and tumor promoters. Prevention can be directed at both processes. Lung cancer cells produce many peptide hormones and express receptors for these hormones, which can act to stimulate tumor cell growth in an ▶ "autocrine" fashion.

Highly ▶ carcinogenic derivatives of ▶ nicotine are formed in cigarette smoke. Lung cancer cells of all histologic types (and the cells from which they are derived) express nicotinic acetylcholine receptors. Nicotine activates signaling pathways in tumor and normal cells that block ▶ apoptosis. Thus, nicotine itself could be directly involved in lung cancer pathogenesis both as a mutagen and tumor promoter.

Clinical Presentation

There are multiple ways by which lung cancer presents, including weight loss, chronic cough, persistent lung infection, malaise, chest pain, hemoptysis, etc. ▶ SCLC, in particular, may present with a wide variety of paraneoplastic syndromes. With the advent of computer tomography (CT) screening programs, a modest number of mostly early stage cancers are being detected as asymptomatic tumors.

Treatment

Two important guiding principles in the treatment of lung cancer are: (1) histologic diagnosis (especially the distinction between ▶ small cell lung cancer or ▶ non-small cell lung cancer, NSCLC) and (2) staging. For early stages of NSCLC, complete resections offer relatively high 5-year survivals. Patients unsuitable for surgery may be offered curative intent radiotherapy. ▶ Adjuvant therapy may be given to more advanced resected cases. For late-stage cases, chemotherapy with or without ▶ palliative radiotherapy are the conventional options, although the long-term survival rates are very low.

Small cell lung cancer (SCLC) is considered a chemotherapy-sensitive disease. Patients with limited stage disease have high response rates to chemotherapy (60–80%), including a 10–30% complete response rate, although the response rates in extensive disease patients is somewhat lower (50%), and almost always consists of partial responses. Tumor regressions usually occur quickly, within the first two cycles of treatment, and provide rapid palliation of tumor-related symptoms. Thus, chemotherapy is the backbone of treatment of patients with SCLC.

Given the poor prognosis of most patients with lung cancer, ▶ targeted therapies offer the best long-term prospects. ▶ Tyrosine kinase inhibitors (▶ erlotinib and ▶ gefitinib) have been associated with responses and improved survival in specific subsets of NSCLC, specifically adenocarcinoma histology, female gender, East Asian ethnicity, and never smoking status. The presence of EGFR mutations (and/or increased gene copy number in some studies) in the tumors are often associated with dramatic responses and lengthened good quality life, although

most responders eventually relapse. Many other targeted therapies are currently undergoing clinical trial.

Prevention

As lung cancer is one of the most preventable of diseases, antismoking campaigns have been initiated in many countries. Avoidance of smoking for never smokers and cessation for current smokers offer the best hopes for prevention, although these aims have proven more difficult to implement than expected. However, as pointed out earlier, about 15% of lung cancer occurs in lifetime never smokers. The very different molecular changes in cancers arising in smokers and never smokers suggests that ▶ environmental tobacco smoke ("passive smoking") can only account for a minority of cancers arising in never smokers. Thus lung cancer will remain an important cause of morbidity and mortality long after a complete ban on the use of tobacco products is implemented on a worldwide scale.

- ▶ Cancer Causes and Control
- ▶ Chronic Obstructive Pulmonary Disease and Lung Cancer
- ▶ Extrapulmonary Small Cell Cancer
- ▶ Lung Cancer and Smoking Behavior
- ▶ Lung Cancer Clinical Oncology
- ▶ Lung Cancer Epidemiology
- ▶ Lung Cancer Staging
- ▶ Lung Cancer Targeted Therapy
- ▶ Mesothelioma
- ▶ Non-Small Cell Lung Cancer
- ▶ Small Cell Lung Cancer
- ▶ Uranium Miners

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Lung Cancer and Smoking Behavior

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Definition

▶ Lung cancer is a major cause of cancer-related death in developed countries and the overall survival rate is still extremely poor. Principal histological types of lung cancer according to the International Classification of Diseases for Oncology, second edition, (ICD-O-2) are ▶ adenocarcinoma, ▶ squamous cell carcinoma, small cell carcinoma, and large cell carcinoma. Formerly, cases of lung cancer were divided into two broad categories based on the relationship between smoking and lung cancer risk, Kreyberg type I and II. The Kreyberg type I lung cancer (smoking related) included squamous cell carcinoma, large cell carcinoma, and small cell carcinoma, while the Kreyberg type II lung cancer (less smoking related or smoking unrelated) included adenocarcinoma.

Currently, the two main groups are ▶ small cell lung cancer (SCLC) and ▶ non-small cell lung cancer (NSCLC). The adenocarcinoma, squamous cell carcinoma, and large cell carcinoma are grouped together as NSCLC because their prognosis and management are similar. NSCLC accounts for approximately 80–85% of all cases of lung cancer. All four histological types are probably caused by smoking, although the risk tends to rise less steeply with extent of smoking for adenocarcinoma.

Cancer is the result of a series of DNA alterations in single cell or clone of that cell, which lead to loss of normal function, aberrant or deregulated cell growth, and often ▶ metastasis. DNA alterations can be caused by exposure to tobacco smoke, environmental and endogenous carcinogens. Although tobacco smoking is an established risk factor for lung cancer, only approximately one in ten smokers develop lung cancer in their lifetime indicating an interindividual variation in susceptibility to tobacco smoke. Lung cancer is a common disease that results from a complex interplay of genetic and environmental risk factors just like

other common multifactorial diseases, such as cardiovascular disease, diabetes mellitus, and mental disorder.

Characteristics

Incidence and Mortality

Although the incidence has peaked in the USA and most of Europe, many more developed and less developed countries are having increasing incidence and mortality from lung cancer. An estimated 1,239,000 (902,000 males and 337,000 females) new cases of lung cancer were diagnosed worldwide in 2000, accounting for 12.3% of all new cases of cancer, and 1,103,000 (810,000 males and 293,000 females) died from the disease, accounting for 17.8% of all deaths of cancer. The disease is the biggest cancer killer in males and the second biggest in females.

For males, incidence rates have varied up to 20 times between high-risk areas and low-risk areas. The highest world age-standardized incidence rate per 100,000 (ASR) has been recorded among African-Americans from New Orleans, USA (ASR = 96.6) and has been followed by African-Americans from Louisiana, USA (ASR = 91.7). The lowest incidence rates areas are Kyadondo county, Uganda (ASR = 4.8); Trujillo, Peru (ASR = 5.9); Poona, India (ASR = 6.1); and Nagpur, India (ASR = 7.5). For females, incidence rates have varied up to 49 times between high-risk areas of North America, such as Kentucky, USA (ASR = 50.3); and low-risk areas of Africa, such as Setif, Algeria (ASR = 1.7); and Sousse, Centre, Tunisia (ASR = 1.7); and Southern Asia, such as Trivandrum, India (ASR = 1.7). The case fatality (ratio of mortality to incidence), which is an indicator of prognosis, is 0.89 for lung cancer (the third worst). Other cancers with bad prognosis are pancreas (0.99, the worst) and liver (0.97, the second-worst) cancers.

Worldwide, lung cancer incidence and mortality is approximately three times higher in males than in females. Lung cancer mortality among males is now abating in several countries, while the mortality in females continues to climb in most countries, as predicted by later onset tobacco abuse.

Cigarette Smoke

There are two main types of cigarette smoke: mainstream cigarette smoke and sidestream cigarette smoke. Mainstream cigarette smoke is a smoke that is directly inhaled by the smoker while sidestream cigarette smoke is a smoke that is emitted from burning cigarette. Environmental tobacco smoke (ETS), also known as secondhand smoke, is the combination of two forms of smoke: sidestream cigarette smoke (85% of ETS) and an exhaled mainstream cigarette smoke, which is the smoke that is first of all inhaled by the smoker upon taking a puff of a cigarette and then breathed out into the air from his lungs (15% of ETS). In other words, smokers are mainly exposed to mainstream cigarette smoke but nonsmokers are predominantly exposed to ETS. Mainstream cigarette smoke contains more than 4,000 chemicals, including over 40 known cancer-causing (carcinogenic) compounds and 400 other toxic compounds. Tobacco-specific N-nitrosamines (TSNAs) are formed by N-nitrosation of nicotine and other minor alkaloids during processing tobacco and during smoking. Since nitrate is the major precursor for nitrogen oxides, increased nitrate content leads to higher yields of 4 (methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in the smoke. Polycyclic aromatic hydrocarbons (PAHs), aromatic amines (AAs), N-nitrosamines (NAs), TSNAs, and trace metals comprise major classes of carcinogenic compounds in cigarette smoke. Nitrogen oxides, acrolein, ammonia, formaldehyde, and nicotine are toxic compounds. ETS contains many of the same chemical constituents as those in mainstream smoke, but the physiochemical nature of sidestream smoke compared with mainstream smoke leads to differences in the ratio of constituents, which may be higher in sidestream smoke. For example, concentrations of benzo(a)pyrene (BP) (a type of PAHs, 3.4 time higher), 4-aminobiphenyl (4-ABP) (a type of AAs, 31 times higher), NNK (a type of TSNAs, ten times higher) are higher in undiluted sidestream cigarette smoke than in mainstream cigarette smoke.

Active Smoking

The tobacco product is manufactured from the leaf of tobacco plant (scientific name: *Nicotiana tabacum* L.). The main types of the tobacco product are cigars, cigarettes, pipe tobacco, and smokeless tobacco (snuff and chewing tobacco). Cigar or pipe smoke, like cigarette smoke, contains toxic chemicals and carcinogens. While 28 carcinogenic agents have been identified in smokeless tobacco, TSNAs are the most

harmful carcinogens. Cigarettes account for the largest share of manufactured tobacco products in the world (96% of total value sales).

The association between lung cancer and cigarette smoking behavior is probably the most intensively investigated relation in epidemiology. Pipe and cigar smokers have less than half the risk of lung cancer as cigarette smokers. The higher prevalence of inhalation in cigarette smokers than in pipe and cigar smokers leads to higher levels of exposure to tobacco combustion products. The lack of an increased risk of lung cancer among smokeless tobacco users has been observed. Abundant evidence indicates that cigarette smoking induces all major histological types of lung cancer. An increase in tobacco consumption is paralleled some 20 years later by an increase in the incidence of lung cancer, and a decrease in consumption is followed by a decrease in incidence. In 1950, the first large-scale case-control study on the association between lung cancer and cigarette smoking was published. Also in 1950, a critical article confirmed the strong association between smoking behavior and lung cancer.

The risk among smokers relative to the risk among lifelong nonsmokers is 8–15 times in males and 2–10 times in females. The overall relative risk reflects the contribution of the different aspects of tobacco smoking: average consumption, duration of smoking, time since quitting, age at start, type of tobacco product, and inhalation pattern.

The association between lung cancer and smoking is more dominant in males compared to females. However, several epidemiological studies indicate that for a given number of cigarette smoked, females may be at higher risk of lung cancer compared with males. Females who smoke appear to be at higher risk of developing small cell lung cancer than squamous cell lung cancer, whereas males who smoke have a similar risk for the two histological conditions. Molecular epidemiological studies have also indicated sex differences in the genetic and biochemical alterations in lung cancer. In several epidemiological studies, the risk for lung cancer is consistently higher in females than in males at every level of exposure to cigarette smoking; the risks for an association of lung cancer with smoking are 1.4–1.9 times higher for females than for males, depending on the histological type of lung cancer. Differing distributions of histological types of lung cancer between males and females may be associated with differences in smoking patterns, but this does not fully explain the observed sex differences. Given many risk factors that have been identified for lung cancer, a practical question is the relative contribution of these factors to the summary burden of lung cancer. The population attributable fraction (PAF) takes into account the magnitude of relative risk that is associated with an exposure along with the likelihood of exposure in the general population. As shown in Table 1, the PAF of lung cancer mortality due to smoking was 79% in males and 48% in females. A lower prevalence of smokers contribute to the lower PAF of active smoking in females. Endogenous factors related to sex may also contribute to sex differences in lung cancer risk.

Lung Cancer and Smoking Behavior. Table 1 Population attributable fraction for lung cancer

Risk factor	Population attributable fraction
Active smoking	79% in males and 48% in females
Environmental tobacco smoke exposure	0.7% (0.2% in males and 2.5% in females), 2.5%
Dietary factor	20%
Air pollution exposure	1–3.6%
Radon exposure	1%
Carcinogen exposure	9–15%

Because of the interactions between exposures, the combined population attributable fraction for lung cancer can exceed 100%

Smoking cessation does significantly reduce lung cancer risk, but the relative risk remains 1.5–2.0 times the risk of lifelong nonsmokers. Some have shown that in the initial few years of quitting smoking, the lung cancer risk may actually increase as many patients quit smoking because of symptoms that might be caused by a lung cancer. The risk of lung cancer does decrease with the duration of cessation and typically plateaus at 20 years. As compared with continuous smokers, the excess risk sharply decreases in ex-smokers approximately 5 years after quitting.

Squamous cell carcinoma predominated in males and adenocarcinoma predominated in females. However, the histological characteristics of lung cancer in a number of developed countries have changed in recent decades so that the frequency of adenocarcinoma has risen and that of squamous cell carcinoma has declined. Recently, the incidence of adenocarcinoma has exceeded that of squamous cell carcinoma

Lung Cancer and Smoking Behavior. Table 2 Squamous cell carcinoma: adenocarcinoma incidence (age-standardized, world) rate ratios for lung cancer in selected developed countries

Country, registry	Lung cancer	Squamous cell carcinoma (SCC)	Adenocarcinoma (Ad)	Ratio (Ad/SCC)
Male				
USA, New Orleans, Black	96.6	28.1	22.5	0.80
USA, New Orleans, White	69.6	16.0	23.7	1.48
USA, Louisiana, Black	91.7	27.5	22.3	0.81
USA, Louisiana, White	70.3	18.4	20.2	1.10
Germany, Munich	56.7	9.5	8.2	0.86
Germany, Saarland	59.6	18.2	14.5	0.80
Italy, Milan	57.8	10.0	15.9	1.59
Italy, Romagna	58.5	17.3	15.4	0.89
UK, England, Thames	46.0	10.9	6.1	0.56
UK, England, Northern and Yorkshire	53.4	17.1	7.6	0.44
Denmark	46.2	12.7	12.2	0.96
France, Bas-Rhin	59.2	22.3	13.3	0.60
France, Isere	49.5	15.9	13.7	0.86
Japan, Miyagi	40.6	10.7	13.2	1.23
Japan, Osaka	43.3	10.0	13.0	1.30
Female				
USA, New Orleans, Black	44.4	6.6	11.5	1.74
USA, New Orleans, White	43.6	6.4	15.3	2.39
USA, Louisiana, Black	33.5	7.0	9.9	1.41
USA, Louisiana, White	39.7	6.8	12.8	1.88
Germany, Munich	13.6	1.5	4.6	3.07
Germany, Saarland	17.3	3.3	5.7	1.73
Italy, Milan	16.7	1.4	6.2	4.43
Italy, Romagna	14.1	2.2	5.6	2.55
UK, England, Thames	23.1	3.9	4.4	1.13
UK, Scotland	30.0	6.6	5.3	0.80
Denmark	31.6	5.0	11.9	2.38
France, Bas-Rhin	11.1	2.0	4.7	2.35
France, Isere	8.7	1.4	3.7	2.64
Japan, Miyagi	12.5	0.8	7.8	9.75
Japan, Osaka	13.9	1.3	6.8	5.23

Data from cancer incidence in five continents, 2007

among males as well as females in developed countries, especially in American and Japanese registries (Table 2). The changes in the ratios of these histological types of lung cancer are not ascribed to differences in sex or age. The increase in incidence of adenocarcinoma could be partly explained by an increase in filtered cigarette smoking. Filter cigarettes with low tar and low nicotine have replaced nonfilter cigarettes. One key characteristic of such changes over time was an increased nitrate content of the tobacco blend from about 0.5% to 1.3%. To satisfy a craving for nicotine, a smoker of low-yield nicotine filtered cigarette may tend to compensate by increasing the number and depth of puffs but the risk of lung cancer is slightly lower among smokers of low-tar and low-nicotine cigarettes than among other smokers. A 1.5–3 times difference in the relative risk of lung cancer has been found in several studies between smokers who deeply inhale cigarette smoke and smokers of comparable amounts of tobacco who do not inhale or inhale

slightly. Therefore, the peripheral lung, where adenocarcinoma generally arises, is exposed to higher amount of smaller particles such as NNK. Indeed, NNK is a systemic carcinogen that induced lung carcinoma in laboratory animals independent of the site of application, whereas intratracheal instillation of BP and other PAHs preferentially induced squamous cell carcinoma. It is biologically plausible that TSNAs such as NNK causes adenocarcinoma in humans. Over the past two decades, the incidence of adenocarcinoma of the lung increased much more rapidly than that of squamous cell carcinoma in both males and females. During this period, filter cigarettes had taken the place of nonfilter cigarettes. The increase in incidence of adenocarcinoma could be partly explained by an increase in filtered cigarette smoking.

Among smokers, quite striking racial differences in the effect of smoking on lung cancer risk were observed. Some Japanese studies have shown that the magnitude of lung cancer risk associated with cigarette smoking is lower than those in Western countries. For males, the risks of lung cancer among current smokers compared to nonsmokers are estimated to be about five in Japanese and over ten in Americans and Europeans. For females, the risk of lung cancer is estimated to be less than four in Japanese while the corresponding figures range from 2.7 to 12.8 in Americans and Europeans. The differences may be modified by not only smoking but also other factors such as genetic, dietary, occupational, and educational factors.

Exposure to Environmental Tobacco Smoke

Nonsmokers are exposed to ETS diluted by several orders of magnitude compared with the mainstream inhaled by active smokers. It has also been become obvious that exposure to ETS in the home, public places, or workplaces is more than a simple nuisance to nonsmokers. Of particular concern is the question whether ETS exposure from husbands increases the risk of nonsmoking females to develop lung cancer. In 1986, the International Agency for Research on Cancer (IARC) concluded that there was some risk of lung cancer from exposure to ETS. Today ETS has been judged to be a cause of lung cancer in nonsmokers by IARC. Likewise, Environmental Protection Agency (EPA) concluded that ETS was a human carcinogen. It is now widely accepted that ETS may also cause lung cancer. A meta-analysis showed that ETS exposure from husbands conferred a 1.20 times increase in the risk of lung cancer among nonsmoking females. As shown in Table 1, the PAF for lung cancer deaths due to ETS exposure accounts for 0.7% (0.2% in males and 2.5% in females) or 2.5%. Slightly lower risks were found in studies conducted in the USA (1.18 times increase) as compared with European (1.27 times increase) and Japanese ones (1.30 times increase). Racial differences may exist in the risk modification by metabolic polymorphisms in the association of ETS and lung cancer.

Genetic Basis of Smoking Behavior

Evidence for genetic determinants affecting the smoking phenotype has steadily accumulated both from studies of substance abuse in animals and from analysis of the contributions of genetics and personality to substance abuse in humans. An appreciation of the neurotransmitter-related mechanisms involved in reward circuits in the human brain had suggested many candidate loci potentially associated with smoking behavior. Genetic difference is known for substances in tobacco smoke absorption, their metabolism, and for their interactions with receptors. Nicotine dependence is one of the major determinants of smoking behavior because the vast majority of cigarette smokers are daily smokers and, of these, the majority are nicotine-dependent smokers as evinced by the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, (DSM-IV) criteria. Several specific genes related to nicotine dependence have been identified. Genes of dopamine receptors (DRs), serotonin receptor (5-HTRs), monoamine oxidases (MAOs), neuronal nicotinic acetylcholine receptors (CHRNs or nAChRs), serotonin transporter (5-HTT or SERT), dopamine transporter (SLC6A3 or DAT), cholecystokinin (CCK, modulates the release of dopamine), CCK A receptor (CCKAR), and nicotine metabolizing enzymes (cytochrome P450 (CYP) 2A6, CYP2D6) are considered to be responsible for nicotine dependence. The 15q24-25.1 region encompasses CHRNA3 and CHRNA5 (as well as CHRNB4), which have a defined role in nicotine dependence. The 15q24-25.1 region has been reported to affect both smoking behavior and lung cancer risk.

Genetic Basis of Lung Cancer

Lung cancer is a multifactorial disease that results from complex interactions between many genetic and

environmental factors. This means that there will not be single gene or single environmental factor that has affected on lung cancer susceptibility. Tobacco smoke and other environmental factors add to carcinogenic load that humans are exposed, but exact numbers are generally less well established. Obviously, cigarette smoking is the strongest independent risk factor for lung cancer. Most of the compounds in cigarette smoke (procarcinogens) require metabolic activation by Phase I enzymes such as CYPs to their ultimate (activated) forms and then bind to DNA, forming aromatic-DNA adducts that are thought to be an early step in carcinogenesis. CYP1–CYP4 are primarily involved in the drug metabolism. Other Phase I enzymes are myeloperoxidase, NAD(P)H quinone oxidoreductase 1 (NQO1), microsomal epoxide hydrolase 1, and alcohol dehydrogenase. Following the Phase I reaction, Phase II enzymes like glutathione S-transferases (GSTs) are responsible for detoxifying the activated forms of PAH epoxides. The major isoforms, which involve the metabolic activation of carcinogens derived from tobacco smoke or the detoxification of the respective activated carcinogens, are GSTM1, GSTM3, GSTT1, and GSTP1. Other Phase II enzymes are microsomal epoxide hydrolase 1, NQO1, N-acetyltransferases, UDP-glucuronosyltransferase, aldehyde dehydrogenase, sulfotransferase, and superoxide dismutase.

DNA damage itself is a balance between activation and detoxification of carcinogens that involve Phase I and II metabolic enzymes. The capacity to repair DNA damage induced by chemical carcinogens appears to be another host factor that may influence lung cancer risk. At least four pathways of DNA repair operate on specific types of damaged DNA. Base excision repair (BER) operates on small lesions, while the nucleotide excision repair (NER) pathway repairs bulk lesions. DNA mismatch repair (MMR) removes nucleotides that have been misincorporated into the newly synthesized DNA strand during replication. Double-strand DNA break repair (DSBR) actually consists of two pathways, homologous recombination (HR) and nonhomologous end-joining (NHEJ). The NHEJ repair pathway involves direct ligation of the two double-strand break ends, while HR is a process by which double-strand DNA breaks are repaired through the alignment of homologous sequences of DNA. Potentially important and frequently investigated BER and NER genes are 8-oxoguanine-DNA glycosylase 1 (OGG1), X-ray cross-complementing group 1 (XRCC1), apurinic/apyrimidinic exonuclease 1 (APEX1), xeroderma pigmentosum A (XPA), and the excision repair cross-complementing group 1 (ERCC1) and ERCC2. Furthermore, cell-cycle control genes (TP53, cyclins, etc.), genes that influence smoking behavior and genes involved in development of the immune system (interleukins, tumor necrosis factor, etc.,) may have the potential to substantially affect lung cancer risk. Most genetic polymorphisms are related to a 10–20% decrease/increase in lung cancer risk. An improved understanding of the interplay of environmental and genetic polymorphisms at multiple loci may help identify individuals who are at increased risk for lung cancer.

Environmental Factors Other Than Cigarette Smoke

Dietary Factors

Convincing evidence shows that a diet rich in vegetables and fruits exerts a protective effect against lung cancer. The evidence is most abundant for green vegetables and carrots. Many studies have addressed the risk of lung cancer according to estimated intake of either β-carotene or total carotenoids (which in most cases correspond to the sum of α-carotene and β-carotene). The overwhelming evidence of a protective effect from observational studies has been challenged by the results of four randomized intervention trials based on β-carotene supplementation. An evaluation of IARC concluded that the available evidence suggests a lack of preventive activity of β-carotene used as a supplement in high doses and inadequate evidence with regard to its activity at usual dietary levels. Carotenoids contained in foods of plant origin are probably protective, whereas carotenoids other than β-carotene (e.g., α-carotene, β-cryptoxanthin, lutein, lycopene) are insufficient for an evaluation. A possible mechanism of cancer promotion by β-carotene by smokers might involve the production of oxidative metabolites. Diets in high vitamin C and vitamin E possibly reduce the risk of lung cancer. On the contrary, dietary constituents such as cholesterol, total fat and saturated/animal fat, and alcoholic beverages possibly increase the risk of lung cancer. With regard to alcohol use, the possibility of residual confounding by tobacco smoking has not been completely ruled out, however. No conclusive evidence between a positive association between retinol,

selenium, and other dietary factors, including meat, dairy products, and coffee, and lung cancer risk has been provided. However, the effect of retinol could not be distinguished from that of β-carotene. Approximately 20% of lung cancer deaths in the USA were potentially avoidable by diet modification (Table 1).

Occupational Exposure

The risk of lung cancer is increased among workers employed in a number of industries and occupations. The responsible agents have been identified for several, but not all, of these high-risk workplaces. Evidence for the carcinogenicity of many occupational agents has been reviewed in the IARC Monographs. Exposure to certain kinds of metals (arsenic, chromium (VI), nickel, cadmium, and beryllium) has provided sufficient evidence of carcinogenicity for lung cancer. Asbestos is an important occupational lung carcinogen; all the different forms of asbestos (chrysotile, crocidolite, amocite, and tremolite) are carcinogenic to the human lung, although the potency of chrysotile might be lower than that of other types. A characteristic of asbestos-related lung cancer is its synergistic relation to cigarette smoking: Risk is increased multiplicatively among subjects who are both smokers and exposed to asbestos. Such a phenomenon has been recorded in relation to other occupational lung cancers. Synthetic fibers (vitreous fibers, mineral wool fibers, continuous glass filament, and refractory ceramic fibers) have been suggested to have an association with lung cancer, although no conclusions can be drawn at present. In the USA, occupational exposure to carcinogens accounts for approximately 9–15% of lung cancer cases (Table 1).

Air Pollution

Some lung cancer patients have not been exposed to either cigarette smoke or occupational carcinogens. Abundant evidence indicates that lung cancer rates are higher in cities than in rural settings. Urban air pollution is a risk factor for lung cancer and the excess risk may be in the order of 50%. Residential proximity to certain industries is associated with exposure to lung carcinogens. Hence, those living near a factory can be exposed to known or purported carcinogens. This potential relation between outdoor environmental exposure and lung cancer is poorly researched and few studies are able to generate the power required to identify what is potentially a much smaller effect than that of smoking or occupational exposure. The effect of air pollution on lung cancer may be greater than zero but the epidemiological evidence was weak because of poor confounder adjustment and the studies being of a descriptive nature. Evidence for a relation between lung cancer and air pollution was modest, with intermediate consistency of findings, limited dose-response evidence, and crude adjustment for important potential confounders. The PAF for lung cancer deaths due to outdoor air pollution accounts for 1–3.6% (Table 1). Indoor pollution is caused by the use of coal burning heaters without proper exhaust emission and high-temperature cooking using unrefined vegetable oils. Indoor levels of BP are reported to be very high in such circumstances. Indoor pollution is a major cause of lung cancer in Chinese females, who experience very high lung cancer rates despite a low prevalence of smoking.

Residential Radon Exposure

Various forms of ionizing radiation, including radon-decay products, are important occupational lung carcinogens. Underground miners exposed to radioactive radon and its decay products, which emit α-particles, have been consistently found to be at increased risk of lung cancer. It has been estimated an apparently linear, approximately 6% risk increase per working-level year of exposure, and the risk is greater for lower rates over a longer period, which modifies the carcinogenic effect of radon. It has been also indicated radon exposure at 150 Bq/m^3 gave an overall risk of 1.14–1.24. Radon may be responsible for only 1% of lung cancer deaths (Table 1).

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Lung Cancer Clinical Oncology

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Synonyms

Bronchogenic carcinoma; Cancer of the lung; Malignant neoplastic changes of the lung or bronchus

Definition

► Lung cancer refers to malignant neoplasia of the lung or bronchus.

Characteristics

Lung cancer is the leading cause of cancer deaths globally. Traditionally, lung cancer is composed of four major histologic types:

- Adenocarcinoma
- Squamous cell carcinoma
- Large cell neuroendocrine carcinoma
- Small cell carcinoma

Adenocarcinoma is the most common histology comprising close to approximately 40–50% of all lung cancer, followed by squamous cell carcinoma (approximately 25%), small cell carcinoma (approximately 15–20%), and large cell neuroendocrine carcinoma (approximately 10%).

For treatment purposes, lung cancer traditionally has been grouped into:

- Non-small cell lung cancer (NSCLC) which comprises adenocarcinoma, squamous cell carcinoma, and large cell neuroendocrine carcinoma
- Small cell lung cancer (SCLC)

However, treatment of NSCLC has become more sophisticated involving treatment based on histology (adenocarcinoma vs non-adenocarcinoma) and molecular alterations (presence or absence of ► EGFR mutations or ► ALK translocations).

Another rare but deadly lung cancer is ► mesothelioma which is related to asbestos exposure in the past and also recently simian virus 40 (► SV40).

Screening Strategies for Lung Cancer

Currently there is no screening technique that has been shown to decrease lung cancer mortality. Chest X-ray with or without the addition of sputum cytology had not shown to decrease lung cancer mortality. A large-scale clinical trial in the USA (National Lung cancer Screening Trial – NLST) that has completed enrolment of 50,000 former or current smokers comparing baseline and then annual ► CT scan for 2 years to chest X-ray performed at the same scheduled. The primary endpoint of the NLST is 50% reduction in lung cancer mortality, and results are pending. A similar randomized trial in being conducted in Europe comparing CT to standard of care will complete enrolment of 20,000 high-risk patients in 2010. Compounding the difficulty of lung cancer screening is that only about one out of nine current of former smoker will develop lung cancer in their lifetime and identifying those really high-risk former or current smokers who will go on to develop lung cancer by molecular profiling will decrease the number of patients to be screened.

Staging Workup of Lung Cancer

Required examinations:

- History (including detailed active and passive smoking history)
- Physical examination
- ► CT chest, abdomen, and pelvis with contrast
- CT or ► MRI of the brain
- Bone scan or ► PET scan

Staging of Lung Cancers

The ► staging of lung cancer has been updated in 2010 by the International Association for the Study of Lung Cancer (IASLC) and adopted by the American Joint Committee on Cancer (AJCC) and ► UICC. The major changes are a finer classification of the Tumor (T) descriptor based on tumor size. The previous sixth edition of this staging system included only one cut-off for tumor size (<3 cm versus >3 cm) in the previous staging system, while in the current staging system there are four cut-offs for tumor size (2, 3, 5, and 7 cm). Additionally, changes to the T descriptors include satellite tumor nodules within the same lobe (T4 to T3), within the same lung (M to T4), or within the opposite lung (still M). Previous T4 descriptors such as malignant pleural and pericardial effusions

Lung Cancer Clinical Oncology. Table 1 The current seventh edition of TNM staging descriptors for lung cancer

	T descriptor
TX	Primary tumor cannot be assessed, or tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy
T0	No evidence of primary tumor
T1	Tumor <3 cm in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of more proximal than lobar bronchus (i.e., not in the main bronchus)
T1a	Tumor ≤2 cm in greatest dimension
T1b	Tumor >2 cm but ≤3 cm in greatest dimension
T2	Tumor >3 cm but <7 cm
	OR
	Tumors with any of these features: involves the main bronchus >2 cm distal to the carina, invades visceral pleura, associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung
T2a	>3 cm and <5 cm
T2b	>5 cm and ≤7 cm
T3	>7 cm (previous T2)
	OR
	Additional tumor nodules in the same lobe (previous T4)
	OR
	Tumor that invades any of the following chest wall (including superior sulcus tumors), diaphragm, phrenic nerve, mediastinal pleura, parietal pericardium
	OR
	Tumor in the main stem bronchus <2 cm distal to the carina but without involvement of the carina
	OR
	Associated atelectasis or obstructive pneumonitis of the entire lung
T4	Ipsilateral pulmonary metastasis (previous M1)
	OR
	Tumor of any size that invades any of the following: mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, and carina
	N descriptor
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2	Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
N3	Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral, or contralateral scalene or supraclavicular lymph node(s)
	M descriptor
MX	Distant metastasis cannot be determined
M0	No distant metastasis
M1	Distant metastasis
M1a	Malignant pleural effusion (previous T4)
	OR
	Malignant pericardial effusion (previous T4)
	OR
	Separate tumor nodules in a contralateral lobe
M1b	Extrapulmonary (distant) metastasis

have been re-classified as metastatic (M) disease. The staging system of lung cancer is applicable to both ►NSCLC and ►SCLC. The nodal staging for lung cancer has not been changed. The M staging has been divided into M1a and M1b though the stage IV has not been divided into stage IVa and stage IVb (Tables 1, 2).

Lung Cancer Clinical Oncology. Table 2 The current seventh edition of ▶ TNM stage grouping of lung cancer

	T1a	T1b	T2a	T2b	T3	T4
N0	IA	IA	IB	IIA[1]	IIB	IIIA[2]
N1	IIA	IIA	IIA[2]	IIB	IIIA	IIIA[2]
N2	IIIA	IIIA	IIIA	IIIA	IIIA	IIIB
N3	IIIB	IIIB	IIIB	IIIB	IIIB	IIIB
M1a	IV	IV	IV	IV	IV	IV
M1b	IV	IV	IV	IV	IV	IV

[1]Indicated stage grouping has been upstaged

[2]Indicated stage grouping has been downstaged from previous stage grouping

Non-small Cell Lung Cancer (NSCLC)

Early Stage NSCLC (Stage I–II)

Stage I ▶ Randomized controlled trials have not demonstrated any overall survival benefit from the use of four cycles of ▶ platinum-based ▶ adjuvant chemotherapy when compared to observation alone. Retrospective analysis of the CALGB9633 trial revealed that for tumors 4 cm or larger there was a survival benefit with the use of four cycles of ▶ carboplatin/▶ paclitaxel combination versus observation. Thus an ongoing intergroup adjuvant trial is comparing four cycles of ▶ cisplatin-based chemotherapy with or without the addition of ▶ bevacizumab (▶ monoclonal antibody against the circulating ▶ vascular endothelial growth factor-A [VEGF-A]) that included tumor >4 cm.

Japanese investigators also arrived at the conclusion comparing the use of ▶ UFT, a ▶ prodrug of 5-▶ Fluorouracil (5-FU), to observation in resected stage I NSCLC that demonstrated only patients with tumor >3 cm benefited from 2 years of adjuvant UFT. For stage IA patients, the standard of care is ▶ lobectomy with careful sampling and resection of N1 and associated N2 nodes with lymphatic drainage from the lobar location of the primary tumor. Because the lymphatic drainage pattern is different for different lobar location and the presence of "skipped" lymph nodes pattern (N2 nodes positive without N1 nodes being positive for cancer), it is important for thoracic surgeons to carefully sample or resect a large number of lymph nodes. In contrast to ▶ colorectal cancer, there is no recommendation of a minimum number of lymph nodes to be resected at the time of curative lobectomy. However, nodal stations that are involved in the drainage of the individual lobe(s) where the tumor is located should at least be biopsied.

Furthermore, often thoracic surgeons will also biopsy the contralateral mediastinal lymph nodes. Previous randomized trials comparing sampling versus complete mediastinal ▶ lymphadenectomy in early stage NSCLC did not show any difference in survival benefit. However, they are limited by heterogeneous stages of patients enrolled, and limited number of patients enrolled. Results from more ongoing trials are pending. However, retrospective analyses have indicated the more lymph nodes resected at the time of surgery, the better the survival outcome likely due to better staging of the patients rather than therapeutic removal of the involved lymph nodes. This issue of how extensive mediastinal lymph nodes should be is critical as curative lobectomy is the only standard of care in stage IA NSCLC, and has implication in the screening of lung cancer. If ▶ CT screening of lung cancer is adopted widely in the future due to positive clinical trial results, most of the tumors detected and resected will be small (<3 cm), and curative lobectomy is the only curative intervention.

Stage II Multiple randomized trials have demonstrated that there is survival benefit with the use of four cycles of ▶ cisplatin-based chemotherapy when compared to observation alone in stage II patients. The role of PORT (postoperative radiation treatment) is controversial in positive N1 nodes. The general consensus and retrospective analysis indicates that PORT to N1 nodes did not improve survival outcome.

Locally Advanced Resectable NSCLC (Stage IIIA) Treatment of stage IIIA NSCLC has been controversial in terms of the sequence of chemotherapy (▶ neoadjuvant or ▶ adjuvant) and ▶ definitive ▶ chemoradiotherapy, the role of surgery, the dose of chemotherapy with radiation, and the role of ▶ PORT in N2 positive nodes. With the positive survival outcome associated with adjuvant chemotherapy, the role of neoadjuvant chemotherapy is limited. Patients with multi-nodal stations involvement NSCLC had a poor outcome. Thus, the role of neoadjuvant chemotherapy may be to assess the response to chemotherapy in bulky N2 nodes to see if there is clearance of the N2 nodes. The role of surgery in IIIA NSCLC is controversial. One randomized trial comparing ▶ chemoradiotherapy followed by surgery versus ▶ definitive chemoradiation revealed no difference in overall survival. However, progression-free survival was better in patients who had underwent surgery.

PORT treatment of N2 disease is generally accepted by physicians treating NSCLC. The use of PORT in N2 positive patients appears beneficial but not in N1 or N0 patients.

Locally Advanced Unresectable NSCLC (Stage IIIB) The standard of care for unresectable stage IIIB NSCLC is ▶ concurrent chemoradiotherapy. The maximal efficacy of concurrent chemoradiotherapy is achieved with full dose chemotherapy rather than weekly chemotherapy. There has been debate about the role of neoadjuvant chemotherapy or adjuvant chemotherapy after chemoradiation. The role of adjuvant chemotherapy has been questioned after randomized trial did not show any survival benefit with the addition of adjuvant chemotherapy after chemoradiation as compared to chemoradiation alone. The use of neoadjuvant chemotherapy followed by concurrent chemoradiation has also failed to show survival benefit when compared to chemoradiation alone. However, the neoadjuvant studies were conducted with weekly chemotherapy concurrent with radiation. The use of maintenance epidermal growth factor receptor (EGFR) ▶ tyrosine kinase inhibitor (TKI) has not shown to improve survival in unselected patients. The total radiation dose for treatment of stage IIIB NSCLC ranged from 62 to 66 Gy. Studies are being conducted to see if higher dose of radiation with more modern radiation technique will result in better survival outcome while minimizing toxicities such as pneumonitis.

Metastatic NSCLC (Stage IV) ▶ Platinum-based ▶ doublet chemotherapy is the standard of care for patients with metastatic NSCLC. The largest randomized trial comparing different chemotherapy regimens as first-line treatment has identified histology as an important factor in selecting the optimal chemotherapy agent to partner with platinum (i.e., ▶ pemetrexed for adenocarcinoma). The addition of monoclonal antibody against the ▶ vascular endothelial growth factor (VEGF; ▶ bevacizumab) or against the ▶ epidermal growth factor receptor (EGFR; ▶ cetuximab) to platinum doublet chemotherapy has shown to improve overall survival.

"Maintenance therapy" whether continuing as part of the original first-line treatment regimen (▶ bevacizumab, ▶ cetuximab) or immediately switching to a non-cross resistant agent (▶ pemetrexed, ▶ erlotinib) significantly improves overall survival. ▶ EGFR ▶ tyrosine kinase inhibitor (TKI; ▶ Epidermal Growth Factor Inhibitors) improves progression-free survival but not overall survival in patients with activating EGFR mutations when compared to platinum-based doublet chemotherapy. For second-line treatment of NSCLC or beyond, currently only single agent chemotherapy (pemetrexed, ▶ docetaxel) or EGFR TKI (▶ erlotinib) has been approved in the USA. Both pemetrexed and erlotinib have also been approved for use in maintenance setting.

Genetics of Non-small Cell Lung Cancer

Overexpression of ▶ epidermal growth factor receptor (EGFR) in NSCLC results in poor outcome in NSCLC. Two small molecule tyrosine kinase inhibitor (TKI) of the EGFR have been approved for use in NSCLC. Some patients had dramatic response to these EGFR TKIs (▶ Epidermal Growth Factor Inhibitors) which led to the identification of activating mutations in the EGFR tyrosine kinase domain. There are two mutations (exon 19 deletion and L858R) that constitute about 90% of all the mutations identified. These activating EGFR mutations are usually found in never-smokers, of Asian ethnicity, female gender, and in adenocarcinoma. Additionally, a T790M mutation exists de novo or may develop as secondary resistance to prolong use of the currently available EGFR TKIs, and second generation EGFR TKIs that can overcome the resistance of T790M are being developed clinically. Patients with EGFR activating mutations treated with first-line EGFR TKI had significantly improved progression-free survival when compared to platinum-based chemotherapy. Asian patients who are never-smokers but without EGFR mutations benefited significantly more from platinum-based chemotherapy than EGFR TKI. Thus in patients who are never-smokers, EGFR mutations should be determined from the tumor tissue.

Another major advance in NSCLC is the discovery of ALK (▶ anaplastic lymphoma kinase) translocation in 2007. The majority of ALK translocation in NSCLC results in various EML4-ALK transcripts. ALK is a member of the ▶ insulin receptor family of ▶ receptor tyrosine kinases, while EML4 is a human homolog of echinoderm microtubule-associated protein-like 4, the major ▶ microtubule-binding protein in dividing sea urchin eggs. Several methods are also being developed commercially to detect ALK translocations in NSCLC including ▶ immunohistochemistry (IHC), reverse transcription-polymerase chain reaction

(RT-PCR), or fluorescence in situ hybridization (► FISH). While many epidemiologic studies are ongoing, ALK positive NSCLC patients are found generally in never-smokers, patients with wild-type EGFR gene, and patients with adenocarcinoma and constitute about 4% of all NSCLC. This indicates the importance of screening for EGFR mutations which may direct subsequent screening for ALK translocation. Currently there is an ALK inhibitor (PF002341066, Crizotinib) that has shown significant clinical activity against EML4-ALK positive NSCLC.

Activating mutations in other signaling pathways such as ► HER2 and PI3K are being discovered. This has led to many inhibitors that can potentially block these mutations are in clinical development. In NSCLC in current or former smokers, mutations in K-► RAS and ► TP53 are commonly found. K-RAS mutations are generally believed to reduce the sensitivity of the tumor to EGFR TKIs. Recent data also suggest that ► sorafenib (a VEGFR and ► B-raf) inhibitor may result in prolonged stable disease.

Small Cell Lung Cancer (SCLC)

With the improving tobacco control globally and especially in the USA, the incidence of SCLC has been decreasing. It now comprises of ~15% of all lung cancers. Among the four major histologies of lung cancer, SCLC is most related to tobacco exposure (► Tobacco-Related Cancers). SCLC is generally diagnosed at an advanced stage with approximately 70% of cases diagnosed as stage IV disease. Brain metastasis is common in SCLC where 50% of the patients will develop brain metastasis during the course of SCLC. Historically SCLC has been staged as limited or extensive stage as determined whether the tumor can be encompassed by a single ► radiation port. However, because there is variability among physicians in defining what can be encompassed in a radiation port (i.e., ipsilateral and contralateral supraclavicular lymph nodes, contralateral lymph nodes, pleural effusion), the IASLC has strongly recommended that SCLC should be staged according to the TNM staging system similar to NSCLC to provide uniformity and allow accurate interpretation of treatment results. Limited stage SCLC is treated with ► concurrent ► chemoradiotherapy, and extensive stage SCLC is treated with chemotherapy alone. Given that approximately 50% of SCLC patients will develop brain metastasis, prophylactic brain irradiation (PCI) has been shown to decrease recurrence in the brain and improve overall survival in both "limited" and "extensive" stage patients who achieved partial or complete response to treatment.

Mesothelioma

► Mesothelioma is an uncommon but uniformly fatal malignancy that is related to ► asbestos exposure. The incidence of mesothelioma in the USA has been decreasing due to the ban of asbestos since 1960s, while the incidence in Europe will peak around 2020. ► Cisplatin and ► pemetrexed combination chemotherapy has been the standard chemotherapy regimen for mesothelioma. The treatment of mesothelioma involves surgery, radiation, and/or chemotherapy. However, many controversies still surround the optimal sequence of treatment, the extent of surgery, and method of radiation given the fact of these patients are old and frail with diminished lung reserve.

Perspective

Lung cancer remains the most common cancer cause of death worldwide and thus a significant health burden globally due to the widespread tobacco use especially in Europe and Asia with still nascent anti-tobacco legislations. While progress is being made in identifying the genetic changes in NSCLC especially among never-smokers such as EGFR mutations and ALK translocations and with effective targeted therapy against these two genetic changes, the bulk of the NSCLC in ever-smokers remained unknown and difficult to treat. In order to decrease the incidence of lung cancer and to improve the survival of lung cancer patients, active tobacco control should be implemented in every country and that cost-effective screening methods for lung cancer be developed. In the meantime, small but significant progress is being made in the treatment of lung cancer daily as more and more genetic changes are discovered in various signaling pathways and provide hope for all those patients who are afflicted with lung cancer.

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Lung Cancer Epidemiology

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Characteristics

Of all cancer types, ▶ lung cancer is the leading cause of cancer-related death both worldwide and in the United States [1].

Types of Lung Cancer

Lung cancer accounts for 13% of all cancers diagnosed worldwide, making it the most commonly occurring malignancy other than non-melanoma ▶ skin cancer [2]. Approximately 97% of primary lung cancers are carcinomas of the lung, arising from deregulated growth of ▶ epithelial cells. The two major lung carcinomas are ▶ non-small cell lung cancer (NSCLC) and ▶ small cell lung cancer (SCLC). Approximately 80–85% of lung cancers are diagnosed as NSCLC, a heterogeneous group of malignancies consisting of three sub types:

- ▶ Adenocarcinoma
- ▶ Squamous cell carcinoma
- ▶ Large cell carcinoma

The remaining 15–20% of lung cancers are diagnosed as small cell lung cancer (SCLC), a highly aggressive tumor. Besides these carcinomas, other cancers of the lung include ▶ carcinoid tumors (synonym ▶ carcinoid) and ▶ sarcoma, accounting for only 1% of all lung cancers.

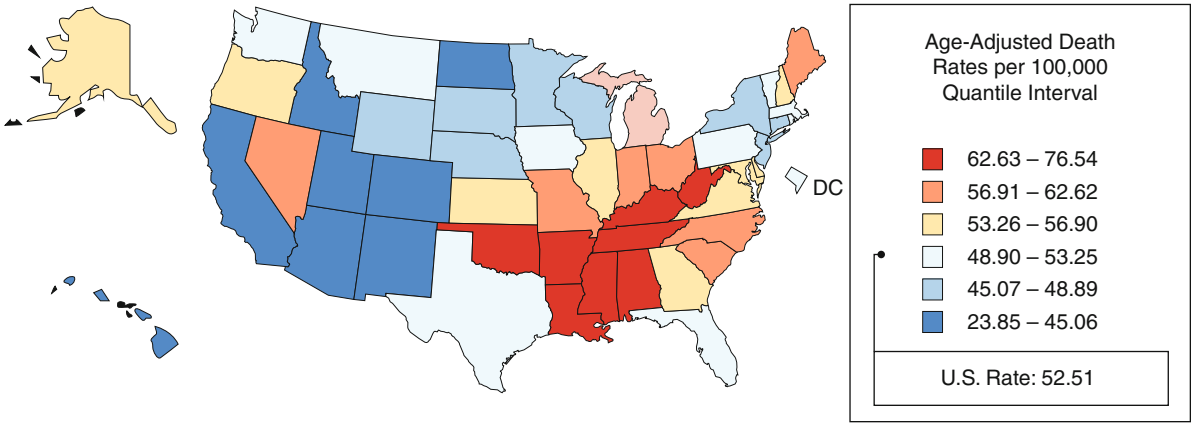
Lung Cancer Incidence and Mortality

Lung cancer is responsible for 12.7% of all new cancer cases diagnosed globally and 18.2% of all cancer-related deaths. An estimated 1.61 million new cases were diagnosed in 2008 and an estimated 1.38 million lung cancer deaths occurred in that same year [3]. Fifty-five percent of new lung cancer cases occur in developing countries, with the lowest incidence rates observed in Central Africa. The highest incidence rates are observed in North America and Europe. In the United States, the age-adjusted incidence rate was 62.5 per 100,000 people per year. In 2010, it is estimated that 222,520 new lung cancer cases will be diagnosed. Lung cancer is the leading cause of cancer-related mortality in the United States, even though the disease is the second most prevalent cancer in men and women, second only to ▶ prostate cancer in men and ▶ breast cancer in women (▶ Cancer Causes and Control). The age-adjusted mortality rate was 52.5 per 100,000 people per year, with some of the highest rates occurring in southeastern states (Fig. 1).

In 2010, an estimated 157,300 people are expected to die from the disease in the United States. Lung cancer is also emerging as a major cause of cancer-related mortality in Asia, primarily because of a substantial increase in the consumption of tobacco products ([4]; ▶ Tobacco Carcinogenesis). In China, for instance, an estimated 351,713 new lung cancer cases were diagnosed in 2008 and 304,020 deaths occurred in that same year [3]. Most lung cancers are diagnosed at an advanced stage, and even with the best treatments, the 5-year survival rates are very low (Table 1). As a result, mortality rates closely parallel incidence rates in both developed and developing countries. An important exception to these dismal survival rates is seen for patients diagnosed with early-stage disease, where 5-year survival is over 50%; however, only 15% of patients are diagnosed before the cancer has spread from its primary site.

Age, Sex, and Race Patterns of Lung Cancer

Lung cancer is most common in the elderly population (Table 2). The incidence of lung cancer remains very low in people under age 40. It then slowly begins to rise and peaks between age 65 and 84. In the United States, the median age at diagnosis for lung cancer is 71 years, with about 90% of diagnoses occurring in people older than 55 years [5]. Not surprisingly, the median age at death is 72 years because most lung cancers are



Lung Cancer Epidemiology. Fig. 1 Mortality rates in the United States. Rates are 100,000 inhabitants (age-adjusted to the 2000 US standard population). Data based on National Center for Health Statistics, Centers of Disease Control and Prevention. (From [6])

diagnosed at an advanced stage and have a poor prognosis. Over 90% of all deaths from lung cancer occur in people older than 55 years.

Lung cancer is more common in men than women. It is the most common cause of cancer among men worldwide, with an incidence of 1.1 million cases in 2008 accounting for 16.5% of all cancers in men. Although lung cancer is the fourth most common cause of cancer in women worldwide, it is the second most common cause of death from cancer. Worldwide, approximately 513,000 women were diagnosed with lung cancer, and 427,000 women died of the disease in 2008. Sex differences in lung cancer incidence vary by geographic region. Among males, the highest incidence rates are observed in North America, Russia, and Eastern Europe. Chinese females have a high incidence of lung cancer despite a very low incidence of smoking. In the United States, it is estimated that 116,750 men and 105,770 women will be diagnosed with lung cancer in 2010, with 86,220 men and 71,080 women dying from the disease. Current trends in lung cancer in both men and women closely parallel historical trends in smoking prevalence. In the early 1980s, the incidence of lung cancer started to decline in males, followed by a decline in mortality in the 1990s. For women, the incidence and mortality rates increased sharply from the 1950s to 1990s as more women began to smoke [6], though these trends appear to have reversed or stabilized in recent years [7]. Approximately 2.89% of men and 2.27% of women will develop lung cancer between ages 50–70 years, and the lifetime risk of developing lung cancer is 44% for men and 38% for women [5]. Recent statistics also show that the age-adjusted incidence rate of lung cancer is 76 per 100,000 men and 52 per 100,000 women in the United States.

Lung Cancer Epidemiology. Table 1 Overall 5-year relative survival in the united states[a]

Stage at diagnosis	Stage distribution (%)	5-year relative survival (%)
Localized to primary site	15	52.9
Regional spread to lymph nodes	22	24
Metastasized to distant sites	56	3.5
Unknown (unstaged)	8	8.7

[a]Rates are based on data collected from 1999 to 2006 for the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute (NCI)

Table 3 summarizes the incidence and mortality rates among various racial and ethnic groups in the United States [5]. The highest age-adjusted lung cancer incidence and mortality rates were reported for black and non-Hispanic white men, but black men have a 50% higher risk of lung cancer than white men. Since the 1990s, the risk of lung cancer in black and white men has declined annually by approximately 2.5%. The lowest incidence and mortality rates were registered for Asian Americans, Pacific Islanders, and Hispanic women. Trends in 5-year survival rates in lung cancer from 1975 to 2006 have improved at comparable rates for whites and blacks; however, current 5-year survival rates for black men (11.8%) and women (15%) are lower than that for white men

Lung Cancer Epidemiology. Table 2 Age distribution at diagnosis and death[a]

Age	≤20	20–34	35–44	45–54	55–64	65–74	75–84	≥85
% at Diagnosis	0	0.2	1.7	8.8	20.9	31.3	29.1	8
% at Death	0	0.1	1.4	7.9	19.7	30.6	30.7	9.6

[a]Rates are based on invasive lung cancer diagnoses and deaths from 2003 to 2007 in the USA (SEER, NCI)

(14.5%) and women (19.4%). These differences in rates of lung cancer are generally thought to be attributable to different patterns of cigarette smoking.

Etiology of Lung Cancer

Overview of Causes of Lung Cancer

Long-term exposure to tobacco smoke is the most common cause of lung cancer. In the United States, about 80% of lung cancer deaths are directly attributable to tobacco use. Lung cancer in nonsmokers accounts for approximately 15% of cases and is often attributable to radon gas, asbestos, air pollution, and genetic factors.

Lung Cancer Epidemiology. Table 3 Lung cancer incidence and mortality rates by race[a]

	Incidence		Mortality	
	Men	Woman	Men	Woman
All races	76.2	52.4	68.8	40.6
White	76.3	54.7	68.3	41.6
Black	101.2	54.8	87.5	39.6
Asian[b]	52.9	28.1	36.7	18.5
American Indian[c]	52.7	39.7	48.1	33.3
Hispanic	41.4	25.4	32.5	14.4

[a]Age-adjusted rates per 100,000 US population
[b]Includes Pacific Islander
[c]Includes Alaska Native

Tobacco Exposure

Epidemiological studies conducted in the 1950s and 1960s demonstrated that the incidence of lung cancer reflects the prevalence of cigarette smoking (▶ Tobacco-Related Cancers). Lung cancer risk increases with the tar content of the tobacco, use of filterless cigarettes, depth of inhalation, and average daily and cumulative amounts smoked. Tobacco smoke contains over 4,000 chemical compounds, 50 of which are known to be potent carcinogens (▶ Tobacco Carcinogenesis). Some of the carcinogens in tobacco smoke and second-hand smoke include ▶ polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines (NAs), aromatic amines, benzene, arsenic, and polonium-210. Both PAHs and NAs induce pulmonary carcinomas in animal models that exhibit the same histological and genetic profiles as human lung cancer.

Prospective studies demonstrated a rising trend in lung cancer death rates with the duration and intensity of smoking. Smoking one pack of cigarettes per day for 30 years increases the risk of dying from lung cancer by approximately 20-fold in men and women, though rates in men are reported to be as high as 60-fold, perhaps because of differences in depth of inhalation or other habits associated with tobacco use [8]. A recent study demonstrated that rates of lung cancer were similar in women and men with comparable smoking patterns and histories of smoking [9]. The duration of smoking is the strongest predictor of the risk of developing lung cancer, increasing exponentially with the number of smoking years. Lung cancer risk increases linearly with the average number of cigarettes smoked per day [10]. Smoking high-tar, filterless tobacco can increase the risk of lung cancer by as much as 40% compared to those smoking medium- or low-tar cigarettes. In addition, exposure to second-hand smoke increases the risk of lung cancer in nonsmokers, especially for spouses of smokers, by as much as 25% [11].

The prevalence of smoking has declined in the United States by nearly 50% since 1965 [12]. In 2008, approximately 21% of adults in the United States were current smokers. Long-term trends in prevalence of smoking have generally drifted downward for both sexes and all ethnic groups in recent years (Table 4). Cigarette smoking prevalence for women rose for the most recent year on record from 17.5% in 2007 to 18.5% in 2008. For adults older than 25 years of age, the prevalence of smoking has steadily fallen since 1991. Among whites, current cigarette smoking rates tend to be higher than rates recorded for Hispanics and blacks, and these rates have declined at a slower pace than that of Hispanics or blacks. Adults aged 25 and older with more than a high school education smoked

Lung Cancer Epidemiology. Table 4 Trends in prevalence of smoking in the United States[a]

Year	1993	1998	2003	2008
Age≥18				
Men	27.3	25.9	23.7	22.8
Women	22.6	22.1	19.4	18.5
Both sexes	25.9	24.0	21.5	20.6
Race/ethnicity				
White	25.6	25.3	23.3	22.6
Blacks	26.3	24.7	21.4	20.8
Hispanics	18.9	17.1	15.8	14.9

[a]*Source:* Centers for Disease Control and Prevention, National Center for Health Statistics. National Health Interview Survey. Data are age adjusted to the 2000 standard population

at the lowest level (15.1%) compared to those with less than a high school education (29.7%) or with a high school education (28.1%). Adults living 200% below the federal poverty level smoked at higher levels (28.6%) than those living 200% above the federal poverty level (17.5%) (▶ Lung Cancer and Smoking Behavior). For all of these educational and socioeconomic groups, the prevalence of smoking has followed a steadily falling trend in the United States.

However, in China, the prevalence of smoking is 67% for men and 4% for women 15 years of age or older. In addition, the intensity of smoking in China – an average of 10 cigarettes per day – is similar to that seen in the United States in the first half of the twentieth century. Over the next several decades, the incidence of lung cancer is expected to increase dramatically, and many of the 450 million Chinese smokers are expected to die from the disease.

Lung cancer risk in smokers declines dramatically over time after smoking cessation. A large cohort study of US veterans demonstrated that the relative risk of lung cancer diminished significantly after 5 years of smoking cessation; however, even after 30 years of smoking cessation, former smokers still have a slightly higher risk of lung cancer than never smokers [13].

Radon Gas and Asbestos

Several occupational and environmental agents have been implicated as carcinogens that increase the risk of developing lung cancer. However, attempts to assess suspected risk factors have been hampered by imprecise quantification of low-level exposure, the long latency period between exposure and development of lung cancer, and the difficulty of isolating the effect of one factor from others, such as smoking. Despite these challenges, epidemiological studies have identified several occupational and environmental risk factors for lung cancer, including radon gas, asbestos, ionizing radiation, a variety of metals, and air pollution. After cigarette smoking, exposures to ▶ radon gas and ▶ asbestos pose the greatest threat to human health.

Radon is an inert, radioactive gas that results from the normal decay of uranium in the earth's crust. Epidemiological studies have demonstrated an increased risk of lung cancer in ▶ uranium miners. Radon gas can accumulate in residential dwellings at low levels, reaching concentrations that are 50–100-folds less than levels in uranium mines. According to the US Environmental Protection Agency, exposure to indoor radon is the second leading cause of lung cancer in the United States, accounting for an estimated 15,000–20,000 deaths per year. In a study conducted in Sweden, the relative risk of lung cancer was 1.3 for average radon concentrations of 3.8–10.8 picocuries/liter (pCi/L) over a period of 30 years. The relative risk increased moderately to 1.8 for concentrations over10.8 pCi/L.

Occupational exposure to ▶ asbestos has been associated with increased risk of lung cancer. Exposure to asbestos may lead to the development of lung cancer 30–40 years after the asbestos fibers are inhaled. Approximately 28 million men and women were potentially exposed to asbestos from 1940 to 1979 [14]. An estimated 4% of all lung cancers diagnosed in the United States are attributable to asbestos exposure [15]. The relative risk of developing lung cancer among those exposed to asbestos is double that of those unexposed and increases linearly with exposure to higher levels of asbestos [16]. Asbestos exposure most commonly results from industrial usage of asbestos or asbestos mining. However, the majority of cases have a much less obvious history of exposure to asbestos. Asbestos exposure can occur as a result of working or living in a building with crumbling asbestos insulation around the pipes. Although exposure to asbestos is expected to decline in the United States, asbestos use throughout much of the rest of world remains the same or is increasing. In many developing countries, there is a sky-rocketing rate of asbestos use. By some estimates, the incidence of lung cancer in countries in Asia and Africa will continue to rise for another 50 years even if immediate steps are taken to limit asbestos exposure.

Future Trends in Lung Cancer Incidence

Current trends in smoking behavior and prevalence will continue to be the most important predictor of future trends in lung cancer incidence. In the United States, reductions in smoking prevalence will result in declines in lung cancer incidence until 2030, but further declines will require continued reductions in smoking prevalence. In some developing countries, the occurrence of lung cancer will increase rapidly over the next several decades due to current rates of smoking prevalence. In China, for instance, the burden of lung cancer is expected to be staggering as many of the 450 million people, most of them men, develop the disease in the coming decades. As smoking decreases, a higher percentage of lung cancers may occur in nonsmokers than in the past. Today, about 10–15% of lung cancers occur in nonsmokers. Epidemiological studies indicate that nonsmokers with lung cancer are more likely to be young, female, receive a diagnosis of adenocarcinoma, and have a significantly better prognosis for survival. Over the next several decades, evidence of these changes is expected to emerge in the lung cancer population in countries where smoking prevalence has fallen to historically low levels.

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Lung Cancer Staging

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Definition

Accurate ▶ staging of tumors remains the cornerstone of ▶ lung cancer management and prognostication. The tumor, node, metastasis (TNM) system (▶ TNM System) currently in use for the classification of ▶ non-small cell lung cancer (NSCLC) was first proposed more than 50 years ago by Denoix, and was later adapted by the American Joint Committee for Cancer Staging in 1974. Subsequently, this staging system was revised in 1986, 1997, and more recently in 2009 resulting in the seventh edition of the TNM classification for lung cancer staging.

The ▶ TNM system considers the anatomical spread of cancer by factors of tumor size and invasion, extent of lymphatic spread, and presence of metastatic disease, thereby dictating current strategies for clinical and surgical staging investigations. Emphasis is on the detection of cytological and histological spread by

means of noninvasive staging, such as imaging, or invasive staging, such as endoscopic or surgical biopsy. New on the horizon include molecular staging and minimal invasive staging. The current standard for staging should include ▶ computed tomography (CT), ▶ positron emission tomography (PET) scan, and a form of appropriate invasive staging for the mediastinum (EUS, EBUS, or ▶ mediastinoscopy). It is increasingly recognized that accurate staging in NSCLC allows appropriate treatment strategy, which is paramount in improving survival for these patients.

Characteristics

Noninvasive Staging

History taking and physical examination may in some cases identify patients with advanced inoperable NSCLC. Routine laboratory tests (including plasma alkaline phosphatase and calcium levels) should also be taken. It is well recognized that chest radiographs are insensitive and unreliable in this role. ▶ Computed tomography (CT) of the chest should be performed in all NSCLC patients except those too ill or infirm to be considered for further evaluation or treatment.

CT of the Thorax

CT scan of the thorax, which usually includes the upper abdomen (liver and adrenals), is useful in defining the location, size, and anatomical characteristics of a tumor, including any local extension or invasion into the chest wall or mediastinum. Liver and adrenal gland metastases may be detected in 3–10% of asymptomatic patients. Three-dimensional CT reconstructions can in some cases provide better appreciation of the tumor and its relation with adjacent organs. The routine administration of intravenous contrast in CT imaging for lung cancer has been questioned. Some have argued that giving contrast does not automatically improve the accuracy of CT scans, and avoiding its routine use reduces the risk of patient adverse reaction. In addition, the reliability of CT scans in differentiating between T3 and T4 lesions in terms of mediastinal involvement and between T2 and T3 lesions in terms of chest wall invasion may only have an accuracy of around 70%. Additional imaging with ▶ magnetic resonance imaging (MRI) or surgical exploration is sometimes needed to clarify the T status. Another limitation of CT is its inability to assess mediastinal lymph node involvement. In general, 21–50% of NSCLC patients have N2 nodal metastases, and if identified preoperatively, they should undergo ▶ neoadjuvant chemotherapy. Conventionally, the most common criterion for a node suspicious of harboring metastases is a short-axis diameter of $\geq$1 cm on transverse CT scan. Identification of N2 mediastinal lymph nodes has a sensitivity of only 57%, a specificity of only 82%, and positive and negative predictive values of only 56% and 83%, respectively. Based on these figures, if clinicians were to rely on CT alone for nodal staging, then 40% of patients would wrongly be excluded from potentially curative surgery, while 20% would wrongly undergo unnecessary or noncurative surgery. The fault lies in the fact that lymph nodes smaller than 1 cm in diameter may contain malignancy, whereas larger nodes may actually be benign. Therefore, additional staging modalities such as PET, together with invasive mediastinal staging are required for accurate assessment.

Magnetic Resonance Imaging of the Thorax

The sensitivity and specificity of MRI (even with contrast agents, such as gadolinium) in detecting mediastinal lymph node involvement is similar to the CT scan. Given the relative lack of availability of MRI and problems with motion artifacts, MRI is rarely used for mediastinal staging. Nevertheless, for patients who are allergic to the intravenous contrast medium used for CT, or in cases where a better appreciation of soft tissue plane is needed, MRI may have advantages over CT scan. Therefore, MRI should not be used as the primary imaging modality for mediastinal staging, but considered for the evaluation of chest wall invasion, or brachial plexus, and vertebral body invasion in superior sulcus tumors.

Positron Emission Tomography Scan

▶ Positron emission tomography (PET) has become an important modality in lung cancer staging. Unlike CT and MRI, PET is a scan of cellular function rather than organ anatomy. The idea of radioisotope imaging for detecting metabolic function in lung cancer is not new. In PET for thoracic malignancies, radiolabeled 18F-fluor-deoxy-D-glucose (FDG) is used as a tracer substrate. FDG is taken up and metabolized by cells in the same initial pathway as glucose, but once FDG is phosphorylated, it is not further metabolized, and becomes trapped intracellularly. It is through this

mechanism that malignant cells with higher rates of glucose metabolism than normal cells accumulate greater amounts of the radiolabeled FDG, which are detected by radiation-sensitive cameras. On PET scan, areas with increased glucose metabolism and hence tracer uptake, such as tumors or regions of infection show up as "hot spots," which are given a standard uptake value (SUV). There is controversy regarding the SUV which is considered abnormal, although a value of >2.5 is accepted by many to be significant. The size of the lesion as well as measurement time following tracer injection can greatly influence the SUV. Some reports have shown the superior accuracy of PET over CT for mediastinal staging. The sensitivity of PET in this role is around 84%, with a specificity of 89% (positive and negative predictive values of 79% and 93%, respectively). Furthermore, when PET is combined with CT for mediastinal staging, the sensitivity may reach 93% and specificity 95% in some series. An added advantage of PET, which is a whole-body scan, is its ability to detect extrathoracic metastases. The current consensus from ACCP is that a whole-body FDG-PET scan be offered to all NSCLC patients eligible for surgery.

Despite the advantages of PET, there is a relatively high false-positive rate, because conditions that cause high metabolism in tissues, other than tumors, can also give a high SUV value, such as, active infection (e.g., tuberculosis, pneumonia, abscesses), active ▶ inflammation (e.g., sarcodiosis, rheumatoid nodules, histoplasmosis, bronchiectasis), tumor necrosis, and postoperative changes after surgery. Therefore, the presence of abnormal PET findings alone that suggest advance stage should not exclude a patient from surgery but instead indicate the need for further preoperative staging with adjuncts such as EUS biopsy, EBUS biopsy, and ▶ mediastinoscopy. Conversely, some argue that mediastinoscopy can be safely avoided when lung cancer staged by computed tomography and PET scan is N2 (and distant metastases) negative because the survival advantage conferred is very small, due to the low prevalence of PET false-negative patients and unproven superiority of induction therapy over adjuvant therapy. In addition, the degree of metabolic activity (i.e., SUV value) required to indicate malignancy has not been consistently defined. In general, an SUV of 2.5 has empirically been taken to denote malignancy in some reports; however, this value is not universally adapted by all centers.

PET has low sensitivity for small lesions, with a lower limit of resolution of around 1 cm. The reliability of PET for detecting lesions that are smaller than 1 cm remains to be proven convincingly. It has also been suggested that the accuracy of detecting small lesions (e.g., lymph nodes) when these are in close proximity to a larger tumor is reduced. Combined PET-CT scans allow the fusion of functional and anatomic images which can improve the interpretation of scan information.

Imaging for Distant Metastases

Patients with distant or extrathoracic metastasis have a very poor prognosis, with 5-year survival of close to zero regardless of the T or N stage. The most common sites for metastasis from lung cancer are the brain, bone, liver, and adrenal glands. Classically, investigations for metastases at these sites include CT and/or MRI for brain lesions, radionucleotide bone scanning for bone metastases, and CT or ultrasound scanning for abdominal metastases. Whole-body PET has gained popularity as a tool in searching for extrathoracic metastases, although PET would not be useful as an imaging modality for brain metastases. The current ACCP guidelines suggest a thorough clinical evaluation for all lung cancer patients, and that further extrathoracic metastatic workup is not needed unless abnormal results are detected. Patients with abnormal clinical findings should undergo scanning appropriate to the site of those abnormalities. In the absence of findings suggestive of extrathoracic metastases on clinical evaluation, the likelihood of positive findings on scanning of the brain, bone, liver, and adrenals is less than 10%. However, it should be emphasized that a scan suggesting the presence of extrathoracic metastases should not immediately exclude patients from curative surgery due to the inaccuracy of these scans. Tissue sampling (or further tests or scans) should be performed to confirm metastatic disease.

Invasive Staging

The advantage of invasive staging over noninvasive staging is that cytological or histological results of suspected metastases can be confirmed.

Bronchoscopy

Fiberoptic bronchoscopy allows the direct visualization of the vocal cords and proximal airways which is useful in defining the T designator in the TNM system,

by evaluating the proximity of the tumor to the carina. Bronchoscopic assessment can also guide the nature and margin of surgical resection needed for the airways more reliably than CT scan. Bronchoalveolar lavage, bronchial brushing, and endobronchial biopsy can be performed through the bronchoscope to obtain diagnosis. Transbronchial needle aspiration (TBNA) may be incorporated into the bronchoscopic examination to obtain tissue from regions of mediastinal lymphadenopathy as detected on CT or PET scan. The Wang needle, commonly used for the procedure, is guided by the bronchoscope (within the working channel) to the area of the tracheobronchial tree overlying the suspect lymph node. The needle is then passed through the bronchial wall to aspirate at the node. Sensitivity and specificity of around 76% and 96%, respectively, can be expected from this method. The relatively low sensitivity of conventional TBNA from a "blind" procedure (based on an estimation of the lymph node position suggested on CT) can be improved when guided by real-time CT, fluoroscopy, endobronchial ultrasound (EBUS), or virtual bronchoscopy (3-D reconstructions from CT scan). The use of rapid on-site cytological evaluation increases the diagnostic yield of TBNA by allowing immediate verification of the adequacy of the lymph node specimen by identification of lymphocytes. The accuracy of TBNA may inherently be limited by the lymph node size criteria used for suspect nodes, potentially missing small metastatic nodes. Currently, conventional TBNA is considered an adjunct to, rather than a replacement for, mediastinoscopy, because of its relatively low sensitivity and negative predictive value of 70%.

Endobronchial Ultrasound

EBUS has evolved to become one of the latest tools for staging of lung cancer: from its origin as a radial probe to evaluate central airway structures, to the small radial probes that can visualize and assist transbronchial biopsies of peripheral lung nodules, to the newest development into the convex probe EBUS (CP-EBUS) consisting of a curvilinear electronic transducer on the tip of a flexible bronchovideoscope. CP-EBUS allows real-time EBUS-guided TBNA, which has access to all of the mediastinal lymph nodes accessible by standard cervical mediastinoscopy as well as N1 nodes. However, the aortopulmonary window, paraesophageal, and inferior pulmonary ligament lymph nodes are not adequately reached by EBUS-TBNA. In a randomized trial, EBUS-TBNA was significantly more successful in obtaining a mediastinal lymph node aspirate (either positive or negative for malignancy) in 80% of patients when compared with only 71% in conventional TBNA patients. In experienced hands, the reported sensitivity and specificity of EBUS-TBNA for detecting mediastinal lymph node malignancy was 80–95% and 100% respectively. In terms of diagnostic performance of EBUS-TBNA for mediastinal pathologies, the accuracy (the patient's final diagnosis matched the EBUS–TBNA result) was between 86% and 98%. The complication rates for the procedure are very low, with numerous studies reporting none. Minor bleeding at the puncture site of some patients were encountered occasionally, and there was a report of asymptomatic pneumomediastinum. Other theoretical adverse events include hemoptysis, pneumothorax, and mediastinitis. Despite its limitations and being somewhat operator dependent, EBUS-TBNA is a minimally invasive approach that is safe and has a good diagnostic yield. Furthermore, the trimodality staging EBUS-TBNA (when the target lymph nodes are successfully sampled) in combination with CT and PET scan is increasingly being accepted as adequate for preoperative lung cancer staging.

Transthoracic Needle Aspiration

The percutaneous transthoracic needle aspiration (TTNA) of a suspicious lymph node is normally performed under CT or fluoroscopic guidance. TTNA by ultrasound guidance may occasionally be performed when the lesion is very close to or abutting the chest wall. Besides mediastinal staging, TTNA can also be used to provide aspiration cytology confirmation of suspected interlobar pulmonary metastases, pleural metastases, and malignant pleural effusions. The procedure is generally well tolerated, relatively safe, and does not require general anesthesia. TTNA for mediastinal nodal staging has a reported sensitivity of around 90%; however, it is a highly operator-dependent procedure. However, TTNA's use in mediastinal staging has been limited by its relatively poor negative predictive value (78%) despite calls for using larger needles, flushing the needles, and having a cytopathologist on site to improve results. Furthermore, only limited mediastinal lymph node stations can be reached by TTNA because of their proximity to the heart or major thoracic vessels. The yield from thoracocentesis for suspicious pleural effusions is lower with conclusive yields on

malignant spread in only 50–65%. An important risk associated with the procedure is iatrogenic pneumothorax, which occurs in 10–30% of cases. Recovery from this complication may be difficult with many lung cancer patients also suffering from chronic airway disease and being smokers or ex-smokers.

Endoscopic Ultrasound

EUS involves the use of real-time ultrasound from a fiber-optic esophagoscope to access posterio-inferior mediastinal (subcarinal, paraesophageal, pulmonary ligament), retroperitoneal, and celiac axis lymph nodes. Using ultrasound guidance, fine needle aspiration (FNA) can be performed by a biopsy needle that is passed through the esophageal wall to aspirate at the suspect lymph nodes. EUS alone can give a qualitative assessment of the suspicious lymph nodes prior to performing FNA. When FNA is performed in conjunction with EUS, the accuracy improves from sensitivity of 78–88% and specificity of 71–91%. EUS for mediastinal staging is limited by the relatively high rate of false-negative results (23%), which may be due in part to the limited depth of ultrasound penetration to the esophagus, interfering with access to the hilar, interlobar, and paratracheal lymph nodes. The use of EUS is further restricted by the lack of accessibility and skilled endoscopists. Nevertheless, one study has found that 59% of NSCLC patients could be excluded from unnecessary thoracotomy based on EUS-FNA results.

Mediastinoscopy

► Mediastinoscopy still remains the "gold standard" invasive staging technique for mediastinal staging. Under general anesthesia, via a suprasternal incision, a mediastinoscope is passed down the pretracheal plane into the mediastinum to the level of the carina. The procedure can expose all of the paratracheal nodal stations bilaterally, as well as the anterior subcarinal station. It is a basic, safe and minimally invasive procedure with a complication rate of 1.7%. Reported complications include pneumothorax, left recurrent laryngeal nerve injury, bleeding, airway and esophageal trauma, and infection. The specificity of mediastinoscopy is very high reaching almost 100%; however, the sensitivity may only be around 80%. Several important lymph node stations, notably the posterior subcarinal, aortopulmonary window, and anterior and inferior mediastinal stations cannot be accessed by standard cervical mediastinoscopy, which can partly explain the relatively high false-negative rate of 9%. A report found that 72% of mediastinal lymph node metastases found on thoracotomy were in stations inaccessible to mediastinoscopy. Nevertheless, mediastinoscopy remains a trusted investigative technique in lung cancer staging. Patients with negative mediastinoscopy findings have 5-year survival rates of up to 53%, compared to the dismal 2-year survival rate of 4% in those with positive findings.

Current ACCP guidelines advise the use of mediastinoscopy in all NSCLC patients otherwise eligible for surgery who have suspicious mediastinal lymph nodes on CT or PET. There is data to suggest that performing routine mediastinoscopy for everyone does not significantly preclude patients from unnecessary thoracotomy. Mediastinoscopy should also be considered in patients with normal CT or PET findings who nonetheless require that mediastinal lymphatic spread be ruled out, for example, patients with large or centrally located tumors, and those who may be marginal surgical candidates for major lung resection.

Extended Mediastinoscopy

The standard cervical mediastinoscopy that most surgeons are familiar with is unable to access the aortopulmonary window (APW) lymph nodes. Particularly, patients with NSCLC of the left upper lobe are prone to tumor spreading to these nodes. EUS-FNA may be utilized to sample (APW) nodes, but it is limited by the relatively high false-negative rate. Ginsberg popularized the technique of extended cervical mediastinoscopy in order to reach these nodes. In the modified approach, the mediastinoscope is passed laterally over the aortic arch rather than down the pretracheal plane, passing between the brachiocephalic and left carotid arteries to reach the APW. The extended procedure carries a small risk of bleeding and embolic stroke, but has the advantage of encompassing a standard mediastinoscopy in the same sitting. When extended cervical mediastinoscopy is performed in combination with standard cervical mediastinoscopy, the sensitivity improves from around 50% to 75%, and the negative predictive value improves from around 70% to 85%.

Anterior Mediastinotomy

An alternative approach to the APW region is via an anterior mediastinotomy (also referred to as

Chamberlain procedure) through the second or third intercostal space at the left sternal border. Anterior mediastinotomy carries a theoretically lower risk of embolic stroke when compared with extended cervical mediastinoscopy. However, an extra incision is required, the pleura may be breached during the approach creating a pneumothorax, and injury to the left internal mammary artery can occur. Used alone, anterior mediastinotomy has a sensitivity of 63–86% for detecting N2 nodal spread, but when coupled with a standard cervical mediastinoscopy, the combined sensitivity reaches 87%.

Cervical Lymph Node Staging

The important areas of cervical lymph nodes for lung cancer are the scalene, supraclavicular, and cervical, which may be collectively referred to as the cervical nodes. It has been estimated that up to 75% of NSCLC patients may have cervical nodal involvement, representing N3 nodal metastases, at the time of presentation. Patients with such metastases are classified as stage IIIb and considered inoperable. The evaluation of palpable cervical nodes by simple percutaneous ► fine needle aspiration biopsy (FNAB) provides reliable biopsy results. However, for impalpable cervical lymph nodes, controversy remains. It was once believed that biopsy of impalpable cervical nodes was unnecessary; nevertheless it is now known that up to half of all cervical nodes harboring metastases in NSCLC patients may be impalpable at the time of presentation. In addition, it has been shown that up to one-third of patients with mediastinal N2 or N3 metastases on mediastinoscopy were also found to have occult metastases to cervical N3 nodes. The challenge for clinicians is to identify those cases with cervical N3 nodes early, thereby potentially avoiding one-third of the invasive mediastinoscopies. Current techniques of noninvasive staging, for example, with PET scan, are still inadequate for detecting these occult N3 nodes because of low sensitivity for small lesions and high false-positive rates. Cervical ultrasonography may be a reliable investigation for detecting suspicious cervical N3 metastatic nodes from lung cancer, allowing targeted FNA biopsy of those cervical nodes in question. The combined cervical ultrasound and FNA approach can detect occult N3 cervical node metastases in 12–31% of lung cancer patients based on the criteria of lymph node size on ultrasonography. By including sophisticated ultrasonography characteristics for metastatic lymph nodes in the scan (e.g., shape, internal architecture, vascular pattern, and echogenicity), the accurate identification of these nodes may be further improved.

Video-Assisted Thoracic Surgery

VATS can be used for lung cancer staging by biopsying appropriate lymph nodes and assessing the extent and invasion by the primary tumor. A potential advantage of using VATS is the option of proceeding with major lung resection during the same anesthesia should the lymph node biopsies show negative results. Interestingly, around 4% of patients are found to be inoperable during routine VATS exploration as a result of extensive but previously unknown local invasion. In the T3 tumors defined by chest wall invasion, VATS can be used to confirm such invasion, as well as to guide the site and extent of potentially curative chest wall resection en bloc with the tumor. VATS can also detect unsuspecting pleural deposits, biopsy those lesions (with intra-operative frozen section), and perform pleurodesis in cases of advanced disease in a single operation. By routinely performing VATS assessment prethoracotomy, 1 in 20 patients may be found to harbor unsuspected pleural metastatic deposits, thereby avoiding unnecessary thoracotomy.

For assessing the mediastinal nodals, VATS allows access to and biopsy of every lymph node station on the operative side, including the pulmonary ligament and paraesophageal stations. VATS APW lymph node biopsy has reported diagnostic accuracy rates of between 92% and 100%, with the added advantage of yielding a better biopsy specimen than FNA. The ACCP guidelines recommend that patients with a primary left upper lobe NSCLC tumor, the APW lymph node region should also be assessed by one of the aforementioned approaches (extended mediastinoscopy, anterior mediastinotomy, VATS) whenever standard cervical mediastinoscopy is indicated. Mediastinal nodal assessment by VATS is also useful for evaluating the response from neoadjuvant therapy in NSCLC patients. In particular, VATS provides an alternative approach to the mediastinal nodes for patients who have previously undergone mediastinoscopy, for whom redo-mediastinoscopy is relatively hazardous. The routine use of VATS immediately before thoracotomy for lung cancer resection has been gaining popularity. By inserting a videothoracoscope as the "final" staging procedure prior to

proceeding with a major incision do not significantly increase operating time, adds negligible morbidity to the surgery, and yet can exclude between 4% and 7% of patients from unnecessary surgery. Furthermore, the video-thoracoscope can be used to identify and forewarn the surgeon of adhesions near the planned thoracotomy site, as well as help select the optimum level (rib space) for the thoracotomy. In patients with suspected contralateral metastasis, VATS can be performed as a quick, safe diagnostic procedure to biopsy the contralateral lesion for frozen section before curative resection of the primary tumor in the same sitting.

Tissue for Staging: What and How Much is Enough?

Pathological staging involving histological examination of the resected tumor and lymph nodes during surgery is the most accurate form of staging. The extent of lymph node clearance required to achieve accurate staging remains a subject of controversy. For example, some advocate the routine resection of all mediastinal lymph nodes by radical dissection during lung cancer surgery, thereby providing a more accurate staging, as well as reducing the likelihood of residual micrometastases responsible for tumor recurrence. However, others argue that systematic lymph node sampling is sufficient and may cause less perioperative morbidity and less disturbances of the immune response that can affect future tumor recurrence. Studies and data to date show that neither the clearance nor the systematic sampling approaches is superior in terms of survival benefit.

The concept of ▶ sentinel lymph node mapping, which is widely applied in the staging of ▶ melanoma and ▶ breast cancer, may become important in lung cancer staging. It has been noted for some time that micrometastases are present in more than 20% of lymph nodes previously thought to be benign when special immunohistochemical staining techniques are employed. Furthermore, the incidence of "skip metastases," where anatomically the N2 nodes appear to harbor malignant cells although the N1 nodes do not, has also been reported to be around 20%. These observations suggest that lymphatic spread in lung cancer can be unpredictable and sentinel node mapping may provide additional information for staging. ▶ Sentinel node mapping is performed by injecting a suitable marker, usually a dye or radioisotope such as technetium-99m (Tc) sulfur colloid, into the tumor or into four quadrants peritumorally at thoracotomy. The marker is allowed time to travel to the sentinel node (s), which is picked up by the dye coloration or handheld Geiger counter and dissected out. Studies have found that this approach was feasible in 82–90% of patients at thoracotomy, with identification of one or more sentinel nodes in 60% of patients. The technique produced an accuracy (no other nodes were positive for cancer if the sentinel node was negative) of around 80%. Based on available data, sentinal node mapping is not recommended as a routine investigation at thoracotomy, although further studies are ongoing.

Molecular Staging: The Final Frontier?

The improved understanding behind the molecular biology of tumor cells in the past few decades and the potential as a tool for lung cancer staging has caused much excitement. The problem with the current TNM system for lung cancer staging is that it is based on tumor tissue and its spread and distribution, but does not take into account the tumor biology or micrometastases. Therefore, there remains a big variation in patient survival within those in the same stage of disease based on current staging techniques. In fact, over the years, molecular markers have already been utilized to help differentiate between primary and secondary, as well as uncommon lung tumors. However, using modern molecular techniques, micrometastases in lymph nodes and pleura, or circulating tumor cells may be more readily detected to help explain "skip metastases" and those with poor outcome following treatment. Furthermore, better characterization of tumor biology and behavior through molecular "fingerprinting," as well as the potential to tailor therapy for individual patient, may improve survival and outcome. Researchers have long suspected that some malignant lung tumors, even of the same histological subtype, behave more aggressively or appear to metastasize much earlier than others. By analyzing at the molecular level the carcinogenic process, mechanisms for growth and evading ▶ apoptosis, and methods of achieving metastasis, researchers can search for molecular indicators for aggressive patterns of growth and spread, using these as potential prognostic indicators allowing tumors to be substaged molecularly in addition to the TNM stage. The prospect of isolating such accurate genetic information regarding lung cancer from a sputum, FNA, or blood sample is being explored.

Thus far, numerous aspects of tumor biology have been studied, including carcinogenesis (▶ oncogenes, e.g., K-ras, erbB-1, erbB-2; ▶ tumor suppressor genes, e.g., p53, p16, ▶ Bcl2; cell growth-regulating proteins), ▶ angiogenesis factors (e.g., VEGF, CD-34, factor VIII), factors affecting tumor invasion (e.g., extracellular matrix metalloproteases, PAI-2, Cathepsin B), and markers of micrometastases (e.g., cytokeratin, MUC1 mRNA, CEA mRNA). Furthermore, the synergistic effect of a combination of molecular mechanisms may be more important than an individual molecular marker in determining prognostic significance. Therefore, testing for an array of molecular markers may yield more accurate prognostic information, especially when testing across the spectrum of NSCLC, which constitutes a heterogeneous group histologically. Previous studies investigating an array of markers in patients with stage I NSCLC have shown the molecular markers K-ras mutation, positive p53 expression, and absent H-ras expression to be predictive of tumor recurrence, and erbB-2, Rb, p53, factor VIII, and ▶ CD44 to be predictive of tumor survival.

To date, molecular staging is not being routinely used in the clinical management of lung cancer. The nonstandardized use of assays, the lack of quantification or scoring system for tumor marker expression, the lack of large controlled trial data, as well as the occasional contradictory results of certain tumor markers (e.g., ▶ p53 expression), have prevented widespread adoption of this staging technique in clinical practice. Nevertheless, many clinicians remain optimistic and eagerly anticipate the emergence of the era of molecular staging to complement the current staging system.

Conclusion

Accurate staging is of paramount importance in guiding appropriate therapy and improving prognosis in patients with NSCLC. Noninvasive and invasive staging techniques available for lung cancer staging continue to evolve rapidly and constantly changing the staging algorithms. For example, with advances and increasing experiences with EBUS and EUS, a trimodality approach for lung cancer staging including CT-PET scan in combination with a form of invasive technique for staging has become the accepted standard. Molecular staging has the potential to substage patients, allowing for more accurate evaluation of prognoses and individualization of therapy. The incorporation of molecular techniques into routine clinical practice may revolutionize lung cancer management.

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Lung Cancer Targeted Therapy

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Definition

Most cytotoxic drugs for ▶ lung cancer are nonselective. They act by damaging cells undergoing ▶ mitosis, which is usually more frequent in malignant cells than in most normal cells. Targeted agents are designed to modulate the activity of signal pathways or proteins or enzymes that are necessary and essential

for oncogenesis and survival of cancer cells, particularly those driving deregulated growth, ▶ angiogenesis, ▶ invasion, and ▶ metastasis characteristics of malignant cells. The different mechanisms of activity result in lower toxicity for cancer patients, particularly in the bone marrow and in the gastrointestinal tract, and in increased effectiveness. Currently, there are two types of targeting agents in clinical use for the treatment of ▶ non-small cell lung cancer (NSCLC): the ▶ epidermal growth factor receptor (EGFR) ▶ tyrosine kinase inhibitors (TKI) and the ▶ vascular endothelial growth factor (VEGF) inhibitors. Numerous additional agents targeting other cancer cell pathways are in clinical development.

Characteristics

Lung cancer is the leading cause of cancer-related death among men and women worldwide. The standard treatment for advanced ▶ non-small cell lung cancer (NSCLC), until now, is ▶ doublet chemotherapy in first-line treatment and single agent in second-line setting. Targeted therapies have been introduced into clinical practice and have improved standard chemotherapy approaches. Single agent or combining targeted therapy in selected patients with NSCLC has increased overall survival with fewer side effects.

Although targeted therapies of NSCLC have increased survival, this increase has remained small. The reason may be due to unselected patients for specific target agents. When all patients were treated with the same drug, normally only a minority of patients would benefit. A future challenge will be to identify a subset of patients with NSCLC for whom targeted treatment will provide a significant improvement in survival.

EGFR TKIs for NSCLC with EGFR Mutation

▶ Epidermal growth factor receptor (EGFR) is a 170 kDa member of the ERB family of EGF-related ▶ tyrosine kinase receptors. Activation of this receptor initiates intrinsic tyrosine kinase activity with signaling through a variety of downstream pathways, including ▶ PI3K/Akt, ▶ Ras/Raf/mitogen-activated protein kinase (MAPK), and STAT3. All these have strong stimulatory effects on tumor growth, including ▶ proliferation, survival, ▶ angiogenesis, ▶ invasion, and ▶ metastasis. EGFR mutations change the 3-dimensional configuration of the receptor protein, and these changes may affect the binding of EGFR TKI. ▶ Erlotinib and ▶ gefitinib are both small molecular targeted drugs that have higher affinity to mutant receptors. These two drugs disrupt EGFR signaling by competing with adenosine triphosphate (ATP) for the binding sites at tyrosine kinase domain, and thus inhibiting phosphorylation, and blocking both ligand-induced activation of the receptor and downstream pathways.

Somatic mutations in the kinase domain of EGFR in lung carcinoma exist in approximately 10% of patients in Caucasians and 30% in East Asians. About 90% of EGFR-activating mutations are clustered in ▶ exon 19 and 21. Patients with these mutations have a better response rate to EGFR TKIs than unselected patients. The phase III ▶ clinical trial of the Iressa Pan-Asia Study (IPASS) compared ▶ gefitinib (609 patients) with ▶ paclitaxel/▶ carboplatin (608 patients) in previously untreated Asian non-/light smokers with adenocarcinoma. Progression-free survival time (PFS) in EGFR mutation-positive patients was longer with gefitinib than with chemotherapy (hazard ratio [HR], 0.48; 95% confidence interval [CI], 0.36–0.64; $P < 0.0001$); in EGFR mutation-negative patients, PFS was longer with chemotherapy than with gefitinib (HR 2.85; 95% CI 2.05–3.98; $P < 0.0001$). Similar data were seen in another Japanese randomized phase III study by Mitsudomi and colleagues where EGFR-mutation patients were randomized to either gefitinib or gefitinib plus ▶ docetaxel/▶ cisplatin chemotherapy. The tumor response rate was 62.1% and 32.2% for EGFR TKI and chemotherapy, respectively, while the PFS was significantly in favor of the EGFR TKI (HR, 0.49; 95% CI, 0.34–0.70; $P < 0.001$). Again, similar to IPASS, there was no significant difference in overall survival (OS) (HR, 1.6; 95% CI, 0.75–3.58).

The observations by IPASS are supported by Rosell and colleagues. They screened 2,105 patients for EGFR mutation and treated 217 mutation-positive patients with ▶ erlotinib. The tumor response rate was 64.0% with a better response rate associated with exon 19 mutation. The median PFS and OS were 14 months and 27 months, respectively.

Based on these results, EGFR TKIs erlotinib and gefitinib were recommended as first-line treatment option for NSCLC with EGFR mutation.

EGFR TKIs for NSCLC with Unknown EGFR Mutation Status

The small molecule EGFR tyrosine-kinase inhibitors erlotinib and gefitinib have been studied in second-line and third-line therapy for unselected patients with NSCLC. The BR.21 trial was a *placebo* control trial in NSCLC in which chemotherapy had been unsuccessful. The response rate was 8.9% in the erlotinib group and less than 1% in the *placebo* group ($P < 0.001$); PFS was 2.2 months and 1.8 months, respectively (HR, 0.61, $P < 0.001$). OS was 6.7 months and 4.7 months, respectively (HR, 0.70; $P < 0.001$), in favor of erlotinib. Erlotinib can prolong survival in patients with non-small-cell lung cancer after first-line or second-line chemotherapy.

INTEREST is a first head-to-head trial that compared gefitinib with docetaxel in previously treated non-small-cell lung cancer patients. Non-inferiority was confirmed for overall survival (HR, 1.02, 96% CI 0.905–1.150), median survival was 7.6 versus 8.0 months, suggesting that gefitinib is a valid treatment for pretreated patients with advanced non-small-cell lung cancer.

Based on these results, erlotinib was recommended as second- or third-line treatment option for NSCLC in the West, and gefitinib was recommended in the East.

EGFR TKIs in Maintenance Treatment for Advanced NSCLC

Based on the US National Cancer Institute's (NCI) concept, maintenance therapy for advanced NSCLC means that use of some agents prevents lung cancer from progressing after being successfully controlled by the doublet platinum-based chemotherapy in first-line setting. There are two kinds maintenance treatment. One is continuation maintenance with treatment using the same drugs used in the induction regimen. The other one is switch maintenance treatment that uses other non-cross-resistant agents not included in the induction regimen. In this setting, maintenance therapy can be defined as an early second-line treatment.

In the Saturn trial, Cappuzzo and colleagues evaluated the benefit of erlotinib as a maintenance therapy in patients who were free of progression at the end of four cycles of ▶ platinum-based frontline therapy. The median PFS was superior at 12.3 weeks with erlotinib compared with 11.1 weeks with placebo, and overall survival was 12 months and 11 months ($P = 0.0088$) in favor of erlotinib. The toxicities associated with the use of erlotinib were tolerated.

Currently available data support the use of erlotinib as maintenance treatment in patients with disease control and EGFR expression. As the survival benefit is relatively modest in an unselected patient population, molecular selection strategies will be necessary to identify a subgroup of patients best suited for erlotinib maintenance therapy. The US Food and Drug Administration (FDA) approved erlotinib for maintenance therapy, and the Committee for Medicinal Products for Human Use of the European Medicines Agency (EMA) recommended maintenance erlotinib in stable disease after 4–6 cycle chemotherapy for approval based on the Saturn results.

Targeted Agents in Combination with Chemotherapy

Small molecular targeting agents, when used in combination with cytotoxic drugs, were expected to produce synergistic effects and increase efficacy for NSCLC treatment. Unfortunately, nearly all phase III clinical trials, including 15 randomized phase III trials with over 12,000 patients, have failed to demonstrate superiority for the combination of targeted agents with chemotherapy in first-line setting. One reason may be that there is antagonism between EGFR TKIs and chemotherapy. Preclinical data had shown that EGFR TKIs cause ▶ cell cycle arrest in G1 phase, which makes the cells relatively insensitive to phase-specific cytotoxic agents. Another reason may be related to the heterogeneity of the disease. NSCLC encompasses a number of histologies, and within the same histology several molecular genetic lesions have been identified. In general, one molecular genetic subtype only accounts for a small number of NSCLC tumors. For instance, EGFR mutation accounts for less than 10% in Caucasian and 30% in East populations. Certain targeted agents may influence the outcome in certain NSCLC molecular subtypes. One single study for all advanced NSCLC patients in which most do not response to experimental targeted compounds is unlikely to reveal positive results that occur only in small subgroup of patients. The key to the future development of targeted therapies is likely to be patient selection, with the aim of enriching the study cohort for patients most likely to respond to the experimental agent.

Resistance to EGFR TKIs

There are two mechanisms for NSCLC resistance to EGFR TKIs:

- Primary resistance
- Acquired resistance

Primary resistance means that a tumor harbors an EGFR sensitivity mutation but does not respond to EGFR TKIs gefitinib or erlotinib. Preexisting ▶ KRAS mutation, ▶ MET ▶ amplification, ▶ ALK fusion, and increased expression of ▶ insulin-like growth factor-1 receptor (IGF-1R) may be related to primary resistance against EGFR TKIs.

Jackman et al. proposed four clinical criteria for the definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in NSCLC.

- Previous treatment with a single-agent EGFR TKI (gefitinib or erlotinib)
- Either or both of the following – a tumor that harbors an EGFR mutation known to be associated with drug sensitivity or objective clinical benefit from treatment with an EGFR TKI
- Systemic progression of disease (RECIST or WHO) while on continuous treatment with gefitinib or erlotinib within the last 30 days
- No intervening systemic therapy between cessation of gefitinib or erlotinib and initiation of new therapy [8]

A newer class of small molecules, the irreversible TKIs, has emerged in recent years. Unlike reversible TKIs ▶ gefitinib and ▶ erlotinib, these agents have demonstrated activity in preclinical studies against T790M mutation that confers resistance to the reversible EGFR TKIs. The fusion between echinoderm microtubule-associated protein-like 4 (EML4) and ▶ anaplastic lymphoma kinase (ALK) has recently been identified in a subset of non-small-cell lung cancers (NSCLCs). EML4-ALK is detected in 4.9–13% of NSCLC. ALK inhibitors have entered clinical development, and remarkably clinical efficacy has been observed in NSCLC patients harboring EML4-ALK translocations.

Target Therapy with Monoclonal Antibodies for NSCLC

▶ Bevacizumab and ▶ cetuximab are two ▶ monoclonal antibodies that are effective against advanced NSCLC. The Eastern Cooperative Group (ECOG) 4,599 was a large phase III trial. The trial accrued non-squamous advanced NSCLC into ▶ carboplatin and ▶ paclitaxel with bevacizumab or without bevacizumab (15 mg/kg). The study found that the addition of bevacizumab led to longer overall survival (12.3 months vs. 10.3 months, $P = .003$) and progression-free survival (6.2 months vs. 4.5 months, $P < .001$); higher response rates (35% vs. 15%, $P < 0.001$). Based on this positive study, bevacizumab in combination with paclitaxel and carboplatin is now approved by ▶ FDA as first-line treatment of advanced and metastatic non-squamous NSCLC in October 2006. The AVAiL trial was a three-arm study where patients were randomized to receive cisplatin and gemcitabine alone, cisplatin/gemcitabine with bevacizumab (7.5 mg/kg) every 3 weeks, or cisplatin/gemcitabine with bevacizumab (15 mg/kg) every 3 weeks in the first-line setting [10]. The results showed an increase in progression-free survival from 6.1 to 6.7 months. This increase was particularly visible in the 7.5 mg/kg bevacizumab arm and was not as evident in the 15 mg/kg arm. There was no difference in overall survival. Based on this second study, the European Medicines Agency (EMEA) approved bevacizumab in combination with platinum-based chemotherapy as first-line treatment for advanced non-squamous NSCLC. The FLEX study was a prospective, randomized phase III trials of the combination of cetuximab with cisplatin and vinorelbine in 1,125 chemo-free NSCLC patients with EGFR immunohistochemistry (IHC) positive tumors [11]. After six cycles of chemotherapy, cetuximab was continued until disease progression. The outcome showed that no difference in ▶ progression-free survival (PFS) between the two arms (4.8 vs.4.8 months), but the cetuximab arm had significantly longer overall survival (11.3 vs. 10.1 months; $P = 0.044$). Another cetuximab trial (BMS099 study) had failed to show any improvement in progression-free survival. So far, cetuximab is not approved in NSCLC by ▶ FDA and ▶ EMEA.

Future Direction

Target treatment improves patient outcomes beyond the plateau effect achieved with chemotherapy in advanced NSCLC. There is overwhelming evidence that selective target population is the most important factor in lung cancer targeted therapy. Patient selection is emerging as the paramount factor in the successful use of targeted therapies in NSCLC. In the future, increased knowledge about pathologic features and

molecular markers will be used to identify those patients that most likely respond to specific forms of therapy. Establishing predictive biomarkers and response monitoring techniques will be essential for optimizing the potential benefit gained from these agents. Personalized therapy for lung cancer is now coming.

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Lung Carcinoma

► Lung Cancer

Lung Colony Formation Assay

Definition

Experimental model used in animals to assess the capacity of tumor cells to seed, survive, and successfully initiate the formation of colonies in the lungs after intravenous (tail vein) injection. Also referred to as "experimental metastasis model" although this latter designation is misleading. ► Metastasis indeed is much more complex and involves many steps at the site of the primary tumor and local lymph nodes that are not covered by the lung colony formation assay.

► Cystatins

Lung Resistance-related Protein

► Major Vault Protein

Lupus Erythematosus

Definition

A chronic autoimmune disease in which the immune system attacks the body's cells and tissues, resulting in inflammation and tissue damage, most commonly in the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system.

► Rituximab

Luteinizing Hormone

Definition

Abbreviation LH; a hormone released by the pituitary gland in response to luteinizing hormone-releasing hormone. Abbreviated LH, it controls the length and sequence of the female menstrual cycle, including ovulation, preparation the uterus for implantation of a fertilized egg, and ovarian production of both

estrogen and progesterone. In males, it stimulates the testes to produce androgen. Also known as interstitial-cell-stimulating hormone (ICSH).

Luteotropic Hormone

► Prolactin

Luteotropin

► Prolactin

Lycopene

Definition

Lycopene is a bright red carotenoid pigment, a phytochemical found in tomatoes and other red fruits. Lycopene is the most potent carotenoid antioxidant in the human body.

► Carotenoids
► Chemoprotectants

Lymph

Definition

A transparent, slightly yellow fluid that carries lymphocytes, bathers the body tissues, and drains into to lymphatic vessels, which transport lymph to the immune organs and into the bloodstream.

► Lymphatic Vessels

Lymph Node

Definition

Lymph node is a component of the lymphatic system filtering the lymphatic fluid for bacteria, viruses, or foreign particles that can be recognized and eliminated by lymphocytes, cells that rapidly multiply in response to infections in the highly specialized lymph node milieu.

► Sentinel Node

Lymph Node Metastases

Definition

Also known as nodal involvement, positive nodes, or regional disease. Cancer cells may spread to the regional lymph nodes near the primary tumor. Localized spread to regional lymph nodes is not normally counted as metastasis, but is a sign of worse prognosis.

► Endothelins

Lymphadenectomy

Definition

Lymphadenectomy, also called lymph node dissection, is a surgical procedure in which lymph glands are removed from the body and examined for the presence of cancerous cells. A limited or modified lymphadenectomy removes only some of the lymph nodes in the area around a tumor; a total or radical lymphadenectomy removes all of the lymph nodes in the area.

Characteristics

The lymphatic system is responsible for returning excess fluid from body tissues to the circulatory system and for defending against foreign or harmful agents such as bacteria, viruses, or cancerous cells. The major components of the lymphatic system are lymph capillaries, lymph vessels, and lymph nodes. Lymph is a clear fluid found in tissues that originates from the circulatory system. Lymph capillaries are tiny vessels that carry excess lymph to larger lymph vessels; these

in turn empty to the circulatory system. Lymph nodes are small, oval- or bean-shaped masses found throughout the lymphatic system that act as filters against foreign materials. They tend to group in clusters in such areas as the neck (cervical lymph nodes), under the arm (axillary lymph nodes), the pelvis (iliac lymph nodes), and the groin (inguinal lymph nodes). The lymphatic system plays an important role in the spread of cancerous cells throughout the body. Cancer cells can break away from their primary site of growth and travel through the bloodstream or lymphatic system to other sites in body. They may then begin growing at these distant sites or in the lymph nodes themselves; this process is called metastasis. Removal of the lymph nodes, then, is a way that doctors can determine if a cancer has begun to metastasize. Lymphadenectomy may also be pursued as a cancer treatment to help prevent further spread of abnormal cells.

http://www.surgeryencyclopedia.com/La-Pa/Lymphadenectomy.html

Lymphadenopathy

Definition

Enlarged lymph nodes.

Lymphangiogenesis

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Synonyms

Development of new lymphatic vessels; Growth of new lymphatic vessels

Definition

Lymphangiogenesis is the process whereby new lymphatic vessels develop within a tissue. Most commonly, lymphangiogenesis refers to the growth of lymphatic vessels by sprouting of new vessels from preexisting lymphatic vessels. Additionally, lymphangiogenesis refers to the initial formation of the lymphatic system during embryonic development.

Characteristics

Lymphangiogenesis, the growth of new lymphatic vessels, occurs during embryonic development and in tumors and lymph nodes of tumor bearing animals. This key process also occurs in wounds and in inflamed tissues. Lymphangiogenesis in tumors has been linked to the formation of lymph node metastases. Importantly, lymph nodes are the initial or frequent sites of ▶ metastasis for many tumors, including human pancreatic, gastric, breast, and prostate carcinomas, ▶ melanomas and other tumors and recent studies indicate that lymphangiogenesis likely plays an important role in driving tumor metastasis.

The lymphatic system is comprised of a network of blind-ended, thin walled lymphatic capillaries, collecting vessels and specialized secondary immune organs, including lymph nodes, tonsils, Peyer's patches, and spleen. This system is connected to the vascular system through the thoracic duct. Lymphatic vessels drain protein-rich interstitial fluid and immune cells from tissues through lymph nodes. A thin network of lymphatic capillaries is found in the outer rim or capsule of the normal lymph node. Lymphatic capillaries are comprised of a single layer of lymphatic endothelial cells, which share many molecular characteristics with vascular endothelial cells. Collecting vessels in the lymphatic system are comprised of endothelia surrounded by a layer of ▶ pericytes. During early embryonic development, the lymphatic system forms by branching off of the cardinal vein and expanding into an alternate network of thin-walled vessels.

Lymphatic vessels differ from blood vessels in several ways. Large collecting lymphatic vessels contain vascular smooth muscle cells in their walls, as well as valves, which prevent the backflow of lymph. However, lymphatic capillaries, unlike typical blood capillaries, lack pericytes and a continuous basal lamina and contain large inter-endothelial valve-like openings. Lymphatic capillaries consist of loosely overlapping cells. Due to their greater permeability, lymphatic capillaries may be more effective than blood capillaries in allowing tumor cells to pass into the vessel lumen.

The recent identification of selective markers of lymphatic versus vascular endothelial cells has

allowed identification of the mechanisms that regulate lymphangiogenesis. Lymphatic endothelia selectively express ▶ LYVE-1, a member of the ▶ CD44 hyaluronic acid receptor family, Prox-1, a lymphatic vessel–specific homeobox transcription factor, ▶ podoplanin, a mucin-type glycoprotein, and ▶ VEGFR3, a receptor for ▶ VEGF-C and VEGF-D. Lymphatic capillaries, unlike typical blood capillaries, lack pericytes and a continuous basal lamina.

Tumor-secreted factors such as VEGF-C and VEGF-A have been shown to promote lymphangiogenesis within tumors. These factors activate VEGFR3, a tyrosine kinase VEGF family receptor that is expressed primarily on lymphatic endothelium. Expression of VEGF-C is correlated with increased lymph node metastasis and poor clinical outcome in a variety of tumors. Indeed, in animal models of metastasis, inhibitors of VEGF-C (soluble VEGFR3) inhibited tumor lymphangiogenesis and tumor metastasis to lymph nodes.

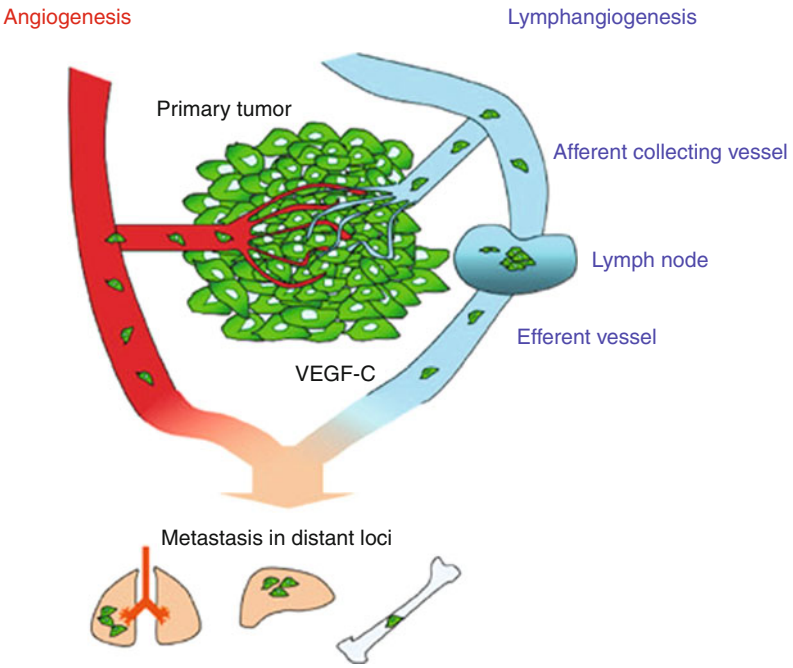
Tumors spread by lymphatic routes to lymph nodes but may also spread by hematogenous (vascular) or lymphatic routes to distant organs. Tumors secrete a number of factors including VEGF-C and others that induce both lymphangiogenesis and ▶ angiogenesis (Fig. 1).

Molecular Regulation

The molecular mechanisms that regulate lymphangiogenesis, the growth of lymphatic vessels, have recently begun to be understood. Two members of the VEGF family, VEGF-C and VEGF-D, stimulate lymphangiogenesis by binding to the receptors VEGFR-2 and VEGFR-3 on lymphatic endothelial cells. Indeed, homozygous deletion of VEGF-C genes in mice leads to a complete absence of the lymphatic system. VEGF-A, FGF, and HGF can also stimulate lymphangiogenesis. The discovery of specific markers of lymphatic endothelium has facilitated analysis of the mechanisms regulating lymphangiogenesis. VEGFR3 is expressed only by quiescent lymphatic endothelial cells and not by quiescent vascular endothelial cells; however, both proliferating vascular and lymphatic endothelial cells express this protein. The homeodomain transcription factor Prox-1 is selectively expressed on lymphatic endothelium and not vascular endothelial cells. It is also expressed by developing neuronal cells in flies and mammals. Podoplanin, a mucin-type glycoprotein, is expressed by lymphatic endothelial cells and a few other cell types such as type I lung alveolar cells and kidney podocytes. Mice with genetic loss of podoplanin display paw lymphedema, or loss of fluid drainage from the paw. Importantly, the CD44 hyaluronic acid–binding protein family member LYVE-1 is expressed only by lymphatic endothelium as well as liver and spleen sinusoidal endothelial cells. Interestingly, LYVE-1 is downregulated on pericyte-lined collecting vessels of the lymphatic system. Although the function of LYVE-1 is currently unknown, there is a clear association of the absence of LYVE-1 with the presence of pericytes. Expression of LYVE-1 is undetectable on lymphocytes, hematopoietic cells, or vascular endothelial cells. LYVE-1 is thus a useful marker to determine the localization of lymphatic endothelium by immunohistochemistry in vivo and to characterize and purify LEC in vitro.

While growth factors and their receptors play critical roles in angiogenesis and lymphangiogenesis, the integrin family of cell ▶ adhesion proteins controls cell attachment to the extracellular matrix and promotes the survival, proliferation, and ▶ motility of many cell types. Angiogenesis, the development of new blood vessels, depends not only on soluble growth factors such as VEGF-A but also on survival and migratory signals transduced by the integrins αvβ3, αvβ5, α5β1, and/or α4β1. In contrast, only integrins α4β1 and α▶ A sentinel lymph node have been shown to play roles in lymphatic vessel development, as animals lacking integrin α9β1 develop ▶ chylothorax and animals lacking α4β1in endothelia do not respond to VEGF-C.

Many tumors express VEGF-C and VEGF-D, growth factors that selectively regulate lymphangiogenesis. While there are three known vascular endothelial growth factor receptors, VEGFR-1, VEGFR-2, and VEGFR-3, only one, VEGFR-3 is expressed predominantly on lymphatic vessel. Importantly, tumor-associated ▶ macrophages can release VEGF-C and can stimulate lymphangiogenesis in the absence of added growth factors. In fact, recent studies showed that macrophage secretion of VEGF-C and VEGF-D induces lymphangiogenesis, as well as lymphangiogenesis in tumors. Importantly, a number of studies have shown that antagonists of VEGF-C suppress tumor lymphangiogenesis and lymphatic metastases in animal models of tumor growth.



Lymphangiogenesis.
Fig. 1 Model of tumor lymphangiogenesis

Clinical Relevance

Congenital or pathologically induced damage to the lymphatic system can result in lymphedema, a condition in which drainage of fluid from tissues is blocked, skin thickens, and adipose tissue accumulates. Mutations in VEGFR3, the forkhead transcription factor ▶ FOXC2 and the transcription factor ▶ SOX18 each induce distinct forms of congenital or ▶ primary lymphedema. Recombinant VEGF-C was able to promote therapeutic lymphangiogenesis in animal models of lymphedema. Additionally, ▶ cancer surgery and radiation therapy, especially in breast cancer therapy, can induce ▶ secondary lymphedema by damaging the lymphatic system in normal tissues, such as the breast. Diseases of the lymphatic system include lymphedema, lymphangiosarcomas, and lymph node metastasis. Primary lymphedema arises from congenital defects in molecules that regulate development of lymphatic vessels, such as VEGFR3, FOXC2, and SOX18. Secondary lymphedema arises as a consequence of surgery, infection (such as filariasis), or radiation therapy. In these disorders, the normal architecture of the lymphatic vessels and/or lymph nodes is disrupted. Removal of fluids from tissues can be disrupted in these disorders and tissue architecture is altered. An understanding of how lymphatic vessels grow and respond to environmental cues could help to develop therapies for these disorders. In over 80% of cancers, malignant tumor cells metastasize to the lymph nodes and travel through the lymphatic system. Tumor cell–derived factors such as VEGF-C stimulate lymphangiogenesis in tumors and studies have shown that increased lymphatic vessel density is associated with increased metastasis. An understanding of the molecular mechanisms that regulate lymphangiogenesis may lead to new therapies for cancer metastasis and lymphedema.

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Lymphangioleiomyomatosis

Definition

LAM is a disorder seen almost exclusively in females and is characterized by bronchiolar smooth muscle infiltration and cystic changes in the lung parenchyma. LAM patients often have angiomyolipoma of the kidneys and/or abdominal and hilar lymph nodes.

Lymphangitic Carcinomatosis

Definition

Diffuse malignant infiltration of the lungs with obstruction of the lymphatic channels that occurs most commonly in patients with carcinoma of the breast, lung, stomach, pancreas, prostate, cervix, or thyroid as well as in patients with metastatic adenocarcinoma from an unknown primary site.

► Carcinomatosis

Lymphatic Mapping

Definition

The process in which a radio-labeled tracer is injected in or near a primary tumor site to outline the way lymph drains to its corresponding lymph nodes. This process is visualized on lymphoscintigrams and serves, with or without the use of blue dye, as an intraoperative guide during sentinel node biopsy.

► Sentinel Lymph Nodes

Lymphatic System

Definition

A circulatory network of lymph vessels that transports lymph fluid and filters it in the lymph nodes. Lymph fluid coming from the entire body is collected in several large trunks that eventually drain into the venous circulation.

The lymphatic system is responsible for returning excess fluid from body tissues to the circulatory system and for defending against foreign or harmful agents such as bacteria, viruses, or cancerous cells. The major components of the lymphatic system are lymph capillaries, lymph vessels, and lymph nodes. Lymph is a clear fluid found in tissues that originates from the circulatory system. Lymph capillaries are tiny vessels that carry excess lymph to larger lymph vessels; these in turn empty to the circulatory system. Lymph nodes are small, oval- or bean-shaped masses found throughout the lymphatic system that act as filters against foreign materials. They tend to group in clusters in such areas as the neck (cervical lymph nodes), under the arm (axillary lymph nodes), the pelvis (iliac lymph nodes), and the groin (inguinal lymph nodes). The lymphatic system plays an important role in the spread of cancerous cells throughout the body. Cancer cells can break away from their primary site of growth and travel through the bloodstream or lymphatic system to other sites in the body. They may then begin growing at these distant sites or in the lymph nodes themselves; this process is called metastasis. Removal of the lymph nodes, then, is a way that doctors can determine if a cancer has begun to metastasize. Lymphadenectomy may also be pursued as a cancer treatment to help prevent further spread of abnormal cells.

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► Sentinel Lymph Nodes

Lymphatic Vessels

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Definition

Lymphatic vessels are the lymphatic capillaries, collecting vessels and ducts that form an integral part of the one-way, open-ended circulatory system known as the lymphatic system. In addition to lymphatic vessels, the lymphatic system consists of lymph nodes and other lymphoid organs.

Lymphatic vessels have three interrelated physiological functions: (1) removal of excess fluids from body tissues, (2) transportation of immune cells (including lymphocytes and dendritic cells), and (3) absorption of dietary fat and fat-soluble vitamins from the digestive system and the subsequent transport of fat (chyle) to the circulatory system.

Characteristics

Lymphatic capillaries are lined by a single layer of endothelial cells with overlapping intercellular junctional complexes (Fig. 1). They are devoid of ▶ pericytes and smooth muscle cells, and are surrounded by a discontinuous or absent basement membrane. Collecting lymphatic vessels typically possess a sparse smooth muscle cell layer, basement membrane, and valves to facilitate unidirectional fluid transport. Lumen patency is maintained by anchoring filaments that connect the abluminal surface of the endothelial cells to the ▶ extracellular matrix. Lymphatic vessels are present in most organs, except avascular structures (such as epidermis and cartilage) and certain vascularized organs (the central nervous system and bone marrow).

Interstitial fluid in tissues drains into lymphatic capillaries and becomes ▶ lymph. It then flows into collecting lymphatic vessels away from tissues, passes through lymph nodes, and eventually reaches either the right lymphatic duct or the largest lymph vessel in the body, the thoracic duct. These vessels drain into the right and left subclavian veins, respectively, to return the processed lymph to the circulatory system.

The regulation of lymphatic vessel formation, growth, and maturation is complex. The lymphatic system was first systematically described by Asellius in the seventeenth century (1627) as "milky veins" in the mesentery of a "well-fed" dog. The developmental origin of lymphatic vessels was first recognized by Sabin in the early twentieth century (1902), who proposed that endothelial cells bud off from veins in the jugular and perimesonephric areas during early embryonic development and migrate to form primitive lymph sacs. Endothelial sprouting from these sacs subsequently forms lymphatic capillaries.

▶ Lymphangiogenesis is the de novo formation of lymphatic vessels, in a manner analogous to blood vessel ▶ angiogenesis. It plays an important physiological role in homeostasis, metabolism, immunity, and wound healing. Aberrant lymphatic vessel formation has been implicated in a number of pathological conditions, including tumor cell metastasis, edema, and inflammatory diseases.

Clinical Relevance

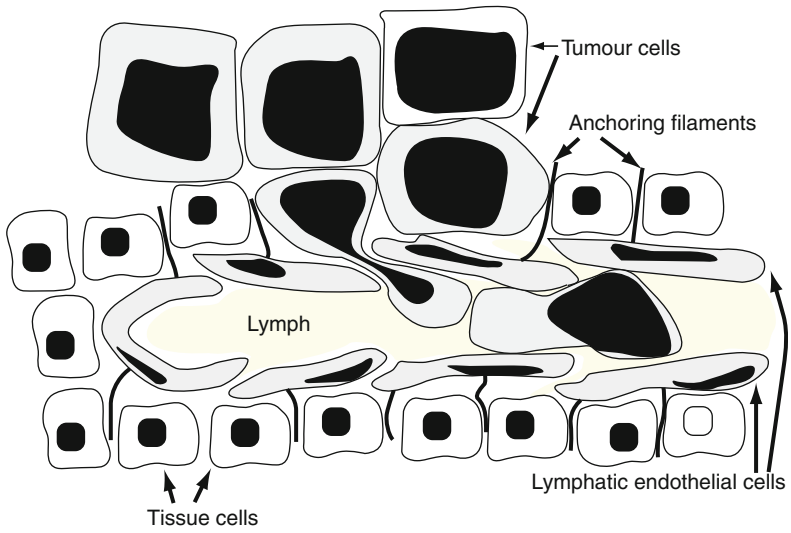
Defective lymphatic vessels result in a number of human pathologies. ▶ Congenital lymphedema is hereditary and is often linked to mutation in the gene encoding vascular endothelial growth factor receptor-3, although other genes may also cause this condition. The hereditary disease ▶ lymphedema-distichiasis is caused by a mutation in the forkhead family transcription factor FOXC2. Lymphatic malformations are composed of defective cutaneous and subcutaneous lymphatic vessels. These lesions are not hereditary and are thought to arise as a developmental defect where part of the developing lymphatic system becomes separated from the rest of the lymphatic system and subsequently becomes cystically dilated.

Secondary lymphedema occurs when the lymphatic system is damaged following trauma. Events that may cause damage include surgery and/or radiotherapy (typically during cancer treatment), infection, severe injury, or burns. Secondary lymphedema may be transitory, recurring, or a chronic condition.

Clinical findings have suggested that tumor-associated lymphatics play a key role in ▶ metastasis by providing a pathway for tumor cell dissemination, (Fig. 1). Indeed, the presence of metastatic tumor cells in regional lymph nodes is an important prognostic indicator for many human cancers.

Members of the ▶ vascular endothelial growth factor (VEGF) family, VEGF-A, VEGF-C, and VEGF-D, signaling through VEGF receptors-2 and/or -3, have been shown to play a critical role in a number of in vitro and in vivo models of lymphangiogenesis. In cancer, the majority of clinical studies show positive correlations between VEGF-C and/or VEGF-D levels and tumor lymphatic vessel density, lymph node status, and, in some instances, poor clinical outcome. In addition, several other growth factors, such as ▶ platelet-derived growth factors (PDFGs), hepatocyte growth factor (HGF), fibroblast growth factors (FGFs) angiopoietins, and ▶ insulin-like growth factors (IGFs), have been demonstrated to promote lymphangiogenesis.

It is currently unclear whether preexisting lymphatic vessels are sufficient to permit tumor cell dissemination, or whether this function requires de



Lymphatic Vessels.
Fig. 1 Schematic representation of tumor cells invading a lymphatic capillary

novo lymphatic formation or an increase in lymphatic size. The relative contribution of each of these processes may also vary with tumor type and subtype. Furthermore, as intratumoral lymphatic vessels are typically collapsed due to the high interstitial pressure found in tumors, it is likely that in the majority of tumors peritumoral lymphatic vessels play a key role in tumor dissemination to lymph nodes.

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Lymphedema

Synonyms

Lymphoedema

Definition

Refers to the building up of lymphatic fluid in the soft tissues of the body, usually in an arm or leg, with the result of a swelling. As the fluid accumulates, the swelling continues. The lymphatic system consists of lymph vessels and lymph nodes that run through the body. Lymph vessels collect a fluid that is made up of protein, water, fats, and wastes from the cells of the body. Lymph vessels carry this fluid to the lymph nodes that filter waste materials and foreign products, and then return the fluid to the blood. If vessels or nodes become damaged or are missing, or when here is a blockage of the lymphatic system, the lymph fluid cannot move freely through the system. The fluids can then build up and cause swelling in the affected arms or legs.

Lymphedema-Distichiasis

Definition

Lymphedema-distichiasis is an autosomal dominant disorder that classically presents as lymphedema of the limbs and double rows of eyelashes (distichiasis).

► Lymphatic Vessels

Lymphoblastoid Cell Lines

Definition

LCLs; ► Epstein Barr virus (EBV) immortalized B cell lines established by in vitro infection or culture

of peripheral blood lymphocytes (PBL) from EBV-infected individuals. These cells are not tumorigenic in ► nude mice.

Lymphocytes

Definition

All adaptive immune responses are mediated by lymphocytes. Lymphocytes are a class of white blood cells that bear variable cell-surface receptor for antigen. These receptors are encoded in rearranging gene segments. There are two main classes of lymphocyte – B lymphocytes (B cells) and T lymphocytes (T cells) – which mediate humoral and cell-mediated immunity, respectively. Small lymphocytes have little cytoplasm and condensed nuclear chromatin; on antigen recognition, the cell enlarges to form a lymphoblast and then proliferates and differentiates into an antigen-specific effector cell.

► Adaptive Immunity

Lymphocytic and Histiocytic Cells

Definition

L&H cells are the tumor cells in the lymphocyte predominant subtype of Hodgkin lymphoma. These are relatively large mononuclear lymphoma B cells, showing strong expression of the B cell maker CD20.

► Hodgkin and Reed/Sternberg Cell
► Hodgkin Disease

Lymphoedema

Synonyms

Lymphedema

Lymphoepithelioma

► Nasopharyngeal Carcinoma

Lymphogenic Metastatic Spread

Definition

Small lymph capillaries surrounding or invading tumors may take up tumor cells and transport them via larger lymph vessels to regional lymph nodes.

Lymphoid Organs

Definition

Are structured tissues where lymphocytes maturate, encounter their antigen, and differentiate. They are divided into primary (bone marrow, thymus) and secondary (e.g., spleen, lymph nodes) lymphoid organs by providing sites for either lymphocyte maturation or activation and differentiation, respectively.

Lymphokine-Activated Killer

Definition

LAK cells are used in ► adoptive immunotherapy for the treatment of malignant diseases. The therapy involves the removal of peripheral blood from a patient, depletion of red blood cells from the blood to produce a lymphocyte-containing white blood cell fraction, incubating the blood fraction in culture medium with ► interleukin-2 (IL-2) to induce their transformation into tumor-destroying LAK cells, and injecting the LAK cells into the patient along with interleukin-2. LAK cells are thought to be similar to NK cells in that they lyse target cells in a nonmajor histocompatibility complex (► MHC)-restricted manner.

► Activated Natural Killer Cells
► Immunotherapy

Lymphokine-Activated Killer Cell

Definition

LAK; a white blood cell that is stimulated in a laboratory to kill tumor cells. If lymphocytes are cultured in the presence of ► interleukin 2, effector cells will develop that are ► cytotoxic to tumor cells.

Lymphokines

Definition

Soluble factors or cytokines that are produced by lymphocytes (mostly T cells), which have effects on the function of other cells expressing lymphokine receptors. Most common lymphokines belong to the interleukin family.

► Peptide Vaccines for Cancer

Lymphoma

Definition

Malignant lymphomas are defined as neoplasms consisting of cells of the lymphoid tissues. 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 tumors, 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 spectrum of aggressiveness may be observed in individual cases, which is either due to disease progression in a multistep process of lymphomagenesis and/or the involvement of specific risk factors.

► Hodgkin Disease
► Malignant Lymphoma: Hallmarks and Concepts

Lymphoscintigraphy

Definition

An imaging technique whereby a radio-labeled tracer is administered, and its whereabouts in the lymphatic system are imaged using a gamma camera.

► Sentinel Lymph Nodes

Lymphotoxin

Synonyms

Tumor necrosis factor-β (TNF-β)

Definition

LT; a cytokine secreted by inflammatory CD4 T cells that is directly cytotoxic for some cells.

Lymphovascular Invasion

Definition

Presence of tumor cells invading blood or lymphatic vessel around the primary tumor.

► Adjuvant Chemoendocrine Therapy

Lymphovenous Shunt

Definition

Part of the lymphatic fluid entering the lymph node may be shunted to the blood capillaries without further transport in the serial system of lymph nodes. This direct access

to the blood stream provides means of metastatic routes, where tumor cells may enter the blood stream at the site of the lymph node and spread by a hematogenously.

► Sentinel Node

Lynch Syndrome

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Synonyms

Hereditary non-polyposis 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, prostate, 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. [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 [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 and 25 and repeated every 1–2 years. In the Lynch syndrome II variant, in addition to the colon, attention for screening is focused 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.

References

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Lysolecithin

► Lysophosphatidylcholine

Lysophosphatidate

Definition

Lysophosphatidate (LPA) is a type of serum-derived lipid growth factor. This bioactive lipid phosphate is present outside the cell and signals through a series of cell surface receptors. LPA can be formed from phosphatidate by the action of phospholipase A1 or A2 or from circulating lysophosphatidylcholine by lysophospholipase D (autotaxin).

► Lipid Mediators
► Lysophosphatidylcholine

Lysophosphatidylcholine

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Synonyms

Lysolecithin

Definition

Lysophosphatidylcholine (LPC) is a major plasma lipid constituent that is produced from phosphatidylcholine (PC).

Characteristics

LPC is produced from PC under a variety of physiological and pathological conditions. LPC is present at high levels (about 100 μM) in plasma under normal conditions and exists mainly in albumin- or lipoprotein-bound forms. The biochemical conversion from PC to LPC is mediated by ▶ phospholipase A1 or ▶ phospholipase A2. Sequentially, LPC is converted to ▶ lysophosphatidate (LPA) by lysophospholipase D (autotaxin) (Fig. 1).

Signaling Mechanisms

LPC is a cell-signaling molecule. It acts as a ligand for a family of ▶ G-protein coupled receptors (GPCRs), e.g., G-protein coupled receptor 4 (GPR4) and G2A. Although our knowledge of LPC-sensitive GPCRs is preliminary, circumstantial evidence suggests multiple roles in a variety of physiological and pathophysiological states, such as the development, regulation of the cardiovascular, immune and nervous systems, inflammation, arteriosclerosis, and cancer.

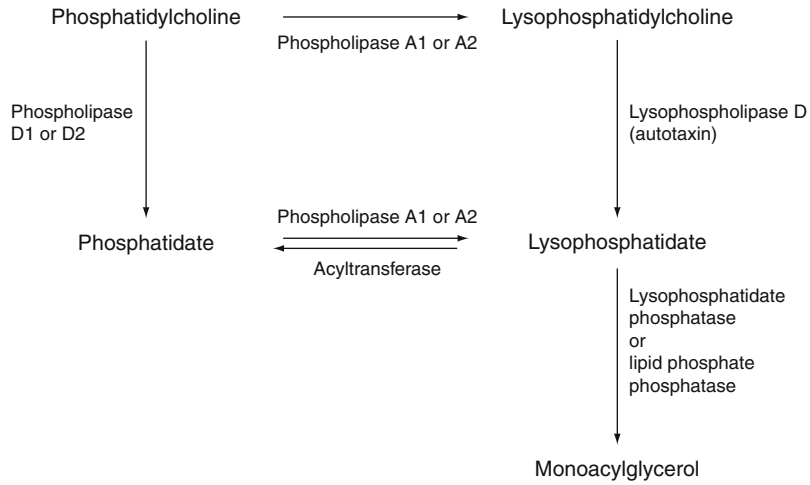
GPR4 shows high-level expression in many tissues such as the lung, liver, kidney, ovary, and lymph node. GPR4 has the Kd value for LPC = 159 nM in the binding experiments of GPR4-expressing cells. LPC induces transcriptional activation of the serum response element (SRE) in GPR4-transfected HEK293 cells. Gi and Rho (▶ Rho Family Proteins) signaling pathways are involved in SRE activation through GPR4. Rho-dependent activities induced by LPC also include actin rearrangement and cell migration. LPC-induced cell migration via GPR4 is sensitive to the C3-exoenzyme. GPR4 is also linked to the activation of the extracellular signal-regulated kinase (ERK) pathway in response to LPC, leading to the stimulation of DNA synthesis and cellular migration in Swiss 3T3 cells. In vascular endothelial cells, LPC upregulates adhesion molecules and growth factors and stimulates the secretion of chemokines and superoxide anions.

G2A is expressed in hematopoietic cells such as T- and B-lymphocytes, monocytes and macrophages, and in tissues such as spleen and thymus. LPC activates the migration of T-lymphoid cell lines and peritoneal macrophages via a G2A/Rho-dependent mechanism. G2A stimulates Rho via Ga13, resulting in actin rearrangement and SRE-dependent transcription activation. LPC-G2A activation also induces cell migration in Jurkat T cells. As a major component of oxidized low-density lipoprotein, LPC exerts a chemoattractive effect on T cells and macrophages in atherosclerosis and other chronic inflammatory diseases. G2A also displays distinct patterns of ERK activation.

As with other GPCRs, GPR4 and G2A induce ligand-dependent increase in $[Ca^{2+}]i$ (in particular, $[Ca^{2+}]i$ release from intracellular stores), by which they regulate cellular Ca^{2+} homoeostasis and the cytoskeleton, adhesion and migration, proliferation, and survival.

Clinical Aspects

Although many lines of evidence support a causal relationship between LPC and atherosclerosis or other inflammatory diseases, its role in carcinogenesis has not yet been extensively investigated. The concentration of LPC (in particular, the ratios of palmitoyl-LPC to linoleoyl-LPC) is elevated in the sera of patients with ovarian cancer or multiple myeloma. In addition, LPC levels are elevated in the bile of patients with ▶ anomalous pancreaticobiliary ductal junction (APBDJ), which is one of the risk factors for biliary tract carcinomas, and that LPC inhibited cellular apoptosis by inducing ▶ cyclooxygenase-2 (COX-2) expression via a Raf-1 (▶ Raf Kinase)-dependent mechanism in human cholangiocytes. Therefore, LPC is considered to be involved in tumor development or progression, although this still needs to be clarified.



Lysophosphatidylcholine.
Fig. 1 Metabolism of lysophosphatidylcholine

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Lysosome

Definition

Is a catabolic organelle in the cytoplasm of eukaryotes. It is characterized by a membrane proto-ATPase that maintains the low pH of 5–6. 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.

► Autophagy

Lyve-1

Definition

Is a transmembrane receptor for extracellular hyaluronic acid. It is a homologue of ► CD44 and mainly expressed on lymphatic endothelial cells.

► Lymphangiogenesis
► Podoplanin