S

S-100 Protein

Definition

Is a low molecular weight protein normally present in cells derived from the neural crest (Schwann cells, melanocytes, and glial cells), chondrocytes, adipocytes, myoepithelial cells, macrophages, Langerhans cells, dendritic cells, and keratinocytes.

► Langerhans Cell Histiocytosis

S1P

Definition

Sphingosine-1-phosphate, an anti-apoptotic, angiogenic sphingolipid. A pro-proliferative sphingolipid that counteracts ceramide effects.

► Sphingolipid Metabolism

S-9

Definition

Is a cell free fraction derived from a chopped up liver that performs mostly ► phase II metabolism on a drug.

► ADMET Screen

SAAB

Definition

Selected and amplification binding (SAAB) is a combinatorial selection method that makes use of preliminary information regarding a ligand's binding specificity when designing a selection template. Unlike other combinatorial selection methods, SAAB does not require subcloning of selected templates but rather obtains binding specificity through direct sequencing of the selected template pool.

► Combinatorial Selection Methods

SAGE

Definition

Serial analysis of gene expression (SAGE) is a method that allows the analysis of overall gene expression patterns with digital analysis.

SAHA

Definition

Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylases.

► Vorinostat

M. Schwab (ed.), *Encyclopedia of Cancer*, DOI 10.1007/978-3-642-16483-5,

Salisburia Adiantifolia

► Ginkgo Biloba

Salisburia Macrophylla

► Ginkgo Biloba

Salivary Agglutinin (SAG, Human)

► Deleted in Malignant Brain Tumours 1

Salivary Gland Malignancies

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Definition

Salivary gland tumors are an uncommon and epidemiologically diverse group of tumors. Though rare, these tumors can pose a diagnostic and treatment challenge for the head and neck surgeon.

Characteristics

Epidemiology/Risk Factors

There are three major paired glands (parotid, submandibular, and sublingual) and numerous minor salivary glands which line the mucosa of the upper aerodigestive tract. The normal function of these glands is to produce saliva, which serves as a lubricant, aids in early digestion, and is important to good dental hygiene.

The incidence of salivary gland tumors is ~5 per 100,000 population, which yields ~2,500 new cases in the United States per year. They comprise less than 0.5% of all malignancies and 3–5% of malignancies of the head and neck region. Most salivary gland tumors (70–80%) arise in the parotid gland and the majority of all salivary gland tumors are benign. Of the tumors originating from the parotid gland roughly 75% are benign, while only 25% are malignant. The percentage of malignant tumors increases for other locations (37–45% for submandibular glands, 75% for sublingual gland, and slightly less than 50% for minor salivary glands). The most common malignant tumor of the parotid gland is mucoepidermoid ► carcinoma, while in the submandibular gland it is adenoid cystic carcinoma. Although the most common malignancy of the minor salivary glands is mucoepidermoid carcinoma as well, when polymorphous low-grade carcinoma is found, it is virtually pathognomonic for a minor salivary gland malignancy.

The etiology of salivary gland malignancies is unknown. Several studies have demonstrated an association between exposure to ionizing radiation and subsequent occurrence of salivary malignancy. Although both alcohol and tobacco use are thought to increase the risk of salivary gland malignancies, the current literature has conflicting data. Various nutritional factors and certain diets (including those high in fruits and vegetables and low in cholesterol) are thought to lower the risk of salivary gland cancer.

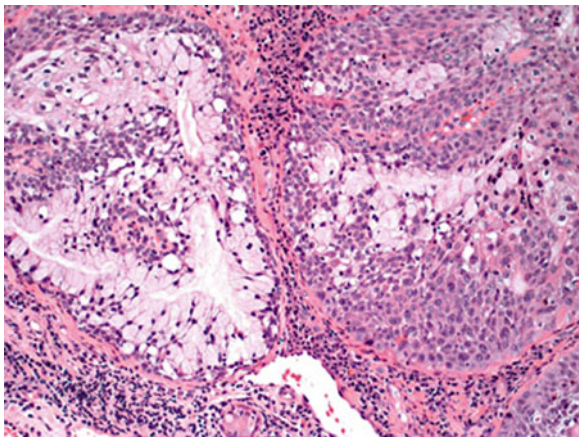
Staging/Classification

Salivary gland cancers constitute a heterogeneous group of tumors with distinct histology and very diverse clinical behavior.

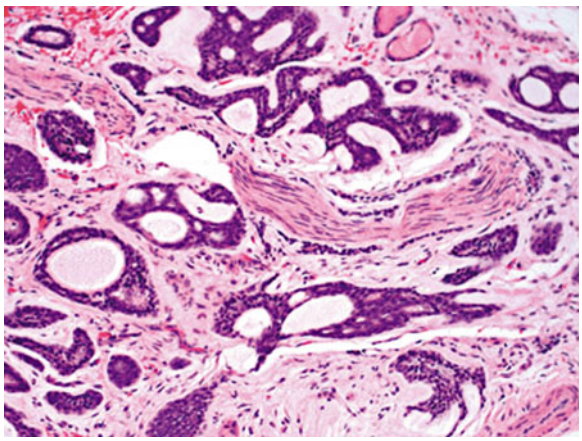
The World Health Organization (► WHO) has described 23 different histologic types of salivary gland cancers (Table 1), most of which are exceedingly rare. Salivary gland malignancies can further be subdivided into high- and low-grade tumors. Low-grade tumors usually exhibit a less aggressive behavior. These include acinic cell carcinoma, cystadenocarcinoma, polymorphous low-grade adenocarcinoma, and other rare tumors. In contrast, high-grade tumors display a significantly more aggressive behavior. The examples of high-grade tumors include salivary duct carcinomas, anaplastic and undifferentiated carcinomas, and carcinosarcomas. Mucoepidermoid carcinoma, adenocarcinoma, and squamous cell carcinoma

Salivary Gland Malignancies. Table 1 WHO (World Health Organization) classification of salivary gland neoplasms

Malignant neoplasms	Benign neoplasms
Acinic cell carcinoma	Mixed tumor (pleomorphic adenoma)
Mucoepidermoid carcinoma	Warthin's tumor
Adenoid cystic carcinoma	Myoepithelioma
Polymorphous low-grade adenocarcinoma	Basal cell adenoma
Epithelial-myoepithelial carcinoma	Canalicular adenoma
Clear cell carcinoma, N.O.S.	Oncocytoma
Basal cell adenocarcinoma	Cystadenoma
Malignant sebaceous tumors	Sialadenoma papilliferum
Cystadenocarcinoma	Inverted ductal papilloma
Low-grade cribriform cystadenocarcinoma	Intraductal papilloma
Mucinous adenocarcinoma	Lymphadenomas and sebaceous adenomas
Oncocytic carcinoma	
Salivary duct carcinoma	
Adenocarcinoma, N.O.S.	
Myoepithelial carcinoma	
Carcinoma ex pleomorphic adenoma	
Carcinosarcoma	
Metastasizing pleomorphic adenoma	
Squamous cell carcinoma	
Small cell carcinoma	
Large cell carcinoma	
Lymphoepithelial carcinoma	
Sialoblastoma	



Salivary Gland Malignancies. Fig. 1 High-grade mucoepidermoid carcinoma (hematoxylin and eosin-medium power magnification)



Salivary Gland Malignancies. Fig. 2 Adenoid cystic carcinoma of parotid origin demonstrating perineural invasion (hematoxylin and eosin-medium power magnification)

of salivary glands can present with various histologic grades (low, intermediate, or high) and, thus, exhibit a variety of biological behaviors (Fig. 1). Certain tumors, such as adenoid cystic carcinoma, while low-grade, exhibit very aggressive behavior due to a propensity for perineural spread (Fig. 2).

The current American Joint Committee on Cancer (► AJCC) staging system takes into account the extent of the primary tumor as well as regional and metastatic disease (Table 2).

At this time, this staging system for salivary malignancies does not take into account histologic grading. Because of the wide variety of histological presentations and resultant diversity of clinical aggressiveness, the treatment of each individual cancer depends on both clinical staging and tumor histology.

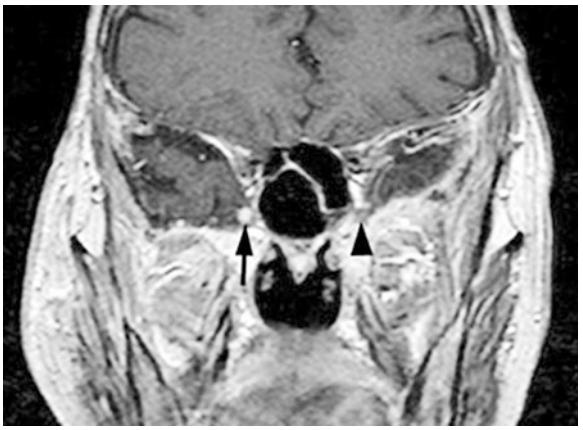
Diagnosis

Detailed clinical history and comprehensive head and neck examination are critical in evaluation of salivary gland neoplasms. Cross-sectional radiologic studies such as computerized tomography (► CT) or magnetic resonance imaging (► MRI) are also very helpful. The real advantage of cross-sectional imaging in evaluating salivary gland masses, which primarily involve the parotid gland, is the ability to accurately reveal the location and extension of a tumor, its relationship to the facial nerve, and to assess for perineural tumor spread (Fig. 3). MRI is the modality of choice for evaluation of parotid masses and there are a few general rules that help in the differentiation of these

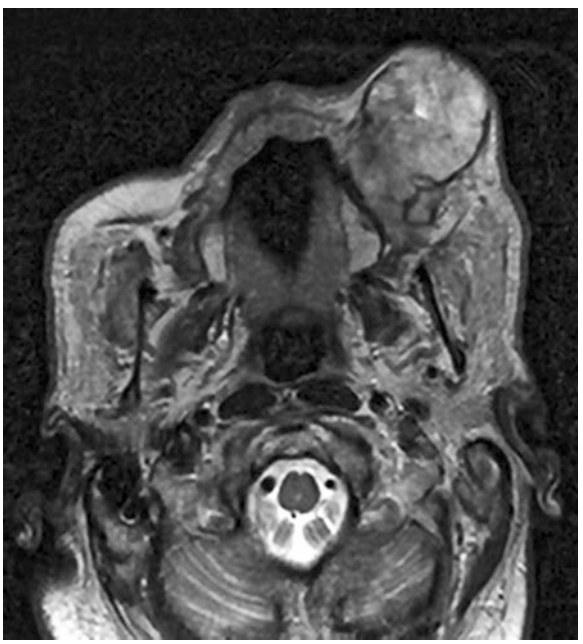
Salivary Gland Malignancies. Table 2 AJCC stage groupings for salivary gland malignancies

Stage I	$T_1N_0M_0$
Stage II	$T_2N_0M_0$
Stage III	$T_3N_0M_0$
	$T_1N_1\ M_0$
	$T_2N_1\ M_0$
	$T_3N_1\ M_0$
Stage IV	T_4 any N M_0
	Any T N_2 M_0
	Any T, any N, M_1
Primary tumor (T)	*Tx* Primary tumor cannot be assessed
	T0 No evidence of primary tumor
	T1 Tumor 2 cm or less in greatest dimension without extraparenchymal extension[a]
	T2 Tumor more than 2 cm but not more than 4 cm in greatest dimension without extraparenchymal extension[a]
	T3 Tumor more than 4 cm and tumor having extraparenchymal extension[a]
	T4a Tumor invades skin, mandible, ear canal, and facial nerve
	T4b Tumor invades skull base and pterygoid plates and encases carotid artery
Regional lymph nodes (N)	*Nx* Regional lymph nodes cannot be assessed
	N0 No regional lymph node metastasis
	N1 Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
	N2 Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension, or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension, or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
	N2a Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
	N2b Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
	N2c Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
	N3 Metastasis in a lymph node more than 6 cm in greatest dimension
Distant metastasis (M)	*Mx* Distant metastasis cannot be assessed
	M0 No distant metastasis
	M1 Distant metastasis

[a]Extraparenchymal extension is clinical or macroscopic evidence of invasion of soft tissues. Microscopic evidence alone does not constitute extraparenchymal extension for classification purposes.



Salivary Gland Malignancies. Fig. 3 Post-contrast T1-weighted coronal MRI showing enhancement of the right maxillary nerve within the foramen rotundum (*arrow*), consistent with perineural tumor spread of an adenoid cystic carcinoma. There is no enhancement of the contralateral nerve (*arrowhead*)



Salivary Gland Malignancies. Fig. 4 T2-weighted axial MRI showing large carcinoma ex-pleomorphic arising along Stenson's duct

lesions. A well-defined parotid mass of very high T2 signal is consistent with benign mixed tumor (BMT) or pleomorphic adenoma. Malignant tumors are, however, frequently also well defined but they tend to be of lower T2 signal (Fig. 4). Positron emission

tomography (▶ PET) scanning has been emerging as a new useful modality in evaluation of metastatic disease of head and neck cancers. Salivary gland tumors, however, belong to a small group of neoplasms for which PET (and PET-CT) cannot reliably distinguish malignant from benign masses, since some carcinomas do not show increased metabolic activity, while benign tumors such as BMT and especially Warthin's tumor may show very high Fluorine-18 2-Fluoro-2-Deoxy-D-Glucose (▶ FDG) uptake.

▶ Fine needle aspiration biopsy (FNAb) has been demonstrated to be a highly sensitive and specific tool in the evaluation of salivary gland neoplasms. In a review of 325 patients with salivary gland neoplasms investigated by FNAb and correlated with histologic review, the technique was found to be 85.5% sensitive and 99.5% specific. While the sensitivity and specificity may vary, especially in the smaller community hospitals, FNAb continues to help with treatment planning, providing the physician and patient adequate preparation for the necessary surgical procedure, potential for complications, and need for ▶ neck dissection or ▶ adjuvant therapy. CT-guided biopsy is the technique of choice for deep (such as in the deep parotid lobe) or poorly localized masses.

Treatment

Surgical resection has been the primary modality for treatment of salivary gland cancers. Commonly, it necessitates removal of the entire gland of origin often in conjunction with removal of involved or at-risk lymph nodes (selective or modified neck dissection). In order to achieve a complete tumor extirpation without oncologic compromise, surrounding structures including cranial nerves, subcutaneous tissues, skin, and muscles may require resection as well. Adjuvant radiation therapy (XRT) has long been used for achievement of better local and regional control of the disease. Frequently, it has been employed postoperatively for advanced T3 or T4 tumors. In addition, there is new limited data available in the current literature for support of adjuvant XRT even for earlier T1 and T2 cancers. In the recent retrospective review from Switzerland, the local recurrence rate was significantly lower for the group with combined treatment as compared to after surgery alone. Fast neutron-beam radiation and accelerated, hyperfractionated photon beam radiation have been reported to be more effective than conventional radiation therapy in treatment of more advanced lesions. Adjuvant XRT is typically recommended for all high-grade tumors and for low-grade tumors with unsatisfactory margins.

There is also a continuing effort to identify more successful systemic therapies for salivary gland cancers. There are several current trials which employ conventional chemotherapeutic agents such as taxol, or cisplatin/carboplatin combined with gemcitabine. Other medical agents have also been tried with variable success in the treatment of salivary gland cancers, though certain histologic types, such as adenoid cystic carcinoma, have consistently demonstrated resistance to systemic therapy. However, new agents that target specific tumor molecules such as ▶ HER2/neu tyrosine kinase and epidermal growth factor receptor (EGFR) are under active investigation.

Overall, treatment of salivary gland cancers constitutes an important component of practice for head and neck surgeons. The wide variety of clinical behaviors usually requires a multidisciplinary team approach with cooperation between otolaryngology/head and neck surgeons, medical and radiation oncologists, oral and surgical pathologists, radiologists, oral/maxillofacial surgeons and dentists, speech pathologists, dieticians and other clinical support personnel.

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Salmonella typhi and paratyphi

Definition

Salmonella typhi and *paratyphi* are parasites, serotypes of *Salmonella enterica*. If invasive, the infections by

Salmonella typhi or *paratyphi* lead to the development of typhoid or paratyphoid fever. The organisms can be transmitted by the fecal-oral route: it is excreted by humans in feces and may be transmitted by contaminated water, food, or by person-to-person contact (with inadequate attention to personal hygiene).

► Gallbladder Cancer

Salpingo-Oophorectomy

Definition

The removal of an ovary together with the fallopian tube is called salpingo-oophorectomy or unilateral salpingo-oophorectomy (USO). When both ovaries and both Fallopian tubes are removed, the term ► bilateral salpingo-oophorectomy (BSO) is used.

Salt Intake

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Synonyms

Edible salt; Salting; Sodium chloride

Definition

The composition of salt is approximately 39.3% sodium and 60.7% chloride by molecular weight. Salt regulates the water content (fluid balance) of the body, and plays an essential role in homeostasis. The nutritional requirement of salt has been estimated to be 1.25 g/day for adults.

Characteristics

Salt intake varies substantially around the world, though in developed countries, typical diets include salt far in excess of requirements. In the INTERSALT study, daily salt intake as determined by 24-h urinary sodium excretion ranged from 5 g in Trinidad and Tobago to 14 g in Tianjin, China. The average intakes were around 8–10 g/day in most western countries and 10–12 g/day in Japan and Korea.

Epidemiological studies have shown that ► nasopharyngeal carcinoma and ► gastric cancer are associated with high salt intake.

Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma is a rare malignancy, with age-adjusted incidence rates of under 1 per 100,000 person-year for both men and women in most parts of the world. In certain geographical regions, however, the incidence of nasopharyngeal carcinoma is dramatically higher. The highest rates of nasopharyngeal carcinoma have been documented among Cantonese residing in the central region of Guangdong Province in Southern China and in Hong Kong (25–30 per 100,000 person-year).

John Ho first suggested in 1971 that Cantonese-style salted fish, a common item in the local diet and a popular weaning food, may be an etiological factor in nasopharyngeal carcinoma. Since then, a large number of case-control studies conducted in various populations residing in different parts of Asia (Hong Kong, Guangzhou [Canton], and Malaysia) have confirmed the association between age at first exposure to salted fish and this type of cancer. It has been estimated that >90% of nasopharyngeal carcinomas in Hong Kong, 50% of those in Guangzhou, and >60% of those in Chinese people in Malaysia are due to childhood consumption of salted fish. Animal data have further strengthened the evidence for Cantonese-style salted fish as a nasopharyngeal carcinogen. For example, rats fed this human food developed cancer of the nasal cavity in a dose-dependent manner, though this cancer rarely occurs in this species. Based on these evidences, the relationship between Chinese-style salted fish and nasopharyngeal carcinoma was determined to be "convincing" by the WHO/FAO (World Health Organization/Food and Agriculture Organization) Expert Consultation on Diet.

However, it may be that salted fish has an oncogenic action not shared by salt alone. Experimental studies have shown that low levels of several nitrosamines/precursors and ▶ Epstein–Barr-virus-activating substances exist in preserved salted fish. Some ▶ epidemiological studies have also indicated that nitrosamines and nitrates included in preserved food play a role in the development of nasopharyngeal carcinoma if such foods are consumed during childhood. On the other hand, the antibody of Epstein–Barr virus has been found in the sera of nasopharyngeal carcinoma patients, suggesting that this virus is associated with nasopharyngeal carcinoma as well as with Burkitt's lymphoma. The consumption of salted fish may lead to the activation of Epstein–Barr virus in the sera. Further investigations are needed to examine the mechanisms through which Chinese-style salted fish increases the incidence of nasopharyngeal carcinoma.

Gastric Cancer

The incidence of gastric cancer also differs by geographic location and ethnicity. In 2002, the age-standardized incidence rates (per 100,000) of gastric cancer in Japan were 62.0 in men and 26.1 in women, and these rates as well as those in Korea (69.7 in men and 26.8 in women) were among the highest in the world. In contrast, among the white population in western countries, the incidence rate was 7.4–12.8 in men and 3.4–6.6 in women, which is clearly far lower than that in Asian countries. In the INTERSALT study, median sodium levels were analyzed in relation to national gastric cancer mortality rates. For the 24 countries studied, the Pearson's correlation coefficient for gastric cancer mortality with sodium was 0.70 in men and 0.74 in women (both $P<0.001$).

Many, but not all, results of case-control studies have shown a positive association between gastric cancer and the intake of high-salt foods such as salted fish, cured meat, and salted vegetables, or the use of table salt. However, in prospective studies among Caucasian populations, the effect of high salt intake or salted foods on the incidence of gastric cancer remains controversial. Several studies have found no positive association. Only a cohort study on 120,852 Dutch subjects and 282 gastric cancer cases showed a weak significant association between salt intake and gastric cancer incidence, though there were no clear trends. In contrast, in the Japanese population, two prospective studies have identified a significant association between dietary salt intake and gastric cancer incidence: Tsugane et al. reported that in a total of 18,684 men and 20,381 women, the quintile category of salt intake and salted food was associated with a risk of gastric cancer in men during a 12-year follow-up. Another Japanese study also investigated the relationship between the amount of dietary salt intake and gastric cancer especially intestinal type, taking into account the effects of other risk factors such as ▶ *Helicobacter pylori* infection and atrophic gastritis, finding that salt intake is an independent risk factor for the subsequent incidence of gastric cancer in the Japanese population. Several reasons for the negative findings in Caucasian studies are proposed. The amount of salt intake in Caucasians may be lower than the threshold for incident gastric cancer. Furthermore, it may also be that the salt intake in the negative studies was not estimated accurately by detailed food frequency questionnaires. Given the lack of definitive evidence in the prospective studies, the WHO/FAO Expert Consultation concluded that salt "probably" increases the risk of gastric cancer.

Several possible mechanisms of gastric carcinogenesis induced by a high-salt diet have been discussed in the literature. High dietary salt intake is believed to alter the viscosity of the protective mucous barrier, leading to mucosal damage in the stomach and making mucosal cells more susceptible to carcinogens in food. Moreover, intragastric high salt concentrations are known to cause mucosal damage and inflammation. Persistent inflammatory changes in the stomach may promote temporary cell proliferation and increase the rate of endogenous mutation. Infection with the bacterium ▶ *H. pylori* is an established risk factor for the development of gastric cancer. In earlier-mentioned latter Japanese ▶ epidemiological study, the effect of high salt intake on gastric carcinogenesis was strong in subjects who had both atrophic gastritis and *H. pylori* infection. The synergistic promoting effects of a high-salt diet and *H. pylori* infection on gastric carcinogenesis have also been observed in an experimental study using a gerbil model. On the other hand, atrophic gastritis is thought to be a precancerous lesion of gastric cancer. Thus, the synergistic promoting effects of a high-salt diet and *H. pylori* infection may appear particularly strongly when gastric atrophy is present. It is generally accepted that gastric adenocarcinoma, particularly of the intestinal type, arises through a multistep process originating with chronic gastritis,

and progresses through stages of atrophy, intestinal metaplasia, and dysplasia, eventually resulting in carcinoma. Based on these results, it is more likely that a high intake of salt is involved primarily in the latter stages of multistep gastric carcinogenesis and thereby promotes gastric carcinogenicity because atrophic gastritis is one of the morphological hallmarks of these stages. These studies suggest that salt per se is associated with incident gastric cancer directly or indirectly.

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Salting

► Salt Intake

Salvage Chemotherapy

Definition

Salvage chemotherapy is when chemotherapy is offered when a cancer recurs or a patient is not responding to other forms of cancer treatment. It is often high dose, with the goal of trying to beat the cancer into remission.

Sanctuary Site

Definition

Any location in the body that is poorly penetrated by drugs, for example, central nervous system, testes, ovary.

Sandwich ELISA

Definition

The technique of sandwich ELISA uses antibody bound to a surface to trap a protein by binding to one of its epitopes. The trapped protein is then detected by an enzyme-linked antibody specific for a different epitope on the protein's surface. This gives the assay a high degree of specificity.

SAPKα

► JNK Subfamily

SAPKβ

► JNK Subfamily

SAPKγ

► JNK Subfamily

Sarcoid

Definition

Locally aggressive fibroblastic skin tumor. It is associated to bovine papillomavirus type 2 infections in horses.

► Bovine Papillomavirus

Sarcoma

Definition

Is a malignant tumor of the mesenchymal tissues, commonly the connective tissues, but includes muscle of all kinds and tendons and bones.

► Cardiac Tumors
► Gastrointestinal Stromal Tumor

Satraplatin

Definition

Satraplatin (Spectrum Pharmaceuticals, Irvine, CA) is a third-generation orally bioavailable platinum-derived chemotherapeutic. Satraplatin forms highly reactive, charged, platinum complexes which bind to nucleophilic groups in DNA, inducing intrastrand and interstrand DNA cross-links, as well as DNA-protein cross-links. These cross-links result in cell growth inhibition and ► apoptosis. Previous phase III ► clinical trials combined satraplatin with ► prednisone in the treatment of high-risk ► prostate cancer (HRPC), resulting in an approximately 33% reduced risk of disease progression, however, lack of statistically significant overall survival rates halted further trial continuance. Current phase III clinical trials however are gauging the efficacy of Satraplatin alone (NCT00450970) or in combination with ► Bevacizumab (NCT00499694) on HRPC patients previously treated under an unlimited ► cytotoxic chemotherapy regimen or patients with metastatic HRPC previously treated with ► docetaxel, respectively.

► Prostate Cancer Experimental Therapeutics

SBMA

► Spinal and Bulber Muscular Atrophy

SBP

Definition

Solitary bone plasmacytoma.

► Plasmacytoma

SCA1

Definition

Spinocerebellar ataxia type 1.

► Cajal Bodies

Scaffold Proteins

Synonyms

Docking proteins

Definition

Coordinate the spatio-temporal activation of signaling pathways by assembling their individual components to a multi-protein complex or ► signalosome.

► B-Raf Signaling

Scatter Factor

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Synonyms

Hepatocyte growth factor; HGF

Definition

Scatter factor (SF), also known as hepatocyte growth factor (HGF), is a multifunctional cytokine that participates in various biologic processes, including: embryonic development (▶ Morphogenesis), oncogenesis (tumor formation), ▶ angiogenesis (new blood vessel formation), and the regulation of ▶ apoptosis (programmed cell death). SF was originally characterized as a protein secreted by mesenchymal cells (e.g., fibroblasts) that disperses (or "scatters") contiguous sheets of epithelium and stimulates cell motility. HGF was identified as a serum-derived protein that stimulates the proliferation of adult rat hepatocytes. Subsequent studies revealed that SF and HGF are identical. HGF is the ligand of a ▶ tyrosine kinase receptor encoded by a ▶ proto-oncogene (c-▶ Met), and HGF binding causes receptor activation.

Characteristics

SF is a heparin-binding glycoprotein composed of a 60 kD α-chain and a 30 kD β-chain. The α-chain is composed of an N-terminal hairpin loop, followed by four ▶ kringle domains (looped structures that mediate protein interactions). The β-chain resembles protein-degrading enzymes such as trypsin, but SF lacks protein-degrading activity due to two key amino acid substitutions at the catalytic center. The binding of heparin to SF modulates its biologic activity, protects it from degradation and allows it to be stored in the extracellular matrix. Several shortened forms of SF containing only the N-terminal loop and the first ▶ kringle domain (NK1) or the first two kringle domains (NK2) are sufficient to bind with high affinity to the c-Met receptor. NK1 and NK2 are produced as a result of mRNA editing, and they can function as partial agonists or antagonists of SF. However, structure–function analyses indicate that the entire SF molecule, including the β-chain, is required to generate the full spectrum of SF's biologic activity. The human SF gene maps to the long arm of chromosome 7 (7q11.2–21).

SF is synthesized as a 728 amino acid precursor (preproSF) that is converted within the cell to its secreted form (proSF) by cleavage of a short segment (▶ Signal Sequence). However, the secreted proSF is not biologically active, and must undergo an internal cleavage within the linker region between the α-chain and β-chain. Cleavage of proSF occurs outside of the cell and results in the production of the mature, two-chain biologically active SF. Thus, the cleavage of proSF to SF is a potential control point for the regulation of SF activity. Several enzymes capable of cleaving and activating SF have been identified. These enzymes include the plasminogen activators (urokinase and tissue plasminogen activator), proteins that convert plasminogen – an enzyme that circulates in blood in inactive form – into its active form, plasmin. Plasmin is the major enzyme responsible for dissolving blood clots. Another enzyme capable of converting proSF into active SF is called "▶ HGF activator." HGF activator is a novel protein-degrading enzyme structurally related to a blood clotting factor (factor XII, or Hageman factor). HGF activator is itself produced in an inactive ("pro-enzyme") form. It may be activated by blood coagulation factors, such as thrombin. The physiologic processes that regulate SF activation have not been fully elucidated, but there is evidence to suggest that an enzymatic cascade that results in activation of HGF activator and then SF is triggered by tissue injury.

The SF Family

SF is not related to classic growth factors (e.g., ▶ Fibroblast Growth Factor or ▶ Platelet-Derived Growth Factor), but is a member of the kringle domain protein family, which includes blood coagulation and fibrin-degrading enzymes (e.g., Plasminogen, prothrombin, factor XII, urokinase, tissue plasminogen activator) and a ▶ macrophage-stimulating protein (MSP). Within this family, SF is most closely related to the plasminogen and MSP, with which it shares a similar αβ chain structure, a similar activation mechanism (i.e., cleavage between the α and β chains), and a high degree of amino acid sequence identity (38% and 50%, respectively). MSP was formerly called HGF-like protein and is the closest relative of SF. The MSP receptor, a tyrosine kinase receptor encoded by the Ron gene, is closely related to the SF receptor, but these two proteins do not cross-activate each other's receptor. MSP circulates in the bloodstream as an inactive profactor (proMSP) which, when activated, causes macrophages to become competent and to undergo chemotaxis and phagocytosis.

SF Receptor (The c-Met Proto-oncogene Product)

The MET proto-oncogene was (c-Met) originally discovered in rearranged form as a carcinogen-induced transforming oncogene (Tpr-Met) generated by a transposition between human chromosomes 1 and 7, resulting in fusion of a powerful promoter from chromosome 1 ("Transposed promoter region") to a portion of the c-Met proto-oncogene (at 7q21–31) that codes for the intracellular region of the receptor. The Tpr-met oncogene product is a membrane-bound tyrosine kinase that is constitutively active (i.e., does not require SF for activation). The full-length c-Met proto-oncogene encodes a growth factor receptor-like tyrosine kinase that consists of an extracellular SF-binding domain, a transmembrane domain, and an intracellular portion containing a kinase domain and sites that associate with various cytoplasmic signaling proteins.

The binding of SF to c-Met triggers molecular events similar to those triggered by the interactions between classic growth factors and their receptors. The receptor undergoes a change in three-dimensional conformation, resulting in:

- Activation of the catalytic kinase domain.
- Dimerization (association of two c-Met receptors).
- Cross-phosphorylation of the two receptors on multiple tyrosines.
- Initiation of a signal cascade ("signal transduction") causing transfer of information from the cell surface to the nucleus. Understanding signal transduction from c-Met will provide the key to understanding SF's biologic actions.

Signal initiation involves the interaction of phosphorylated tyrosines internal to the kinase domain of the activated c-Met with regions known as ▶ SH2 domains (src-homology domain-2) of proteins that act as signaling intermediaries. Most c-Met signaling involves the interaction of these signaling intermediaries with a "multifunctional docking site" involving two tyrosine (Y) residues located at amino acids 1,349 and 1,356:1349YVHVXXX1356YVNV. This unique site associates with many signaling proteins, including phosphatidyl-inositol-3´-kinase (▶ PI3K), phospholipase C-γ, pp60c-src, c-Cbl, and the Grb2/Sos complex, which binds p21Ras. Amino acid sequences similar to the multifunctional docking site of c-Met are found in the two related receptors: Ron and c-Sea (a tyrosine kinase receptor whose ligand has not been identified). Similar sequences are not found in the receptors for the epidermal growth factor, platelet-derived growth factor, or other factors.

The manner in which SF binding to c-Met can result in different physiologic consequences depending upon the cell type and context (see below) is just beginning to become unraveled. For example, it was recently found that SF-induced epithelial morphogenesis (i.e., the formation of a three-dimensional network of branching tubules) specifically requires association with c-Met at cell–cell junctions and phosphorylation of a protein known as Gab1 (the Grb2-associated binder). Grb2 binds to the tyrosine-1,356 site of c-Met via its SH2 domain, while another portion of the Grb2 protein (the SH3, or src-homology-3 domain) binds to Gab1. Gab1 is a member of the family of the "multi-substrate docking proteins," which includes IRS-1 (insulin-responsive substrate-1), a cytoplasmic protein that is a major mediator of the biologic effects of the insulin-like growth factor ▶ IGF-I.

Cellular and Molecular Regulation

SF Producer and Responder Cell Types

In vitro studies initially suggested that SF is produced predominantly by cells of mesenchymal (connective tissue) origin, including: fibroblasts, vascular smooth muscle, endothelial cells, glial cells, macrophages, activated lymphocytes, and other cell types. However, based on subsequent in vivo studies (immunohistochemistry and in situ hybridization), it is now apparent that a variety of epithelial cell types, including keratinocytes, mammary epithelial cells, and many carcinoma cells may also produce SF. For reasons not understood, cultured epithelial and carcinoma cells lose the ability to produce SF when placed in culture, although they often retain the c-Met receptor. A variety of cell types express the c-Met receptor and are biologically responsive to SF, including (but not limited to) keratinocytes, hepatocytes, mammary epithelium, vascular endothelial cells, melanocytes, glial cells, and the corresponding malignant cell types.

Regulation of SF Production

The complexity of the regulatory mechanisms for SF production is becoming increasingly apparent as the list of known and partially characterized factors that regulate SF production continues to grow. In addition to well-known pro-inflammatory (IL-1α, IL-1β, TNF-α) or anti-inflammatory (TGF-β) cytokines that enhance or inhibit production of SF by fibroblasts,

a group of partially characterized scatter factor–inducing factors distinct from IL-1 and TNF stimulate SF expression in fibroblasts and other SF-producer cell types. SF-inducing factors are secreted by various carcinoma cell lines, and they appear in the serum of rats following a subtotal hepatectomy. When co-cultured with epithelial cells, fibroblasts cease to express SF mRNA and protein, again by a regulatory mechanism that has not been elucidated. Heparin and heparan sulfate proteoglycans, which are known to bind to SF, also appear to stimulate its production. However, these molecules may simply function to stabilize the SF protein and to prevent its degradation.

Biologic Responses Induced by SF and Their Regulation

The major biologic responses induced by SF fall into four broad categories:

- Motility
- Proliferation
- Morphogenesis
- Cell survival (or more properly, protection against apoptotic cell death)

The c-Met receptor can transduce each of these biologic functions. These biologic responses may overlap (e.g., morphogenesis involves a component of cell migration through extracellular matrix); and they appear to be determined by the extracellular environment and by cell-specific programs of differentiation. For example, Madin–Darby canine kidney (MDCK) epithelial cells cultured on flat surfaces are scattered, while cells cultured in collagen gels respond to SF by forming networks of branching tubules similar to those found in the kidney.

Activation of specific pathways for motility, proliferation, morphogenesis, and/or cell survival may be determined at the receptor level or more distally. The specific pathways that activate each of these processes are only now beginning to be dissected. For example, recent studies suggest that treatment of various epithelial and cancer cell types with SF induces resistance to DNA-damaging drugs and radiation by a process that involves the sequential activation of c-Met, phosphatidyl-inositol-3´-kinase, c-► Akt (protein kinase B). The latter is a serine/threonine kinase that functions to protect cells against apoptotic death.

The extracellular environment plays a major role in modulating the biologic responses to SF. Studies of SF-induced branching morphogenesis of MDCK epithelial cells provide clues as to how this modulation might occur. Thus, certain extracellular matrix molecules promote forward extension of tubules (collagen I, laminin), while others promote branching (heparan sulfate proteoglycans, collagen IV). TGF-β, a component of the extracellular matrix, inhibits the entire process of branching morphogenesis. The binding of matrix proteins to integrins activates intracellular signaling processes, including tyrosine phosphorylation; and SF may induce the expression of a specific set of integrins that allows the extracellular matrix to modulate intracellular signaling. There is evidence that the extracellular matrix may modulate c-Met signaling by inducing the phosphorylation and dephosphorylation of different sites on c-Met and other signaling proteins.

SF and c-Met Participate in Various Physiologic and Pathologic Processes

Development

An important role for SF in development was suggested by the finding that homozygous deletion of either SF or c-Met results in embryonic lethality in mice. Various studies implicate SF as a mediator of mesenchymal: epithelial signaling during embryogenesis. For example, during mouse development, the SF gene is expressed in mesenchymal cells, while the c-Met gene is expressed in adjacent epithelia. This pattern is observed in multiple developing organs, and appears to be regulated with great precision in space and time. Injection of SF into the developing chick embryo induces abnormalities of the neuraxis, indicating that inappropriate exposure to SF can interfere with normal development. In addition, several studies suggest that SF and c-Met can mediate the conversion of mesenchymal cells to an epithelial phenotype, as judged by its ability to induce morphologic alterations as well as the expression of epithelial-specific markers (e.g., cytokeratins and epithelial-specific junctional proteins). Mesenchymal: epithelial interconversion is commonly observed during embryogenesis, further supporting a role for the SF-c-Met ligand-receptor pair in development.

Oncogenesis

Malignant cell transformation is mediated by the Tpr-Met oncogene, which encodes a truncated and constitutively active form of the c-Met receptor. This finding raises the possibility that SF-mediated overstimulation

of c-Met has similar consequences. The idea that SF could mediate tumorigenesis in vivo is suggested by several considerations. First, SF stimulates the motility, and invasiveness of a variety of carcinoma cell types in vitro. Secondly, SF is a potent inducer of angiogenesis (new blood vessel formation), a process considered to be essential for the continued growth of solid tumors. Finally, SF can overcome apoptosis (programmed cell death) of epithelial cells which is associated with detachment of cells from their substratum. Detachment of carcinoma cells from the underlying basement membrane is an early step in tumor invasion. Studies of experimental animal models and human clinical samples further support a role for SF in tumorigenesis. Overexpression of the SF and/or c-Met genes in a variety of cell types induces or further enhances the tumorigenic phenotype in vivo, by ▶ autocrine and/or ▶ paracrine mechanisms. In studies of human breast cancer, bladder cancer, gliomas, and other tumor types, significantly higher levels of SF and/or c-Met were observed in high-grade invasive cancers than in low-grade noninvasive cancers. And in a study of 258 primary invasive breast cancers, a high SF content in the tumor was strongly predictive of relapse and death. Finally, recent genetic-epidemiologic studies have strongly linked activating mutations of the c-Met gene to a specific type of kidney cancer: hereditary papillary renal carcinoma.

Angiogenesis

The formation of new blood vessels from pre-existing vessels occurs extensively during normal development and tissue remodeling, but occurs only to a limited degree in normal adults. Physiologic ▶ angiogenesis in adults is observed transiently during wound healing, ovulation, and placental implantation. However, persistent and inappropriate angiogenesis contributes to certain pathologic processes, including chronic inflammatory diseases (e.g., rheumatoid arthritis) and cancer. SF induces an angiogenic phenotype in cultured vascular endothelial cells (i.e., stimulates endothelial cell proliferation, ▶ Chemotactic ▶ Migration, and capillary-like tube formation) and induces angiogenesis in vivo in several different experimental animal models. SF may contribute to angiogenesis in AIDS-related ▶ Kaposi sarcoma, a cytokine-dependent neoplasm associated with extensive endothelial cell proliferation, and neovascularization. The observations that both SF content and tumor angiogenesis are powerful independent prognostic indicators for breast cancer suggest a role for SF as a tumor angiogenesis factor. However, a causal relationship between SF and tumor angiogenesis is not yet proven.

Clinical Relevance

The ability of SF (HGF) is to stimulate epithelial cell growth and morphogenesis, to induce angiogenesis, and to protect cells against toxins or environmental conditions that induce apoptosis suggests a variety of potential therapeutic applications for SF. In this regard, there are a number of experimental animal (mouse and rat) studies suggesting that administration of the SF protein can block or ameliorate acute and chronic injury to the liver, kidney, or lung. For example, administration of SF prevents or reduces the loss of renal function caused by toxins such as HgCl2 or ▶ cisplatin in mice. In a rat model, infusion of SF blocked or slowed the development of liver fibrosis and cirrhosis; and SF blocked the development of pulmonary fibrosis induced by bleomycin in the mouse lung. These findings suggest that SF is potentially clinically useful as a hepatotrophic factor for repair of liver damage or as a renotrophic factor for repair of kidney damage. The use of SF in humans presents significant challenges, such as the delivery of sufficient quantities of the factor to the sites where it is needed, in view of its short biologic half-life. Nevertheless, if reliable methods of protein or gene delivery can be developed, there may be a variety of clinical applications for SF to promote organ repair and regeneration.

Several studies suggest that the administration of other angiogenic factors (▶ VEGF and basic ▶ FGF) is potentially useful in restoring the blood supply and preventing tissue damage, in animal models in which coronary or peripheral blood vessels are ligated in order to produce acute ischemic injury. Because of its ability to induce angiogenesis as well as its ability to protect a variety of different cell types against apoptotic cell death, it is anticipated that SF may be particularly advantageous in these settings. There is also the potential for development of small molecule inhibitors of the c-Met receptor that could be used to treat pathologic processes driven by excessive production of SF, such as certain cancers. Such inhibitors have already been developed to inhibit the function of the ▶ EGF receptor. A criticism of this approach is that tumor

growth is driven by a variety of cytokines, growth factors, and angiogenic factors, so that the specific inhibition of a single receptor type will be insufficient to halt tumor growth. Nonetheless, combinations of receptor inhibitors may be clinically useful, and there may be situations in which inhibition of a single receptor is sufficient to inhibit tumor growth due to synergistic interactions among growth factors and cytokines.

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Scavenge Free Radicals

Definition

Work by reducing the action of free radicals and thus preventing cell damage by compounds like polyphenols.

► Polyphenols

Scavenger Cells

Definition

Any of a diverse group of cells that have the capacity to engulf and destroy foreign material, dead tissues, or other cells.

SCF

Definition

Skp1/Culin/F-box protein is a multi subunit complex that ubiquitylates proteins that are phosphorylated at specific sequences known as phosphodegrons. This ► ubiquitination targets proteins to degradation.

► Anoxia and Cancer
► Kit/Stem Cell Factor Receptor in Oncogenesis

SCF-R

► Kit/Stem Cell Factor Receptor in Oncogenesis

ScFv

Definition

Single chain fragment of variable region antibody composed of the V_H and V_L immunoglobulin regions joined via a flexible peptide linker, e.g., $(Gly_4Ser)_3$ peptide.

► Chimeric T Cell Receptors

SCGE

► Single Cell Gel Electrophoresis Assay

SCH52365

► Temozolomide

Schiller-Duval Bodies

Definition

Microscopic papillary structures within a tumor, consisting of a central blood vessel surrounded by

malignant cells. They are a characteristic feature of yolk sac tumors and help differentiate this entity from morphologically similar neoplasms, e.g., clear cell carcinomas.

Schirrous (archaic)

► Desmoplasia

Schistosomas Hematobium

Definition

A trematode species from freshwater snails whose cercaria imbed in human organs such as the urinary bladder, causing chronic inflammation and eventually cancer.

► Urothelial Carcinoma

Schwannoma-derived Growth Factor

► Amphiregulin

Schwannomin

► Merlin

SCID

Definition

Severe combine immunodeficiency disease.

► Childhood Cancer

Scid Mice

Definition

Severe combined immunodeficiency; a mutant strain of mice that has a mutation in DNA-dependent protein kinase gene. Due to the mutation, scid mice show the defective DNA rearrangement of antigen receptor genes and thus develop no functional ► T cells and ► B cells.

► Rap1 and Sipa-1

SCID Mouse Model

Definition

Severe combined immunodeficiency mice, due to a genetic autosomal recessive mutation (SCID), have no functional specific humoral and cellular immune system. Therefore, these animals serve as hosts for many xenograft tumor models.

Scintigraphy

Definition

An imaging modality that involves injection and detection of radioactive substances. A nuclear medicine technique based on the administration of radioactive that are selectively taken-up by specific cells or organs thus giving a two-dimensional imaging based on radiopharmaceutical distribution.

SCLC

Definition

► Lung Cancer
► Small Cell Lung Carcinoma

Sclerosing Angiogenic Tumor

► Hepatic Epithelioid Hemangioendothelioma

Sclerosing Endothelial Tumor

▶ Hepatic Epithelioid Hemangioendothelioma

Sclerosing Epithelioid Angiosarcoma

▶ Hepatic Epithelioid Hemangioendothelioma

Sclerosing Interstitial Vascular Sarcoma

▶ Hepatic Epithelioid Hemangioendothelioma

Screening Toxicity

Definition

Represent informal toxicity studies, usually in very limited numbers of small rodents, often mice, to test small amounts of novel chemical agents to provide an approximate idea of their toxicity and their appropriateness for attempting to develop as a new drug. They may also be used to screen series of chemicals with a view of selecting the least toxic.

▶ Preclinical Testing

SDF-1

Definition

The ▶ chemokine SDF-1 has been isolated from stromal fibroblasts and interacts as a ligand with the ▶ G-protein coupled receptor CXCR4.

▶ Trefoil Factors

SDF-1α

▶ Chemokine Receptor CXCR4

SDGF

▶ Amphiregulin

Sdi1

▶ P21

SEA Domain

Definition

Acronym for *S*ea urchin sperm protein, *E*nterokinase, *A*grin; is an extracellular domain of a number of proteins and associated with ▶ glycosylation. The common module might regulate or assist binding to neighbouring carbohydrate moieties.

Seckel Syndrome

Definition

Is an autosomal recessive disorder characterized by dwarfism, intrauterine growth retardation, bird-like facies, microcephaly, and mental retardation. ATR-Seckel Syndrome has been found in patients with mutations in ▶ ATR. Cell lines from patients with Seckel syndrome, who are normal for ATR, show defective ATR signaling, suggesting that Seckel syndrome can be caused by mutations in other components of the ATR pathway.

▶ S-Phase Damage-Sensing Checkpoints

Second Malignant Neoplasm

Synonyms

SMN

▶ Second Primary Tumors

Second Messenger

Definition

Refers to chemical signals created within a cell in response to a hormonal stimulus from outside. Common second messengers are calcium ions, cyclic AMP, or inositol triphosphate. Second messengers act in a cell to orchestrate metabolic or gene expression responses to a particular stimulus.

► Relaxin

Second Primary Cancers

► Radiation-Induced Sarcomas After Radiotherapy
► Second Primary Tumors

Second Primary Malignancy

Synonyms

SPM

► Second Primary Tumors

Second Primary Tumors

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Synonyms

Second malignant neoplasm SMN; Second primary cancer SPC; Second primary malignancy SPM

Definition

A *second primary tumor*, usually malignant, is defined as a histologically and/or clonally distinct tumor diagnosis that develops after the first cancer.

Introduction

Advances in the management of cancer have substantially improved the prognosis of cancer patients, including their life expectancy. Currently, more than 60% of cancer patients can survive over 5 years posttreatment; cancer survivors constitute 3.5% of those living in the West. However, advances in cancer management are not without adverse consequences, and long-term complications following successful treatment have become increasingly important. Second primary tumor is one of the most devastating issues facing long-term cancer survivors.

Second or higher order primary tumors account for ~6–10% of all cancer diagnoses, and are the fifth most commonly diagnosed cancer in Western countries. For instance, ~100,000 of the 1.4 million new cancers diagnosed in 2006 in the United States are second cancers. Although the aim of the management of second primary tumors assimilates that of the initially diagnosed malignancies, the diagnosis and treatment of SPTs are far more complex, especially when the effects resulting from previous therapy limit such management.

Causal and Risk Factors

Patients with one form of malignancy are known to have a higher risk of developing a second cancer.

The underlying causes and risk determinants are multifactorial; however, the majorities are related to shared etiology such as immunodeficiency, cigarette smoking, and alcohol abuse. Secondly, cancer patients may possess a certain genetic predisposition to cancer. In other words, hereditary susceptibility can explain the development of some second primary cancers. Furthermore, cancer diagnosis and treatment modalities such as surgery, chemotherapy, and radiotherapy are all known carcinogenic factors. In addition, the incidence of second primary malignancy is significantly higher when the first cancer is diagnosed and treatment is delivered at early age. Survivors of ► childhood cancers and adulthood cancers have, when compared with the general population, an increased risk of 3–6 and 1.5–2 times, respectively of developing a second primary tumor. The reasons for this age effect are unknown, but might be related to a higher rate of cell proliferation, susceptibility of tissue to the

carcinogenic effect of treatment, and a longer period of follow-up.

Shared Etiologic Exposure

Most second primary tumor cases are associated with some forms of shared etiologic exposure. Cancer-inducing factors can be lifestyle- or work-related. For example, ▶ asbestos exposure is associated with ▶ lung cancer and mesothelioma, while endocrine and dietary factors are associated with the development of breast, endometrial, and ovarian cancers. Patients with acquired immunodeficiency syndrome (AIDS) or on immune suppression following an organ transplant are prone to develop ▶ non-Hodgkin lymphoma, cervical cancer, and certain forms for skin cancer, i.e., Kaposi sarcoma. Among all of the cancer-inducing risk factors, cigarette smoking is the most common and important. ▶ Tobacco related cancers include non-small cell and small cell lung cancers, ▶ squamous cell carcinomas of head and neck area, pancreatic cancer, ▶ bladder cancer, kidney cancer, and cancer of uterine cervix.

It has been well demonstrated that patients who have been successfully treated for squamous cell cancer of head and neck area have an increased risk of 20% in 5 years of developing a second primary cancer in the upper aerodigestive track. Studies have also demonstrated that lung cancer patients with an extended history of tobacco use are at increased risk of a second lung carcinoma, cancers of the larynx/hypopharynx, bladder, and pancreas. This indicates that tobacco use is a common causative factor.

Excessive alcohol consumption is related to increased incidence of ▶ esophageal cancer, liver cancer, and squamous cell carcinomas of head and neck areas. Recent clinical studies have shown that alcohol may serve as a risk factor for adenocarcinoma of the breast and ▶ colon. In addition, synergistic effects exist for second malignancies of the upper aerodigestive track for cigarette smoking and alcohol use.

The underlying mechanism of the development of second or higher order primary tumors due to exposure to a shared etiology is not well understood. It has been suggested that repeated carcinogenic exposure may cause "field cancerization," where the mucosa accumulates genetic alterations resulting in the induction of multiple, independent malignant lesions. This theory is now widely accepted and supported by research results in molecular biology.

Second Primary Tumors. Table 1 Cancer syndromes and their associated malignancies

Cancer syndrome	Affected gene(s)	Associated malignancies
Li–Fraumeni syndrome	▶ p53	Breast, soft-tissue, bone (osteosarcoma), brain, adrenal gland, hematological (leukemia), etc.
Hereditary non-polyposis colorectal cancer (HNPCC)	MLH1, MSH2, MSH6, CHEK2	Colon, small intestine, endometrium, ovary, stomach, kidney, ureter, etc.
Ataxia-telangiectasia	ATM	Breast
Fanconi anemia	FANC A-G, L	Breast, ovary, head and neck, cervix, esophagus, liver, brain, etc.
Retinoblastoma (RB)	RB-1	Eye, orbit, bone (osteosarcoma), soft-tissue, melanoma, brain, etc.

Genetic Predisposition

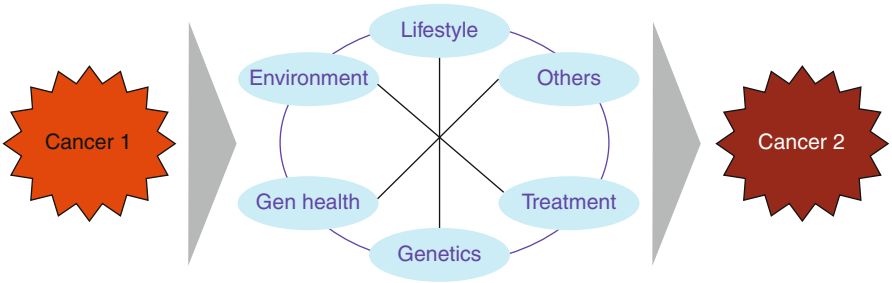
Some types of cancers are related to hereditary genetic abnormalities, and cancer syndromes are most recognizable in familial settings. Numerous cancer syndromes and their related genetic alterations have been identified. Table 1 illustrates several more commonly diagnosed cancer syndromes, the related genetic alterations, and associated malignancies.

With the development of molecular testing technology, more genetic alterations associated with increased risk of cancer or familial cancer syndrome will be discovered.

Therapy-Related Risk Factors

As the survival of cancer patients improves following treatment, identification of long-term treatment-related complications becomes critical. Treatment-related second primary tumor is one of the most devastating side effects. Commonly utilized cancer treatment modalities including surgery, chemotherapy, and radiotherapy are all known to cause cancer. Hence, some second primary tumors are iatrogenic.

Although uncommon, cancer surgery is related to some kinds of second primary cancers. For example, radical mastectomy has been the standard primary treatment of locally advanced invasive ▶ breast cancer, one of the most commonly diagnosed cancers in female patients. Surgical manipulation of the axilla in order to access the axillary lymph node during mastectomy, a necessary surgical procedure, is related to the



Second Primary Tumors.
Fig. 1 Risk factors for second or higher order primary tumors

swelling of the ipsilateral arm due to lymph fluid accumulation (i.e., lymphedema) secondary to the damage to the lymph vessels. The incidence of this complication increases with the addition of radiotherapy. Arm lymphedema has been recognized as the causative factor of angiosarcoma of the affected arm or forearm, known as Stewart-Treves syndrome, named after two American physicians who initially described the cause–effect relation. Improvement in the surgical techniques involved in breast cancer treatment, including the use of lumpectomy with more limited axillar surgery and sentinel node dissection, has significantly reduced the occurrence of angiosarcoma. Some forms of colon and stomach cancer have also been described as surgery-induced cancers. However, second primary cancer is not being considered as a substantial long-term clinical complication of cancer surgery.

Unlike surgery, chemotherapy and radiotherapy are more likely to induce iatrogenic malignancies. Second primary tumors caused by chemotherapy and radiotherapy usually develop after a lengthy latent posttreatment period. This delay, which can take years, can be partly due to the time needed for the ▶ DNA damage that is responsible for malignant transformation of cells to develop.

The carcinogenic effect of numerous chemotherapeutic agents has been repeatedly illustrated. The significance of chemotherapy in the development of second primary cancer was first studied in detail in a group of patients who were successfully treated for ▶ Hodgkin disease (HD). Researchers discovered that the cumulative risk of second primary cancer diagnosis following HD treatment during childhood was 7% at 15 years after diagnosis; the two common diagnoses of second cancer are leukemia (2.8%) and non-Hodgkin's lymphoma (1.1%). Further investigations revealed that certain agents used for HD chemotherapy, such as ▶ alkylating agents, were directly responsible for the increased incidence of leukemia in that group of patients. Other studies aimed at related chemotherapy drugs and cancer showed that many other agents such as ▶ cisplatin or cyclophosphamide are cancer-causing to various degrees.

A radiotherapy-induced primary second cancer is suggested when, after a long latency period of a minimum of 5 years but typically 15–20 years, a tumor with a histology type differs from the first tumor developing within (or close to) the irradiated field. Ionizing radiation can cause most types of cancer. The commonly known radiation-induced second malignancies include: bone or soft-tissue sarcoma, breast cancer, thyroid cancer, leukemia, and brain tumors. Some of the radiation-induced tumors, such as bone or soft-tissue sarcoma, can be more aggressive than their sporadic counterparts and respond poorly to conventional therapy.

The incidence of radiation-induced second primary tumor is related to the radiation dose exposure, but reversely related to the age of radiation exposure, i.e., the risk is higher among young irradiated patients. The underlying mechanism of this phenomenon is unknown, but it is suggested that younger patients have a larger number of dividing stem cells that are more susceptible to radiation-induced malignant transformation.

These risk factors are all associated with second or higher order primary tumors; the mechanisms of SPT development are more likely caused by more than one factor. In other words, many influences and the interactions between these influences may contribute to the development of the second primary malignancies. For example, patients with hereditary retinoblastoma, who have undergone radiation therapy, when compared to sporadic RB cases, are more susceptible to second primary cancer(s) in the irradiated field. Figure 1 illustrates the multifactorial nature of the risk factors contributing to second primary tumors.

It is important to emphasize that although surgery, chemotherapy, and radiation are related to second malignant neoplasm development, the benefits of these treatments usually surpass this complication. Therefore, second primary tumor is rarely a contraindicatory factor associated with these therapeutic modalities.

Diagnosis

Since long-term follow-up is required for most successfully treated cancer patients, the majority of second primary tumors are diagnosed during routine posttreatment follow-ups.

The diagnostic procedures utilized for second primary cancer diagnoses are no different from those used for primary malignancies. Usually, a careful history and physical examination, together with pertinent laboratory and imaging tests are required for the diagnosis of any neoplasm, followed by pathological confirmation. The diagnostic process of any second primary cancer is similar; however, as local and/or distant recurrences are common following cancer treatment, especially those with more advanced disease, differentiation between a second malignant neoplasm with local relapse or distant metastasis is crucial, as the treatments of the two entities differ significantly.

The diagnosis of a second primary cancer can be confirmed if the newly discovered malignancy is histologically different from the previous diagnosis. When features of the tumor and/or cells appear similar under the microscope, a second primary malignancy is likely when premalignant changes such as carcinoma in situ are found within or near the specimen, as these premalignant changes indicate a de novo development process of a malignancy.

However, when the morphologies of the two diseases are identical under the microscope, and features that may suggest a second primary disease are lacking, further investigation with more advanced diagnostic techniques such as molecular diagnostics will be required in order to determine the difference. It is well accepted that all cancers are initiated from a single clone of transformed cell; therefore, pathological test results that can rule out monoclonal nature from the previous cancer diagnosis will be required for a diagnosis of cancer recurrence.

Treatment

The pathological diagnosis, patient's health status and preference, as well as available medical resources determine the management strategy of any type of malignancy. However, although the aim and principle of second primary tumor treatment assimilate those of the original disease, treatment of a SPT is complicated and usually restricted by previous therapy, and any associated complications.

Whenever possible, curative treatment should be considered for second primary tumors. Like their original counterparts, treatment modalities utilized for second primary cancer can include surgery, chemotherapy, radiation therapy, and/or other less commonly used methods such as immunotherapy, hormonal therapy, and molecular targeted therapy.

Usually, surgery and medical therapy can be repeatedly utilized as long as the patient's overall health condition, performance status, and functionality allows. However, if the location of the SPT is within the previously irradiated field, the utilization of radiation therapy, especially external beam photon therapy, is stringently restricted by previous radiation dose, the tolerance dose of irradiated tissues and/or organs, as well as the period between the original and subsequent radiotherapy. The dose limitation of a specific tissue and organ is commonly described as TD 5/5 (the total dose equivalent that may cause severe long-term complications with 5% probability within 5 years), and any treatment dose equivalent above such limitation may cause a high incidence of substantial toxicities. Unfortunately, curative treatment for a second primary tumor usually necessitates high dose radiation that exceeds TD 5/5. Hence, patients should be provided with and fully understand the consequence of such treatment prior to re-irradiation.

The success of second primary tumor treatment depends on ▶ early detection. In a certain sense, early diagnosis is more critical for a second primary tumor because under many circumstances only limited treatment can be utilized due to limitations set by any previous cancer therapy. Therefore, one cannot overemphasize the importance of close follow-up after any cancer treatment.

Prevention

The development of any effective prevention methods of second primary cancers inevitably needs a thorough

understanding of the mechanisms of its development. As multiple risks usually exist for the development of a second primary tumor, and the incidence of a particular type of second primary cancer is relatively low, there is currently no effective preventive approach to the development of second primary cancer. Nevertheless, some confirmed common etiologies of cancer development exist. For example, cigarette smoking has been proven as a strong risk factor of second primary cancers of aerodigestive track for patients with lung cancer or squamous cell carcinoma of the head and neck areas. Studies have shown that after successful treatment of their initial malignancies, those patients that stop smoking can reduce the relative risk of developing a second primary cancer. Therefore, while additional information and knowledge are needed in order to develop more effective prevention strategies for second primary tumors, termination of exposure to known risk factors such as tobacco and alcohol serve as the most effective preventative modality of second primary tumors.

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Secondary Amenorrhea

Definition

Absence of menstruation for >3 months.

► Menopausal Symptoms After Breast Cancer Therapy

Secondary Cancer

► Second Primary Tumors

Secondary Cancer Prevention

Definition

Prevention of tumor progression through early diagnosis of asymptomatic tumors and/or treatments against preneoplastic or early neoplastic lesions.

► Immunoprevention of Cancer

Secondary Cancer Site

Definition

The discontinuous end location where a metastatic cancer cell grows after it spreads from its primary site. For metastatic breast cancer to the bones, the secondary site is the bones.

► Metastatic Colonization

Secondary Cancers

Definition

► Second Primary Tumors.

Secondary Dissemination

Definition

Dissemination following initial treatment.

► Leptomeningeal Dissemination

Secondary Hypogonadism

Definition

The failure of testicular or ovarian function caused by decreased gonadal stimulation from the pituitary/hypothalamic unit (as opposed to primary failure of the gonad itself).

► Prolactin

Secondary Lymphedema

Definition

A condition in which the lymphatic system is physically damaged by surgery (such as to remove lymph nodes) or injury, resulting in interrupted flow of lymph and blocked drainage of fluid from tissues, skin thickening and adipose tissue accumulation.

► Lymphangiogenesis

Secondary Lymphoid Organs

Definition

Secondary (or "peripheral") lymphoid organs include the lymph nodes, spleen, and mucosa-associated lymphoid tissue (MALT). The secondary lymphoid structures function to survey all entering or circulating antigen and to mobilize an immune response against antigen upon its discovery. This is in contrast to the primary (or "central") lymphoid organs. These are the sites where the cells of the immune system are produced including the bone marrow and the thymus required for T cell maturation.

► DNA Vaccination

Secondary Metabolites

► Natural Products

Secondary Structure

Definition

The linear arrangement or topology of α-helices and β-sheets with respect to the amino acid sequence.

► Structural Biology

Secondary Tumor

► Metastasis

Secondhand Tobacco Smoke

Definition

A composite of sidestream smoke and the smoke exhaled by a smoker. Sidestream smoke is the material released into the air from the burning tip of the cigarette plus the material which diffuses through the paper.

► Tobacco Carcinogenesis

Second-Tier/Third-Tier Lymph Node

Definition

After passing through a sentinel node, lymph fluid moves subsequently to second-tier and third-tier nodes.

► Sentinel Lymph Nodes

Secosteroid

Definition

Steroid molecule with a broken B-ring (C9 and C10).

► Vitamin D

Secretagogues

► Gut Peptides

γ-Secretase

Definition

A protease that is responsible for the processing of the cytoplasmic domains of several type I membrane proteins, such as the amyloid beta-protein precursor (in Alzheimer disease), Notch, and CD44. The cytoplasmic regions are translocated to the nucleus where they function as transcription factors.

► CD44

Secreted Phosphoprotein 1

► Osteopontin

Secreted Protein Acidic and Rich in Cysteine

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Synonyms

BM-40; Osteonectin; SPARC

Definition

Secreted Protein Acidic and Rich in Cysteine (SPARC) belongs to a group of non-structural proteins of the extracellular matrix (ECM), termed ► matricellular proteins, which modulate interactions between cells and their environment. It is expressed in developing and remodeling tissues, and regulates cell attachment, deposition of ECM, matrix mineralization, and ► angiogenesis.

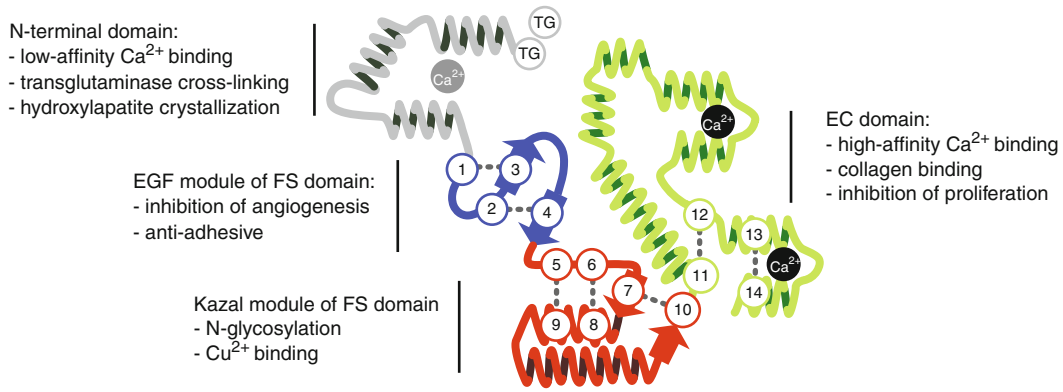
Characteristics

SPARC is a counter-adhesive protein that induces cell rounding, inhibits cell spreading, and mediates focal adhesion disassembly and the reorganization of actin stress fibers. In several cell types, it delays cell cycle in G_1 phase. It is the main non-collagenous component of bone, where binding of SPARC to collagen can induce deposition of calcium. However, inhibition of ► hydroxylapatite crystallization suggests that SPARC prevents matrix mineralization rather than inducing it. SPARC also regulates the production, assembly, and organization of ECM, and accordingly, it is expressed at high levels in developing tissues during embryogenesis and in remodeling adult tissues, such as gut, ovary, testis, mammary gland, bone, and in healing wounds. SPARC is predominantly secreted by non-epithelial cells including endothelial and smooth muscle cells, osteoblasts, and platelets. Fibroblasts and ► macrophages express SPARC in healing wounds, where it is also released by platelet degranulation.

Gene Organization

The SPARC gene spans 25.9 kb on human chromosome 5q31.3–q32. Its first non-coding exon is separated from the following coding exons by a large 10.6 kb intron. Exon 10 contains the entire 3′ non-translated region. This gene organization and the transcription start site are highly conserved in vertebrates. The major human 2.2 kb transcript contains an open reading frame from nucleotides 84–992, followed by 1,137 bp of 3′ non-translated region. The less abundant 3.0 kb transcript has an identical coding region, but utilizes a downstream polyadenylation signal. The SPARC promoter lacks TATA and CAAT boxes, but contains a GGA box 1 between nucleotides −51 and −120 which drives transcription. A GGA box 2 is located between nucleotides −131 and −165. Both boxes are mainly composed of a repetitive GGA motif. They are positive regulators of promoter activity, while a 10 bp spacer of low purine content has a negative impact.

Three ► Retinoid X/► Vitamin D receptor binding sites, immediately followed by an ► E-box in GGA box 1, may be responsible for stimulation of SPARC



Secreted Protein Acidic and Rich in Cysteine. Fig. 1 Domain organization of SPARC. Schematic representation of the structure of each SPARC domain. All domains and modules are shown in individual colors. Ca^{2+} ions bound with high and low affinity are respectively in *black* and *gray*. The numbers in the *circles* represent cysteines, and TG denotes transglutaminase cross-linking acceptor residues. The N-glycosylation site is located immediately after cysteine 7

expression by ▶ retinoic acid and dexamethasone. Histone acetylation is essential for the activation of transcription by retinoid receptors. Sodium butyrate, an inhibitor of ▶ histone deacetylase, has been shown to stimulate SPARC expression. The activation of SPARC transcription by cAMP is consistent with several cAMP consensus sequences found in the promoter region and in the first intron. The presence of a heat shock element correlates with heat- and stress-induction of SPARC expression. Jun and ATF family members repress SPARC transcription in fibroblasts, but stimulate expression in epithelial cells, although this regulation appears to be indirect.

Protein Structure

The structure of SPARC is highly conserved among different species. All cysteine residues are found at identical positions from human to nematode, indicating that protein folding is critical for function. After the 17 amino acid signaling peptide is removed, the human protein is secreted as a polypeptide of 286 residues with a calculated mass of 32 kDa, which migrates at 40–43 kDa on SDS-PAGE due to ▶ glycosylation and possibly other posttranslational modifications.

The protein is divided into three distinct domains (Fig. 1). The loosely structured N-terminal acidic domain (Ala^{1}-Glu^{52}) has an α-helical character, which depends on binding several Ca^{2+} molecules with low affinity. Clusters of glutamic acid in this region resemble the γ-carboxyglutamic acid (Gla) domain of vitamin K–dependent proteins of the blood clotting system. Gln^{3} and Gln^{4} are the amine acceptor residues for tissue transglutaminase-catalyzed cross-linking, of which SPARC is the major substrate in maturing cartilage. This domain also mediates the interaction with hydroxylapatite, indicating that it may play role in stabilization of connective tissue. A peptide from this domain inhibits endothelial cell spreading.

The follistatin-like (FS) domain (Asn^{53}-Pro^{137}) consists of two loosely linked modules. The N-terminal epidermal growth factor–like (EGF) module is a highly twisted β-hairpin with two disulfide bonds. Peptides from the EGF module have anti ▶ angiogenic properties. They inhibit endothelial cell ▶ migration and proliferation, and promote the disassembly of focal adhesions of endothelial cells. An adjacent small hydrophobic core of mixed α/β structures stabilized by three disulfide bonds has high structural homology to the serine proteases of the Kazal family. A peptide from the Kazal module, which contains the copper-binding sequence KGHK, can stimulate angiogenesis. This module carries an N-linked carbohydrate at Asn^{99}, which causes a ~2 kDa shift in electrophoretic mobility. SPARC glycosylation is tissue-specific and has functional significance. Bone SPARC, which carries high-mannose and biantennary glycans, binds collagens with high affinity. Platelet and recombinant SPARC have bi- and triantennary structures and bind collagens with low affinity. SPARC glycosylation is apparently sensitive to neoplastic transformation, as tumor-produced SPARC has a unique hybrid pattern of glycosylation.

The C-terminal extracellular ▶ calcium-binding (EC) domain (Cys^{138}-Ile^{286}) folds into a compact globular and predominantly α-helical structure. It carries a canonical pair of ▶ EF-hand motifs, which bind Ca^{2+} with high affinity. A peptide corresponding to the second EF-hand motif inhibits endothelial cell proliferation. A long α-helix at the N-terminus of the EC domain and a short loop, which connects two EF-hand motifs, form a collagen-binding site.

This domain organization is shared by a family of proteins that have common FS/EC domain pairs, but different acidic N-termini and sometimes C-termini. Other members of the family include hevin, tsc36, testicans, and SMOCs.

Functional Properties

SPARC binds and modulates the activities of structural and soluble components of the ECM, often in a Ca^{2+}-dependent manner. Ca^{2+} enhances binding to collagens, but inhibits interaction with multimeric vitronectin. In the α-granules, the major storage organelle for platelet-secreted proteins, SPARC binds to ▶ thrombospondin with high affinity in the presence of Ca^{2+}.

The action of various growth factors is modulated by SPARC. SPARC co-localizes with PDGF in platelet α-granules and binds with high affinity to PDGF-AB and PDGF-BB, but not PDGF-AA. This interaction results in interference of PDGF binding to its receptor on fibroblasts and inhibition of PDGF-stimulated proliferation of human vascular smooth muscle and mesangial cells. SPARC has also been shown to bind ▶ VEGF and antagonize its pro-angiogenic effect on endothelial cells. Although SPARC does not directly bind ▶ bFGF, it antagonizes its effect on the proliferation and migration of endothelial cells. SPARC can also suppress ligand-induced autophosphorylation of the bFGF receptor and inhibit both bFGF- and VEGF-induced ERK activation in endothelial cells.

▶ TGFβ and SPARC induce one another's expression in a reciprocal manner. In mesangial cells, SPARC binds the TGFβ/TGFβRII complex. Accordingly, in these cells, treatment with SPARC has no effect, but in combination with TGFβ it causes stimulation of ▶ SMAD phosphorylation, ▶ JNK activation, and an increase in total and phosphorylated c-jun. Treatment with SPARC stimulates SMAD phosphorylation in TGFβ-responsive epithelial and endothelial cells, but inhibits TGFβ-induced activation in fibroblasts.

Integrin-linked kinase (ILK) was identified as a SPARC binding partner. SPARC can induce Ser^{473} phosphorylation of ▶ AKT through ILK and ▶ Focal Adhesion Kinase (FAK) in glioma cells. In contrast, in ▶ ovarian cancer, SPARC significantly suppresses activation of AKT and ERK signaling.

A scavenger receptor, stabilin-1, was identified as another SPARC binding partner. Binding results in receptor-mediated ▶ endocytosis of SPARC, followed by its targeting for degradation in macrophages.

Animal Models

In the nematode *C. elegans*, SPARC is expressed in muscle cells along the body wall and in the sex muscle, with no evidence of expression in other cell types. SPARC overexpression leads to the *Unc* phenotype consisting of a lack of coordinated movement or paralysis, accompanied by frequent disorganization of gonad and vulval protrusions. The offspring embryos are deformed and not viable.

During *Xenopus laevis* development, SPARC is expressed in the notochord and mesoderm prior to appearance of the first somites, and later in the neural tube. Disruption of normal SPARC function causes a broad spectrum of developmental abnormalities including embryonic axes deformities associated with disorganized myotomes, lack of intersomitic boundaries, and defects in eye development.

SPARC knockout mice do not have significant developmental abnormalities, but display multiple defects associated with abnormal ECM deposition. Animals develop early-onset cataracts accompanied by abnormal collagen IV deposition in the lens capsule. A reduced number of osteoblasts and osteoclasts leads to decreased bone remodeling and profound osteopenia. Animals have an enlargement of fat pads due to an increase in the number and diameter of adipocytes. The accumulation of adipose tissue compensates for the body weight loss caused by osteopenia. The skin of adult transgenic mice has decreased tensile strength and reduced collagen content with smaller diameter collagen fibrils compared to wild-type mice. SPARC-null mice also show significant acceleration of wound healing in vivo.

SPARC in Cancer

The role of SPARC in tumorigenesis is complex and appears to be cell-type-specific due to its diverse function in a given ▶ microenvironment. In some types of

cancer, high levels of SPARC expression have been shown to correlate with disease progression and poor prognosis. In ▶ melanoma cells, high levels of SPARC expression induce ▶ epithelial to mesenchymal transition (EMT), and increase ▶ invasion and tumor progression. High levels of SPARC are also associated with invasive meningioma and ▶ osteosarcoma. In glioma, SPARC promotes invasion, but delays tumor growth.

In other types of cancer SPARC functions as a tumor suppressor. It inhibits the proliferation of ▶ breast cancer cells and induces apoptosis in ovarian cancer cells. In the majority of primary lung adenocarcinomas, SPARC is silenced by ▶ methylation, and this epigenetic aberration is associated with poor outcome. In ▶ non-small cell lung cancer, SPARC is not ▶ methylated, but its expression is frequently downregulated due to methylation of the recently identified ▶ tumor suppressor gene RASSF1A. Similarly, in breast and ▶ prostate cancers and ▶ neuroblastoma, the majority of neoplastic cells do not express SPARC. In neuroblastoma, high levels of SPARC are expressed in Schwannian stromal cells, which is associated with a favorable outcome.

Stroma-associated fibroblasts are commonly SPARC-positive, which in some types of cancer correlates with poor prognosis. However, other studies indicate that SPARC plays a role in creating a microenvironment that is inhibitory to tumor progression. SPARC has been characterized as a potent inhibitor of angiogenesis. It also induces the formation of tumor stroma and prevents the activation of fibroblasts. Enhanced growth of Lewis lung carcinoma and ▶ Pancreatic Cancer xenografts in SPARC-null mice is associated with altered production and organization of ECM within and surrounding the implanted tumors.

SPARC has also been found to have tumor suppressive activity in a number of animal studies. Significantly lower numbers of ▶ Metastases are seen following injection of ▶ adenoviral SPARC-infected breast cancer cells compared to controls. In SPARC-null mice, enhanced tumor growth and extensive dissemination have been reported following inoculation of ovarian cancer cells. Continuous infusion of SPARC into nude mice has also been shown to potently inhibit the growth of neuroblastoma xenografts. Recent studies have demonstrated that SPARC sensitizes ▶ colon carcinoma cells to ▶ radiotherapy and ▶ chemotherapy. In nude mice with xenografted colon carcinoma tumors, SPARC enhances the antitumor effects of cytotoxic agents, resulting in improved survival.

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Secretor Enzyme

Definition

A product of the Secretor gene, also named fucosyltransferase2 (FUT2), that catalyzes addition of fucose in α1,2 position onto type 1 chains. The name of the gene and enzyme stem from the presence of the gene product in secretions, namely in saliva.

▶ Lewis Antigens

Securin

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Synonyms

EAP1; hPTTG1; Human ESP1–associated protein 1; Human pituitary tumor-transforming gene 1; Pituitary tumor-transforming gene 1; PTTG1; Tumor-transforming 1; TUTR1

Definition

Securin is a 22 kDa protein that is crucial for the stability of the cells' genome. By preventing premature ▶ sister-chromatid separation during mitosis,

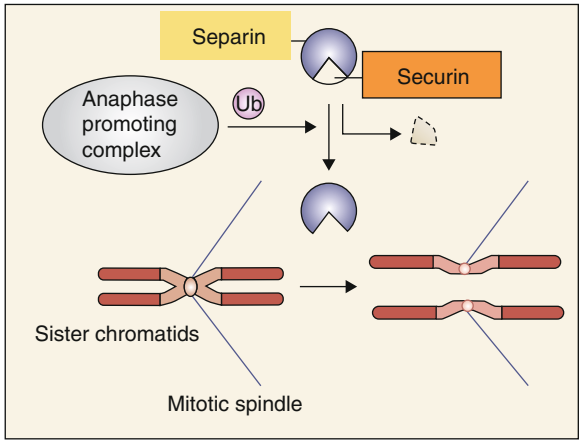
securin is involved in the regulation of accurate cell cycle progression.

Characteristics

The proto-oncogene *PTTG* was first isolated from rat pituitary tumor cells. The human homolog of *PTTG*, *securin* (hPTTG1), was found to be overexpressed in Jurkat T cells (human lymphoma T cell line) and in leukocytes from patients with different types of hematopoietic neoplasms or myelodysplastic syndromes. High levels of securin protein expression have been reported for various other tumors, including tumors of the pituitary gland, adrenal gland, kidney, endometrium, uterus, and ovary as well as esophageal and colorectal cancer. Hence, securin has been implicated in cell transformation and tumor development. In contrast, most normal adult tissues express little securin with the exception of tissues with highly proliferating cells, e.g., testis and thymus. Thus, the level of securin expression correlates with cell proliferation in normal tissue. In fact, the securin protein is expressed in a cell cycle–dependent manner.

Securin Function

The cell division cycle (▶ Cell Cycle Targets for Cancer Therapy) is a tightly regulated process to ensure the correct division of the genome into the daughter cells. Low securin expression is characteristic for noncycling cells and for cells at the beginning of the cell cycle (G1-phase). As the cell cycle proceeds, the securin level rises continuously, peaking in mitosis (M-phase). During M-phase the ▶ sister-chromatids are connected by cohesin proteins and aligned at the metaphase plate while being attached to the mitotic spindle. In this phase of the cell cycle, securin acts as sister-chromatid separation inhibitor by binding to separin (human ESP1), which is responsible for the destruction of the cohesin complexes (Fig. 1). In late M-phase, the metaphase–anaphase transition, securin is ubiquitylated (▶ Ubiquitination) by the activated ▶ anaphase-promoting complex or cyclosome (APC/C). This triggers securin destruction by the proteolysis machinery enabling separin activation and sister-chromatid separation, followed by cell division. In addition to regulating cell cycle progression, securin is involved in processes like DNA repair, ▶ apoptosis, angiogenesis, and tumor development.



Securin. Fig. 1 During M-phase of the cell cycle, chromosomes are aligned at the metaphase plate with the centromers linked to the mitotic spindle. The sister-chromatids are connected in the centromeric region by cohesin proteins. Securin binds to separin, thereby inhibiting the cohesin degradation activity of the protein. In late M-phase when all chromosomes are accurately aligned, the activated anaphase-promoting complex induces securin degradation by ubiquitylation (Ub). Activated separin cleaves the cohesin proteins enabling an even distribution of the sister-chromatids to the daughter cells (Adapted from Malumbres and Barbacid [5] with permission)

The multifunctional protein securin consists of 202 amino acids and contains, like many APC/C substrates (e.g., cyclins), a destruction box motif (D-box) within the N-terminus. The D-box is recognized by the APC/C complex at the end of metaphase leading to securin ubiquitylation and subsequent proteolysis. Furthermore, a central ▶ transactivation domain, a DNA-binding domain, and proline-rich motifs representing potential Src homology 3 domain (SH3)-binding sites (▶ SH2/SH3 Domains) have been identified in securin. Securin (hPTTG1) is the only member of the PTTG protein family that has been studied in detail. The protein family consists of at least three members that have no significant homology to other known proteins. The different expression pattern of the *hPTTG1*, *hPTTG2*, and *hPTTG3* genes in normal and tumor tissue may suggest a tissue-specific expression and diverse functions of the encoded proteins.

Securin Function in Tumor Development

Overexpression of *securin* can transform mouse and human cells (NIH3T3, HEK293), enabling them to form tumors in ▶ nude mice, thus specifying securin as an oncoprotein. Mutation of the proline-residues of the SH3-binding sites abrogates the transforming

(in vitro) and tumor-inducing (in vivo) activity. However, securin seems to be involved in tumor development in several ways, although the precise mechanisms remain unknown. First, as securin is essential for sister-chromatid separation during mitosis, it regulates cell proliferation and chromosome stability. Loss of securin function in tumor cells increases abnormal mitosis resulting in ▶ aneuploidy and apoptosis. The antiapoptotic function of securin is partly mediated by the interaction with the tumor suppressor protein p53 (▶ p53 Protein), blocking its DNA-binding and transactivating activity. Despite this effect, overexpression of securin can also cause aneuploidy by failure of the cells to divide the chromosomes evenly between the daughter cells. The induction of chromosomal instability in combination with the inhibition of apoptosis possibly accounts in part for the oncogenic activity of securin. Consequently securin overexpression results, depending on the degree of expression, in elevated cell proliferation and nontumor cell transformation, which may also be mediated by the induction of *c-Myc* oncogene expression (▶ Myc Oncogene). Recently an additional mechanism for the induction of genetic instability by securin in tumor cells has been suggested. Securin binds to DNA repair proteins (p53 and ▶ Ku70) and, when overexpressed, inhibits ▶ double-strand break DNA repair activity in colorectal cancer cells.

Second, high securin expression levels induce angiogenesis in vitro and in vivo possibly by activating the expression of basic fibroblast growth factor (▶ BFGF) and ▶ vascular endothelial growth factor (VEGF), both potent mitogenic and angiogenic factors. This activity of securin is dependent on the proline-rich domain of the protein suggesting that securin may function through SH3-signal transduction pathways.

Third, securin expression correlates with tumor cell ▶ invasion and has been implicated to serve as prognostic marker for poor prognosis of pituitary, thyroid, colorectal, breast, esophageal cancers, and squamous cell carcinoma of the head and neck. The proinvasive and angiogenic effect of securin may be facilitated by its induction of ▶ matrix metalloproteinase 2 (MMP-2) expression. Taken together, securin appears to contribute to at least three important hallmark features of cancer: cell transformation, angiogenesis, and cell invasion.

However, the regulation of securin expression in tumors is largely unknown. The growth factors ▶ HGF, TGFα (Transforming Growth Factor), ▶ EGF, IGF-1 (▶ Insulin-like Growth Factors), and the hormone insulin have been implicated in securin regulation. Recently, in colorectal cancer and esophageal squamous cell carcinoma, the β-catenin/TCF-signaling pathway (▶ APC/β-Catenin Pathway) has been found to control securin expression. In the process of tumor development, this crucial signaling pathway is deregulated, leading to the accumulation of the oncogenic protein β-catenin, which acts as transcriptional activator in the β-catenin/TCF4 protein complex. The constitutive activity of β-catenin causes at least in part the overexpression of securin and other target genes with the potential to contribute to tumor initiation and progression. Although the molecular mechanisms of securin participation in tumor development are currently poorly understood, further research will reveal whether it is relevant as potential diagnostic or therapeutic target.

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"Seed and Soil" Theory of Metastasis

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Definition

A theory proposed to explain the metastatic preference of ▶ cancer cells for specific organs is called the

"seed and soil" theory, the cancer cells being the "seeds" and the specific organ ▶ microenvironments being the "soil." Interaction between the "seeds" and the "soil" determines the formation of a secondary tumor.

Characteristics

Historical Development of the "Seed and Soil" Theory

▶ Metastasis is the spread of a cancer from its primary location to distant sites in the body, forming the secondary tumors. When cancer cells metastasize, they usually do so to preferential organs, depending on the type of cancer. For example, breast cancer cells usually metastasize to the lymph nodes, bones, lungs, liver, and brain; colon cancer cells often metastasize to the lymph nodes and liver.

The propensity of certain organs to harbor metastatic tumors was noticed in the middle of nineteenth century. When Fuchs studied the metastasis pattern of uveal melanoma in 1882, he found that a favorable site for secondary tumor development should be taken into account. Paget, a surgeon, reported the autopsy results of 735 cases of breast cancer and summarized the studies of others, and clearly proposed the "seed and soil" theory in 1889, pointing out that metastasis depends on interaction between cancer cells and specific organ microenvironments. The theory has had a great influence on the field of cancer research.

Recent Evidence Shows that the "Seeds" Can Even Prepare the "Soils"

A primary tumor can induce reorganization of the vasculature and lymph channels in the ▶ Sentinel Lymph Node before metastasis, that is, before the cancer cells arrive. The dramatically remodeled vasculature of the sentinel lymph node can then integrate into the tumor vascular system after metastasis and nurture the fast growing metastatic tumor. The molecular basis for this pre-metastatic remodeling is not yet known.

The preparation of a pre-metastatic "niche" in bone marrow before the arrival of cancer cells has also been reported. The bone marrow–derived hematopoietic progenitor cells that express ▶ vascular endothelial growth factor receptor 1 are responsible for creating a favorable niche for incoming tumor cells.

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Seer

Definition

Surveillance epidemiology and end results.

▶ Childhood Cancer

SELDI-TOF MS

Definition

▶ Surface-enhanced laser desorption/ionization time-of flight mass spectrometry.

Selectins

Definition

A family of cell-adhesion molecules mediating inflammatory cell arrest. E-selectin is found on activated endothelium. P-selectin (CD62-P) is expressed by endothelium and platelets upon stimulation with, e.g., thrombin. L-selectin is expressed on leukocytes. Adhesion molecules contain a lectin-like domain that recognizes and binds carbohydrates. Relevant examples for adhesion mediated by sialyl-Lewis antigens are E(endothelial)-selectin and P(platelet)-selectin.

▶ Lewis Antigens
▶ Tumor-Endothelial Cross-talk

Selection Template

Definition

An oligonucleotide, typically double-stranded DNA, that is used either directly or through a derivative (e.g., RNA transcript) in a combinatorial selection method. Selection templates usually contain a region of selectable randomized sequence flanked by defined sequences used minimally in ▶ PCR amplification and also containing additional information for use in the selection process (e.g., bacteriophage promoter for RNA transcript production in ▶ SELEX, IISRE binding sites for IISRE cleavage in REPSA).

▶ Combinatorial Selection Methods

Selective Estrogen Receptor Modulators

Definition

SERMs; block the effects of estrogen in the breast tissue. Used in ▶ hormonal therapy of ▶ breast cancer. SERMs work by binding o the estrogen receptors in breast cells. If a SERM blocks the estrogen receptor, estrogen cannot attach to the cell. Consequently, the cell does not receive estrogen signals to grow and multiply. A characteristic that distinguishes these substances from pure receptor agonists and antagonists is that their action is different in various tissues, thereby granting the possibility to selectively inhibit or stimulate estrogen-like action in various tissues. Cells in other tissues in the body, such as bones and the uterus, also have estrogen receptors. But each estrogen receptor has a slightly different structure, depending on the kind of cell it is in. Breast cell estrogen receptors are different from bone cell estrogen receptors and both of those estrogen receptors are different from uterine estrogen receptors. SERMs are "selective" – this means that a SERM that blocks estrogen action in breast cells can activate estrogen action in other cells, such as bone, liver, and uterine cells.

▶ Breast Cancer Anti-Estrogen Resistance

Selective Serotonin Reuptake Inhibitors SSRIs

▶ Fluoxetine

Selenium

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Definition

Selenium (Se) is an essential trace element having multiple effects in many functions of the normal human organism and inducing disturbances when deficient. Thus, it has an important role in health maintenance, being furthermore implicated, when in low levels, in various chronic pathological conditions such as rheumatoid arthritis, diabetes mellitus, cardiovascular diseases, renal insufficiency, and cancer.

Characteristics

More than 10 million new cancer cases each year are recorded worldwide, with cancer being one of the leading causes of death. Much progress has been made in the quest of the etiology and pathogenesis of cancer in humans; however, there are still a lot of issues to be elucidated. Identifying cancer risk factors is critical both in prevention and treatment. Epidemiological and genetic studies suggest that factors such as smoking, bioactive food components, and hormones can influence the incidence and mortality of this disease. Among them a lot of attention has been drawn in those that indicate a strong relationship between low Se concentration in the serum and increased risk of various types of cancer in humans.

Trace elements, such as Se, zinc, arsenic, cadmium, and nickel, found naturally in the environment, are delivered to humans from a variety of sources including air, drinking water, and food. In the human body, Se is absorbed by the gastrointestinal tract, skin, and respiratory system. Concentrations of this trace element in the air are low. Thus, diet and drinking water

supplies are the primary sources of Se intake. Se enters the food chain through plants, which uptake it from the soil. Notably, there is a wide geographic distribution of Se in the soils varying from high concentrations in the former USSR and USA to low concentrations in China, and some other parts of Asia, as well as in many European countries. Se is present in a wide range of foods such as grains, meat, fish, eggs, as well as in some vegetables. Average selenium consumption from foods ranges from 71 to 152 μg daily. The suggested daily Se uptake in adults varies between scientists groups, ranging from 60–120 μg to 200–300 μg daily. Se intake from dietary sources is assessed by 24-h and weekly dietary recalls, diet histories, and food frequency questionnaires. The latter is the least preferred method, since potential differences in Se absorption from the intestinal lumen, various food preparation methods, and variations depending on geographical Se distribution impair the results. In humans, high Se concentrations are detected in the thyroid gland, kidneys, genitals, and liver, while the lowest Se concentrations are detected in pancreas and lymph nodes.

Organic Se is present in foods mainly in the form of selenomethionine, selenocysteine, and selenium-methylselenocysteine, while inorganic Se either in the form of selenite or selenate is found infrequently and in very low amounts. Both organic and inorganic Se are utilized with similar efficacy in the human body, producing selenoproteins (SPs), although Se enters at different points in the metabolism processes depending on the chemical form. Twenty-five SPs have been identified until now, out of a possible 50 thought to exist. Glutathione (GSH) reduces the inorganic forms (selenate and selenite) of Se. The originally discovered cytosolic glutathione peroxidase GSH-Px or GPx-1, the phospholipid hydroperoxide GSH-Px, and the secretory GSH-Px represent three isoenzymes of glutathione peroxidase. The latter, ubiquitously expressed, is the first and best characterized SP in mammals. Other selenoproteins such as thioredoxin reductase (TrxR) and selenoprotein P (SeP) also contain molecular Se in their active center and act in a similar fashion. Through all of them Se regulates the cellular antioxidant defense system, DNA damage, and protein function. Se also controls cell-mediated immunity and B-cell function. TrxR is considered to be a key enzyme in Se metabolism, reducing Se compounds and controlling the intracellular redox state, and SeP appears to protect endothelial cells against damage from free radicals. The varying degree of anticarcinogenic activity of different forms of selenium may be attributed to their metabolism in vivo. High Se concentration can lead to either cytotoxic effects or possibly to carcinogenesis, due to DNA strand breaks. Thus, in the first half of the twentieth century, Se was considered an undesirable element for higher organisms. In the second half of the twentieth century, the role of Se in human nutrition and biology was appreciated, since there was growing evidence that Se has multiple roles in many biological systems. Among them, Se controls cell-mediated immunity and B-cell function. Se role as an antioxidant, as well as a cancer-preventing factor nowadays is well established by accumulating evidence. A bulk of studies strongly supports the issue that Se supplementation is effective in the reduction of cancer incidence when nontoxic doses of the element are provided with the diet, possessing thus, anticancer properties. Experimental data have shown that the chemopreventive effect of Se is due, at least partly, to its inhibitory effect on cell growth, DNA, RNA, and protein synthesis in transformed cells. Furthermore, changes in stress-related cellular proteins are widely implicated in explaining the protective role of Se. Several reports have described the inhibitory effect of Se on kinase enzyme activity. Cell cycle cyclin-dependent-kinase 2 (cdk2) and/or cell signaling protein kinases and/or some redox regulated proteins with critical transcription factors have been proposed as targets against which Se exerts its chemopreventive actions. An increase in cyclin B expression as well as phosphorylation of the cdk2 coincidentally with the cell cycle arrest has been demonstrated. In addition, Se exerts an antiproliferative effect by modulating cellular proliferation in G1 phase in both normal and neoplastic cells and possibly impairing the expression of c-fos and c-myc oncogenes.

Furthermore, human defense mechanisms against reactive oxygen species (ROS), which induce oxidative damage are amplified by Se. Cellular oxidative damage is a general mechanism for cell and tissue injury. Selenocystein reduces the levels of hydrogen peroxide and a number of organic hydroperoxides, thereby, acting as antioxidant. Oxidative stress in the target tissue has been suggested to play an important role in carcinogenic process. Thus, Se may act as an antitumoral agent although more studies are needed to investigate the actual role of antioxidants and their

possible relationships with trace elements alterations, and Se in particular, in the pathogenesis of various cancer types. It has also been shown that in colorectal cancer cells, Se supplementation decreases the COX-2 protein and PGE-2 levels.

Se plasma and tissue concentration is regulated by incompletely understood homeostatic mechanisms. For estimation of the body Se amount levels, whole blood, serum, plasma, erythrocytes, urine, hair, and nails represent biological specimens suitable for sampling. Each of these differs in terms of the exposure period represented. Plasma and serum measures tend to reflect short-term exposure, while Se levels in erythrocytes represent a long-term exposure. Longer-term exposure is measured by toenail samples.

Early epidemiological studies ~40 years ago suggested an inverse association between Se levels and risk of cancer. Studies from about 30 countries showed a significant inverse correlation with age-adjusted mortality for colorectal (CRC), prostate, breast, ovary, and lung cancer as well as for some hematological malignancies, while only a weak association was found for pancreatic and skin cancer. Low Se is associated with risk of lung, colorectum, esophagus, stomach, liver, breast, prostate, and urinary bladder cancer. Results from many chemoprevention trials strongly suggest that Se supplementation may have some protective effects against the above mentioned cancer types in populations where average dietary Se levels are low.

There are a few data regarding the association between Se and CRC or adenomas. These results depending mainly on small studies are inconclusive and probably new prospective studies with large series of individuals are needed. However, epidemiological studies have reported an inverse association between Se and CRC, cancer stage and survival, while some others failed to detect this association reporting null results. A pooled analysis reported by Jacobs et al. indicates that individuals with high blood Se concentration (median value 150 ng/ml) had 34% lower odds ratios (OR 0.66, 95% CI 0.50–0.87, P_{trend} 0.006) for developing a new adenoma throughout the follow-up time period, in comparison with those individuals with lower plasma Se levels (median value 113 ng/ml). Thus, new prospective studies regarding Se alone or in combination with other trace elements must be designed in the near future. In breast, contradictory studies have been reported. Although there is some evidence indicating the chemopreventive role of Se in breast cancer, rigorous retrospective and prospective studies are needed to confirm this issue.

The urinary bladder mucosa differs from other tissues, being exposed to Se directly, via the urine, and indirectly, via the blood. An inverse association between serum Se levels and bladder cancer has been reported in the literature. Serum Se levels and glutathione peroxidase activity in patients suffering from transitional cell carcinoma were inversely correlated with tumor grade. Similarly, this strong inverse association has also been detected in prostate cancer. In recent reports, individuals taking Se supplementation exhibited a significant reduction in the risk of prostate cancer. Regarding the other cancer types the usual reverse association between serum Se levels and neoplastic development has been observed, although this phenomenon is not universally accepted, since there are also not confirmatory studies.

By several studies it has been indicated that the process of the underlying tumor development can lead to an uptake of trace elements by neoplastic cells explaining, thus, the increased levels of Se in tumor mass. The same phenomenon is also observed with some other trace elements such as Zn, Fe, Cu in neoplastic tissue. It is not clear whether the increased Se concentration in the cancerous tissues is responsible for the decreased serum Se levels found in these patients or if the decreased serum Se levels precede the development of cancer.

In conclusion, Se is found naturally in the environment and is uptaken by humans through a variety of sources, including food, drinking water, and air. Intake and plasma levels differ depending on geographical distribution and genetic factors. Se chemopreventive action has been demonstrated in some types of human cancers. More clinical trials are needed to investigate the role of Se supplementation in reducing cancer incidence in humans.

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Selenocysteine

Definition

A ► selenium analog of cysteine, where a selenium atom in selenocysteine has taken the place of sulfur in cysteine. Selenocysteine is naturally present in 25 known human proteins, of which thioredoxin receptor (TrxR) isoenzymes constitute three.

► Thioredoxin System

Selenoenzyme

Definition

An enzyme containing selenocysteine as one of the amino acids of the protein.

► Thioredoxin System

SELEX

Definition

Systematic evolution of ligands by exponential enrichment (SELEX) is a combinatorial selection method that identifies oligonucleotide aptamers exhibiting high affinity and binding specificity to a variety of ligands, including proteins and small molecules.

► Aptamer Bioconjugates for Cancer Therapy
► Combinatorial Selection Methods

Self-Renewal

Definition

Is the ability to undergo multiple rounds of cell division while maintaining an undifferentiated state.

► Stem Cell Markers

Self-Sufficiency in Growth Signals

Definition

Capacity of tumoral cells, which refers to the ability that they have to proliferate in the absence of stimulatory growth signals. Normal cells require growth signals in order to actively proliferate. However, tumor cells are characterized by a greatly reduced dependence on exogenous growth stimulation. The explanation is that tumor cells generate many of their own growth signals, inducing a growth signal autonomy.

► Funnel Factors

Sella Turcica

Definition

The bony cavity in the skull that houses the pituitary gland.

► Prolactin

SEMA

► Semaphorin

SEMA Domain

Definition

Is commonly found in ► semaphorins – a large family of secreted and transmembrane proteins. Some

semaphorins function as repellent signals during axon guidance.

Semanová II syndrome

► Nijmegen Breakage Syndrome

Semaphorin

Claus Christensen
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Synonyms

Collapsin; Growth guidance cue; Neuropilin ligand; Plexin ligand; SEMA

Definition

The semaphorins (abbreviated SEMA) constitute a family of genes encoding secreted and membrane-associated proteins which share a common domain called the sema domain.

The name semaphorin is derived from the Greek words "*sema*" meaning "signal" and "*phor*," which means "to carry." This name was given to semaphorins owing to the function of the first semaphorins in ► axon guidance.

Characteristics

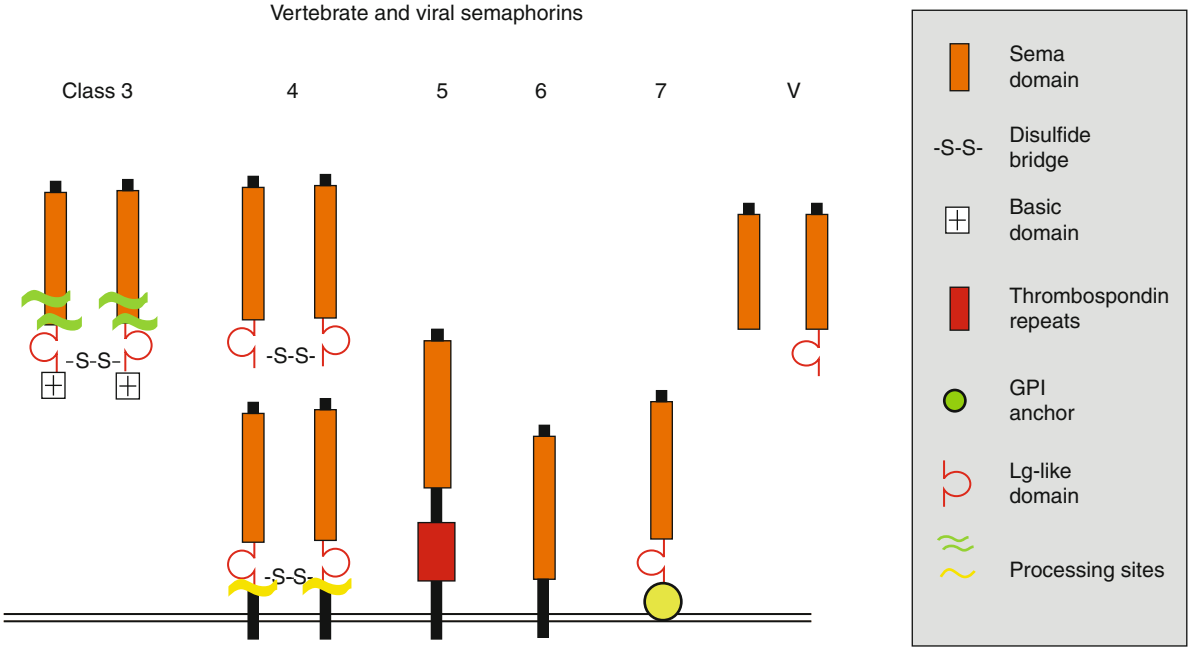
Subclasses

To date, the semaphorin family comprises at least 20 different members in vertebrates and at least three different members in invertebrates. All semaphorins contain an approximately 500 amino acid extracellular sema domain and a class-specific carboxy terminus that may encompass additional sequence motifs. At present the known semaphorins are divided among eight subclasses on the basis of these structural features and phylogenetic analysis. The characteristics of individual subclasses are depicted in Fig. 1. With respect to cellular localization semaphorins fall into three categories: secreted (subclass 2, 3, and V), transmembrane (subclass 1, 4, 5, and 6), and glycosylphosphatidyl (GPI)-linked (subclass 7).

Other protein families including ► plexins and the ► receptor tyrosine kinases MET and ► RON contain sema domains, but on the basis of phylogenetic analysis these proteins are only distantly evolutionary related to semaphorins.

Structure and Processing

To date, crystal structures of sema domains have been provided for a small number of semaphorins (SEMA4D, SEMA3A, and Sema6a). Overall, the sema domain is structured as a seven-bladed beta-propeller, i.e., a ring-like protein structure comprising seven beta-sheets each containing four anti-parallel beta-strands. The beta-propeller structure is found in a large number of proteins including integrins, and hence semaphorins and integrins show structural homology in addition to functional interaction (see below). Unique to the sema beta-propeller is an insert of approximately 77 amino acids within the fifth beta-sheet. The seventh beta-sheet is followed by a cysteine-rich domain common to semaphorins, plexins, and integrins (PSI domain), which forms a highly compact structure abutting the side of the beta-propeller. Following this domain, an Ig-like domain is found in semaphorins belonging to class 3, 4, 7, and V (Fig. 1). Semaphorins undergo various posttranslational modifications that affect their activities. These modifications involve glycosylation, dimerization, and proteolytic processing. Consequently, semaphorins are typically found as mixtures of isoforms representing monomers, dimers as well as fully or partially processed proteins. Notably, class 3 semaphorins contain basic amino acid motifs following the consensus sequences RXXR or KRRXRR, which serve as cleavage sites for furin and related serine endoproteases. The KRRXRR site is found within the PSI domain in all class 3 semaphorins and upon processing of this site, an amino-terminal fragment is generated which largely consists of a sema domain. This particular processing has been shown to cause a loss of the repellent activity of class 3 semaphorins in neurobiology. However, such processing has different consequences in cancer biology depending on the type of semaphorin as well as



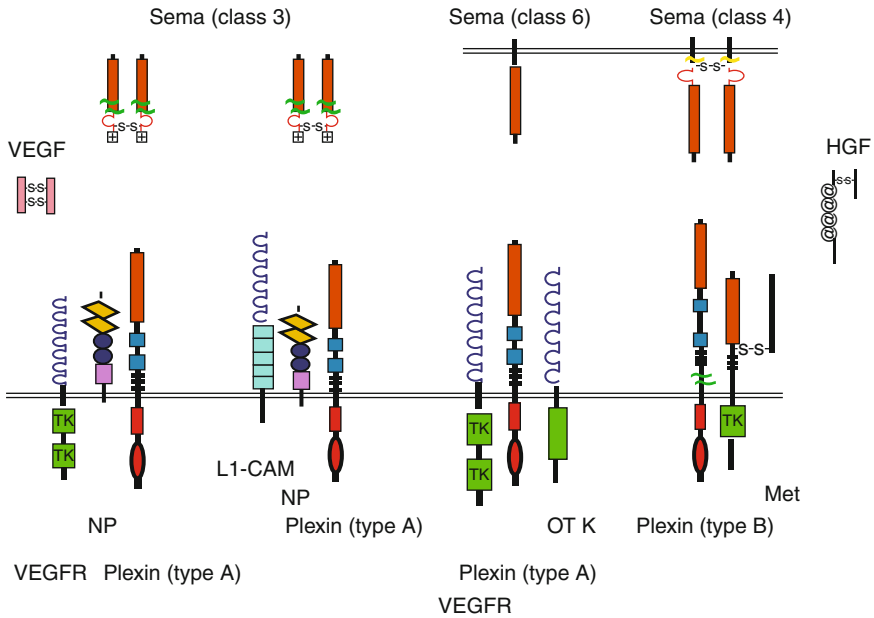
Semaphorin. Fig. 1 The structure of vertebrate and viral semaphorin proteins. Semaphorins are secreted, transmembrane or linked to the surface by GPI anchor. They are found as dimers which may undergo proteolytic processing. This processing may cause shedding of the extracellular part of transmembrane semaphorins

time and place during tumor progression. In the case of Sema3E, furin-dependent processing results in the generation of a pro-metastatic isoform, known as p61-Sema3E, whereas it results in downregulation of the anti-angiogenic and tumor-suppressive activity of Sema3B.

In the case of membrane-associated semaphorins, proteolytic processing may cause shedding of the extracellular domains from the cell surface, as shown for the class 4 semaphorin Sema4D.

Brief History of the Semaphorin Family

The first discoveries of semaphorins were reported in 1992 and 1993 by Alex Kolodkin and co-workers in Corey Goodman's lab at the Howard Hughes Medical Institute, Berkeley, University of California. In the attempt to identify molecules involved in the fasciculation of nerve axons in Grasshoppers, they isolated a transmembrane protein, which they first called Fasciclin IV, and which guided nerves in developing insect limp buds through repulsion. Meanwhile, in 1993, Yuling Luo and co-workers working in Jonathan Raper's lab at the University of Pennsylvania isolated a protein from chicken brain extracts responsible for a growth cone collapsing activity observed when such extracts were added to sensory ganglion neurites in vitro. The gene encoding this activity was named Collapsin-1. At the time of its discovery, the sequence of Fasciclin IV was unique but through the design of PCR primers, degenerate in third base positions, Kolodkin et al. were able to clone additional genes from different species encoding the same amino acid motifs as found in Fasciclin IV. These genes included a human sema gene, two sema genes in Drosophila, and two viral sequences. The human gene, initially called Sema III and later to be renamed SEMA3A, was realized as the human counterpart to chick Collapsin-1. On the collective basis of these genes, the semaphorin/collapsin family was first described by Kolodkin et al. in a Cell paper in 1993. The common family trait was a ~500 amino acid region, named the sema domain, constituting the better part of these proteins and containing a number of unique and highly conserved motifs (see Characteristics). In the following years a number of new semaphorin members were isolated from different species. Semaphorins isolated from chick continued to be named collapsins until the nomenclature of semaphorins was changed by initiative of the semaphorin nomenclature committee in 1999. To date, the semaphorin family comprises at



Semaphorin. Fig. 2 Semaphorin receptor complexes. The core of semaphorin receptor complexes is made of plexins or plexins in combination with neuropilins (NP). Plexins bind semaphorins directly. In the case of class 3 semaphorins, it is generally so that a neuropilin is required in order to bind this class of semaphorins. Neuropilins also act as co-receptors for ▶ vascular endothelial growth factors (VEGFs). Other proteins may be involved in semaphorin receptor complexes including vascular endothelial growth factor receptors (VEGFRs), the off-track kinase (OTK), the cell adhesion molecule L1-CAM, or the receptor tyrosine kinase Met. Hepatocyte growth factor (HGF) is the ligand for Met

least 18 different members in vertebrates and at least three different members in invertebrates. In the years, following the initial discovery of the semaphorin family, functional studies were conducted on different semaphorins in neurobiology. These studies were unanimously showing that semaphorins act as chemotactic cues, which guide migrating axonal growth cones as the complex wiring of the nervous system is established. Semaphorins were typically found to be secreted by neighboring cells and at first considered to act primarily as repelling factors. This notion was changed in 1999 when, for the first time, a semaphorin was shown to be able to act as an attractive guidance cue. Today semaphorins are considered to be bidirectional guidance factors, exerting repelling or attractive guidance depending on the composition of receptor complexes on the responding cells. Today semaphorins are furthermore known to take part in different aspects of biology outside the nervous system including angiogenesis, immunobiology, and cancer. In cancer biology, semaphorins appear to be bifunctional acting either as suppressors or promoters of tumor progression. Recent studies of Sema3B and Sema3E even suggest that the same semaphorin may be responsible for both tumor-suppressive and pro-metastatic activities within the same tumor model, resulting in smaller and less vascularized primary tumors that are nevertheless more metastatic.

Semaphorins and the Composition of Their Receptors

Although many different types of receptors and adhesion molecules are today implicated in semaphorin receptor complexes, two distinct types of receptors form the core of most of these complexes: the neuropilins and the plexins (Fig. 2). Members of the membrane-anchored class 1, 4, or 7 semaphorins bind plexins independent of neuropilins. Hence, Plexin-B1 and Plexin-C1 function as receptors for the transmembrane Sema4D and the GPI-linked Sema7A, respectively. On the contrary, the secreted class 3 semaphorins have for long been considered to require a complex of neuropilin and plexin. Hence, the earliest studies addressing this question showed that plexin-A1 combined with NP-1 and NP-2 constituted functional receptor complexes for Sema3A and Sema3F,

respectively. But, this concept was challenged in 2004, when Sema3E and plexin-D1 were shown to control vascular development together independently of neuropilins. The reason for this discrepancy is unknown, but at least in some tissues SEMA3E appears to exist predominantly as a processed isoform, known as p61-Sema3E, which is incapable of binding neuropilins.

Neuropilins

The neuropilin family consists of neuropilin-1 (NP-1) and neuropilin-2 (NP-2), the latter existing in different splice variants. Neuropilins are transmembrane proteins that extracellularly possess two CUB (complement-binding) domains, two domains of homology to coagulation factors V and VII, and an oligomerization domain, abbreviated MAM (short for meprin, A5,μ). The CUB domain is involved in the binding to class-3 semaphorins. NP-1 binds Sema3A but not Sema3F; whereas NP-2 binds Sema3F but not Sema3A. The other class 3 semaphorins Sema3C, Sema3D, and Sema3E bind both neuropilins. The intracellular domains of NP proteins are very short and devoid of classical signaling motifs. Yet, they are highly conserved and bind possible adapter molecules with a ▶ PDZ domain.

Neuropilins may facilitate clustering of neuropilin–plexin receptor complexes but neuropilins also serve as bridges to other families of adhesion- and receptor molecules. Notably, neuropilins bind members of the ▶ vascular endothelial growth factor receptor (VEGF-R) family. In addition, NP-1 binds to L1-CAM, which is a member of the immunoglobulin (Ig) superfamily of ▶ cell adhesion molecules (Fig. 2).

Plexins

Nine plexins have been identified and they are grouped into four subclasses (plexin-A, -D). There are four A-type, three B-type, one C-type, and one D-type plexin. Like semaphorins, sema domains are a characteristic of the extracellular part of plexins. In addition, plexins have two to three ▶ Met related sequences (PSI domains), and three IPT domains. Intracellularly plexins have two highly conserved stretches, separated by a variable linker region. The conserved domains include motifs distantly related to ▶ GTPase-activating proteins (▶ GAPs). In type-B plexins, the carboxy terminus contains PDZ-domain-binding motifs.

Plexin Signaling

At least some plexins may be locked in an autoinhibited configuration when not binding semaphorins. As shown for Plexin-A1 this involves the binding of the plexin sema domain to the rest of the extracellular part. It is believed that the plexins upon binding to semaphorins change configuration in a manner that allows phosphorylation of tyrosine residues in the intracellular domain as well as binding to various ▶ small GTPases and GTPase exchange factors (▶ GEFs).

The phosphorylation of the tyrosine residues in the intracellular part of the plexins is not because of autokinase activity inherent to the plexins but occurs because plexins are coupled to tyrosine kinases. Upon the binding of the semaphorin ligand, the plexins become phosphorylated by the tyrosine kinases with which they interact. Hence, Plexin-B1 binds the receptor tyrosine kinase Met, and when Plexin-B1 is stimulated by Sema4D, Met causes phosphorylation of Plexin-B1. A similar interaction has been reported between the receptor tyrosine kinase ErbB-2 and Plexin-B1, as well as between ErbB-2 and Plexin-D1. Other examples of plexins interacting with tyrosine kinases are Plexin-A1 and Plexin-A2, which bind the intracellular tyrosine kinases Fes and Fyn, respectively. Plexin-A1 also interacts with the Off-track receptor, which possesses a kinase domain yet no kinase activity, as well as the VEGF receptor KDR/VEGFR-2. The binding of these two receptors to Plexin-A1 appears to have opposing effects on heart morphogenesis (Fig. 2).

The GAP-related domains in the cytoplasmic part of plexins have been shown to serve as docking sites for small GTPases such as Rac1 and Rnd1 (see GTPase and Rho Family Proteins). Furthermore the activation of RhoA is implicated in the axonal collapse mediated by B-type plexins. However, Plexin-B1 does not bind RhoA directly. Instead it binds to leukemia-associated Rho-GEF (LARG) and PDZ-Rho-GEF (PRG) through the PDZ-domain-binding motifs that are specific to B-type plexins. Despite the presence of a segmented GAP domain in plexins, at first plexins were considered not to have any intrinsic GAP activity. However, Plexin-B1 does exert GAP activity when Sema4D binds to the extracellular domain and Rnd1 at the same time binds to the linker region. Under such circumstances Plexin-B1 exerts a GTPase activation activity on R-Ras (see ▶ RAS). A similar mechanism

has recently been proposed for Plexin-D1. R-Ras appears to function mainly in the regulation of Integrins (see ▶ Integrin Signaling and Cancer).

The small GTPases Rho, Rac, and Rnd are known for their regulatory function with respect to the actin filament assembly and actin-myosin contraction, which constitute the basis for cellular structure and locomotion. Integrins couple this machinery to the extracellular matrix and provide much of the stability during ▶ migration (see also ▶ Motility). By recruiting activated forms of small GTPases or affecting integrin function plexins may influence the shape and migratory behavior of cells and axonal growth cones.

Brief History of Semaphorins in Cancer Research

SEMA3B and SEMA3F: The first discoveries of semaphorins in cancer biology were made in 1996 when the SEMA3B and SEMA3F genes were identified at the chromosomal region 3p21.3, which is deleted in small cell lung cancers. To date, tumor-suppressive activities have been described or suggested for SEMA3B and SEMA3F in a range of cancer types, including lung cancer, malignant melanoma, colorectal and ovarian cancer. Both semaphorins are thought to act as tumor suppressors through their ability to compete with vascular endothelial growth factors for the binding to neuropilin co-receptors. An alternative model predicts that SEMA3F acts by reducing expression of β1 integrin in cancer cells. However, in 2008, Sema3B was shown to simultaneously promote metastasis of experimental human tumors, although at the same time reducing primary tumor growth. The pro-metastatic activity was mediated by Sema3B-induced expression of interleukin-8, which in turn recruited tumor-associated macrophages and generated a pro-metastatic microenvironment.

SEMA3A, SEMA3C, and SEMA3E: Meanwhile, SEMA3F and SEMA3B are located together on chromosome 3, and the other subclass 3 semaphorins, SEMA3A, SEMA3C, and SEMA3E, are located on chromosome 7. Up to now, no chromosomal alterations have been shown to inactivate SEMA3A, SEMA3B, or SEMA3E in cancer cells; and concomitantly several studies suggest that these three semaphorins may add positively as well as negatively to tumor progression.

The first to be associated positively with cancer was SEMA3C. In 1997, SEMA3C was identified in cis-diaminedichloroplatinum (CDDP) (see ▶ Cisplatin and Platinum Drugs) resistant ovarian TYKnuR cells as a gene capable of conferring chemoresistance to other cells upon ectopic expression. The underlying mechanism is still unknown. Later SEMA3C has been shown to be upregulated in metastatic human lung adenocarcinoma cells and in malignant glioma cells, and latest, in 2010, SEMA3C was shown to promote the migration of human breast cancer cells. The latter activity was shown to rely on proteolytic processing by the metalloprotease ADAMTS1.

In 1998, Sema3E was identified in murine mammary adenocarcinoma cells (see ▶ Breast Cancer) and reported to be expressed predominantly in metastatic cancer cells; and later, in 2005, Sema3E was shown to stimulate experimental lung metastasis when overexpressed in a nonmetastatic cell line of the same origin. Surprisingly, the pro-metastatic activity was shown to be associated with an isoform, known as p61-Sema3E, generated from furin-dependent processing of an internal KRRFRR sequence, whereas processing-deficient full length Sema3E did not promote experimental lung metastasis. In contrast, in 2008, overexpression of full length Sema3E was reported to inhibit primary tumor growth of human breast cancer cell lines in mice. These seemingly contradictory observations were reconciled in 2010 in the context of spontaneous metastasis models using human cancer cell lines. As for Sema3B, expression of Sema3E was shown to reduce primary tumor growth and at the same time increase metastasis. The tumor-suppressive activity relied on the inhibition of angiogenesis through binding of Sema3E to Plexin-D1 expressed by endothelial cells, resulting in the inactivation of R-Ras and reduced motility. Conversely, the pro-metastatic activity relied on autocrine signaling by p61-Sema3E involving binding to Plexin-D1 and transactivation of the ErbB2/Neu oncogenic kinase. Importantly, this latest study also showed that coexpression of Plexin-D1 and Sema3E correlated with poor prognosis in malignant melanoma patients. Another report, also from 2010, has shown higher expression of SEMA3E and Plexin-D1 in human prostate cancer tissue compared to normal tissue. In 1999, SEMA3A became the first semaphorin, for which an antagonistic activity was demonstrated toward VEGF signaling. This led to the anticipation that SEMA3A should act as an anti-angiogenic factor and hence a tumor-suppressor. Since then, several reports have

demonstrated or indicated anti-angiogenic and tumor-suppressive activity of SEMA3A in a range of cancers, including breast cancer, malignant glioma, prostatic cancer, meningioma, multiple myeloma, leukemia, and mesothelioma. However, in 2007, increased expression of SEMA3A was demonstrated in pancreatic cancer tissue and linked to late stage and shorter patient survival. Furthermore, in 2009, increased expression of SEMA3A was detected in glioblastoma multiforme, and associated with increased invasion and migration in a NP-1-dependent manner. Hence, SEMA3A seems capable of acting also as a proinvasive factor, depending on cancer type. Most recently, in 2011, targeted as well as systemic delivery of SEMA3A to metastatic murine mammary adenocarcinoma was shown to inhibit both tumor growth and metastasis. The inhibition of primary tumor growth was achieved through action on the tumor vasculature whereas inhibition of metastasis resulted from the direct action on the cancer cells. Surprisingly, only the latter effect was NP-1-dependent. SEMA3D and SEMA3G: to date, limited information exists concerning the role of SEMA3D and SEMA3G in cancer, the latter semaphorin gene having been identified just recently (2005). However, in 2008, data were presented, which indicate a tumor-suppressive activity of these semaphorins. In one report, both semaphorins were shown to function as anti-tumorigenic agents in neuropilin-expressing human breast cancer cell lines. In another report, SEMA3D expression was shown to be reduced in high-grade as compared to low-grade gliomas; meanwhile higher expression of SEMA3G in gliomas was associated with better overall survival.

Sema4D: In 2002, Sema4D was shown to stimulate invasive growth in vitro through a receptor complex consisting of Plexin-B1 and Met. Later, in 2005, Sema4D was shown to promote angiogenesis in vitro and in vivo in a manner that likewise requires a coupling between the Plexin-B1 and Met receptors. Experimental evidence for a role of Sema4D in tumor angiogenesis has been reported in the context of head and neck squamous cell carcinoma. Notably, in 2007, it was shown that the pro-angiogenic activity was generated through release of Sema4D from the tumor cell surface through proteolytic processing. The protease involved was the metalloproteinase MT1-MMP (membrane-tethered collagenase membrane type-1 matrix metalloproteinase). In another study, reported in 2008, it was demonstrated that a tumor microenvironment lacking Sema4D was incapable of supporting tumor growth and metastasis; and that tumor-associated macrophages were in fact the main producers of Sema4D within the tumor stroma. High expression of Sema4D has also been found in lymphoid and myeloid leukemia cell lines as well as in some T-cell and B-cell non-Hodgkin lymphomas (see ▶ Hematological Malignancies; Hodgkin Disease and Malignant Lymphoma: Hallmarks and Concepts). Whether Sema4D contributes to the progression of these cancers is not known. In parallel, Sema4D was shown to be upregulated and play a role during the maturation of monocytic lineage cells. Here, both Plexin-B1 and CD72 have been shown to participate as receptors for Sema4D.

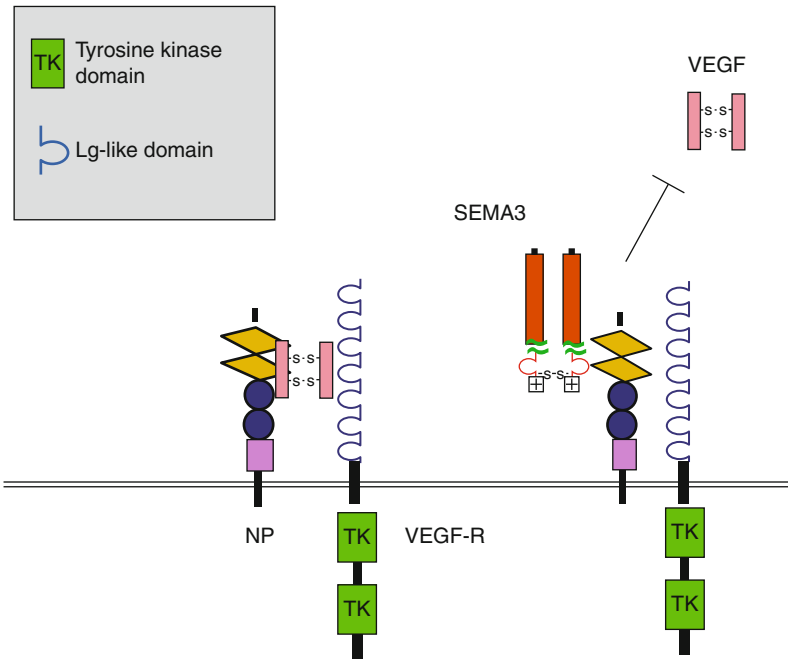
Sema5A and Sema5C: In 2003, Sema5C was identified in a genetic screen for genes that would suppress a tumor phenotype in Drosophila, that arises from inactivation of the lethal giant larvae l(2)gl gene. This work showed that inactivation of Sema5C blocked tumor growth in such flies, and pointed to a mechanism involving a TGF-beta-like signal pathway. With respect to SEMA5A, different reports data suggest contradictory roles in tumor progression, depending on cancer type. In 2010, the downregulation of SEMA5A in tumor tissue was associated with poor survival among nonsmoking women with non-small cell lung carcinoma (NSCLC). However, in 2007, human SEMA5A was identified in a search for genes upregulated in aggressive pancreatic cancer cell lines; and subsequently, in 2009 and 2010, SEMA5A was shown to promote angiogenesis as well as spontaneous metastasis of pancreatic cancer cells.

Other semaphorins: The class 5 semaphorins SEMA5B and SEMA5D and the class 6 semaphorins SEMA6A and SEMA6B have also been associated with different human cancer cells in terms of aberrant expression, but the significance of these findings is unknown. Table 1 summarizes the expression and alleged functions of semaphorins in cancer according to published material.

Different mechanisms may account for semaphorin functions in cancer biology. The same semaphorin is capable of engaging different receptor–complexes and different cell types within the tumor microenvironment. Also, the same semaphorin may exist in different isoforms due to posttranslational modifications and these isoforms may have different receptor predilections. Therefore, the same semaphorin may exhibit

Semaphorin. Table 1 The expression and alleged functions of semaphorins in cancer

Semaphorin	Type of aberrant expression	Tumor type(s)	Function(s)
SEMA3A	Downregulated	Breast cancer, malignant glioma, prostatic cancer, meningioma, multiple myeloma, leukemia, and mesothelioma	Inhibition of angiogenesis and migration
	Increased expression in a subset	Glioblastoma multiforme tissue	Mediates spreading though increased migration
	Increased expression in late stage tumors and metastases	Pancreatic cancer	Increased invasiveness
SEMA3B	Maps to 3p21.3 deleted in lung cancer	Lung cancer	Inhibition of angiogenesis
	Downregulated	Various lung, ovarian, and breast cancer cell lines	
	Expressed	Malignant melanoma clinical samples versus nevi	Inhibition of primary tumor growth, yet increased metastasis
		Various lung, colorectal, malignant melanoma, and neuroblastoma cell lines	
SEMA3C	Overexpression	Ovarian, lung, glioma cancer cell lines	Promotes cancer cell survival (chemoresistance)
	Differentially expressed (only seen in metastatic cell line)	Lung adenocarcinoma cell lines	
SEMA3D	Higher expression in high-grade versus low-grade	Malignant glioma	SEMA3D
SEMA3E	Differentially expressed (only seen in metastatic cell lines)	Breast carcinoma cell lines	Promotes metastasis
	Increased expression	Clinical samples of breast adenocarcimona and malignant melanoma	Inhibition of primary tumor growth, yet increased metastasis
	Increased expression	Prostate cancer tissue and cell lines	Inhibition of adhesion/migration
SEMA3F	Maps to 3p21.3 deleted in lung cancer	Lung cancer	Inhibition of angiogenesis
	Downregulated	Malignant melanoma	Inhibition of adhesion and migration
SEMA3G	Expressed	Malignant glioma	High expression associated with better prognosis
SEMA4D	Expressed	Head and neck squamous cell carcinoma	Promotes angiogenesis and invasive growth
	Differentially expressed	Lymphoid and leukemia cell lines non-Hodgkin lymphoma	Unknown
SEMA5A	Expressed	Malignant Melanoma cell line	Unknown
	Overexpressed	Uterine leiomyomata	
	Downregulated in cancer	NSCLC	
	Tissue		
	Upregulated	Pancreatic cancer cell lines	Pro-angiogenic and pro-metastatic
SEMA5B	Differentially expressed	Renal cell carcinoma	Unknown
Sema5C	Inactivation (experimentally by P element insertion)	Drosophila l(2)gl tumor model	Necessary for tumor growth
SEMA5D	Expressed	Malignant melanoma cell line	Unknown
		Ovarian cancer cells	
SEMA6A	Maps to 5q21-22 deleted in lung cancer	Lung cancer	Unknown
SEMA6B	Downregulated when cells treated with retinoids	Glioblastoma	Unknown



Semaphorin. Fig. 3 Model for how semaphorins may suppress tumorigenesis by blocking VEGF signaling. Neuropilins (NP) are co-receptors for both class 3 semaphorins and vascular endothelial growth factors (VEGFs). When class 3 semaphorins bind neuropilins they prevent the binding and activation of VEGF-receptors (VEGF-R) thereby blocking the pro-angiogenic signaling of VEGF

different and even opposing modes of action during tumor progression.

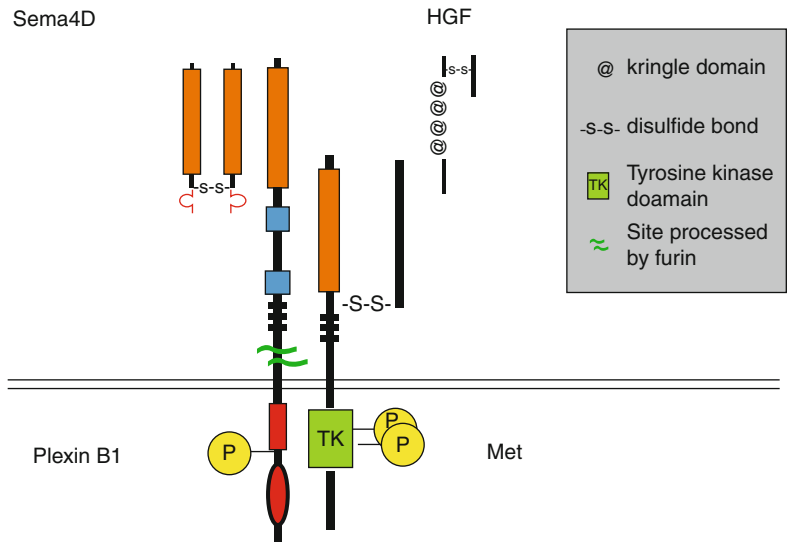
Model I: Semaphorins May Act as Antagonists of Vascular Endothelial Growth Factor (VEGF) Signaling

Both class 3 semaphorins and vascular endothelial growth factors are known ligands of the neuropilin receptors, meanwhile neuropilins exist in complexes with either plexins or VEGF-receptors (VEGFR). This creates the basis for a mutually antagonistic relationship between class 3 semaphorins and VEGF ligands (see Fig. 3). Hence, NP-1 is a co-receptor of VEGFR-2 (also known as KDR) mediating the activity of certain VEGF-A isoforms in angiogenesis. However, SEMA3A binding to NP-1 blocks this activity in endothelial cells. Likewise, NP-2 is a co-receptor for VEGFR-1 and VEGFR-2, and binding of NP-2 to SEMA3F or SEMA3B may influence signaling through these two receptors. NP-2 was also shown to act as a co-receptor for VEGFR-3 mediating the activity of VEGF-C and VEGF-D during lymphangiogenesis.

Initially, class 3 semaphorins were considered to be tumor suppressors due to their antagonistic activity on VEGF signaling in endothelial cells (see ► Anti-angiogenic and ► Anti-angiogenesis). Today, the picture is made complicated by the fact that both endothelial cells and many cancer cells express class 3 semaphorins and VEGF ligands, and both cell types may express any combination of neuropilins, plexins, and VEGF-receptors dependent on tumor type and localization. The effect these expressions have on the overall migratory, adhesive, and proliferative capabilities of the EC and cancer cells within a given tumor may depend upon the ratio between the different receptors and between ligands. One updated model depicts that downregulated expression of class 3 semaphorins such as SEMA3F and SEMA3B shifts the balance in favor of VEGF ligands and their pro-migratory activity in cancer cells or pro-angiogenic activity in EC.

Model II: Semaphorins May Act Through Complexes of Receptor Tyrosine Kinases and Plexins

The reason for both tumor-suppressive and tumor-promoting activities of semaphorins may originate from the interaction between different plexins and tyrosine kinases. One of the most prominent examples of a potential tumor growth promoting mechanism is the interaction between Plexin-B1 and Met (see Fig. 4). The Met receptor is a known oncogene, and both Met and its ligand hepatocyte growth factor (HGF; see ► Scatter Factor) contribute to increased invasiveness



Semaphorin.
Fig. 4 Semaphorins may promote tumorigenesis because of the synergistic interaction between Plexins and receptor tyrosine kinases. A prominent example of this is the interaction between Plexin-B1 (the receptor of Sema4D) and Met (the receptor of hepatocyte growth factor (HGF)). Met causes phosphorylation of Plexin-B1, when Sema4D binds Plexin-B1

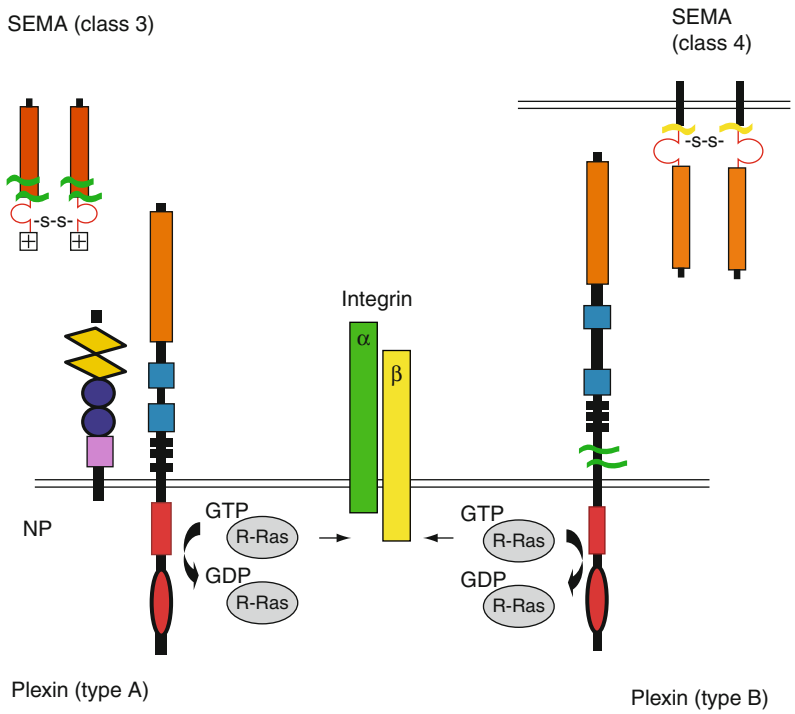
and angiogenic activity in tumors. Sema4D and HGF have been shown to act in synergy to cause activation of Plexin-B1 and Met and facilitate invasive growth in vitro and angiogenesis in vivo. Another prominent example of a semaphorin-mediated coactivation of a Plexin and a receptor tyrosine kinase is the binding of p61-Sema3E to Plexin-D1 and the transactivation of the ErbB2/Neu oncogenic kinase. This mechanism accounts for the autocrine signaling of p61-Sema3E in certain cancer cells leading to increased metastatic spread.

Model III: Semaphorins May Act Through Signaling to Integrins

Integrins constitute a family of adhesion molecules of major importance to the migration of cells through the extracellular matrix. At the same time migration is a crucial aspect of the behavior of endothelial cells during angiogenesis as well as the behavior of cancer cells during ► invasion and metastasis. By affecting the function of integrins semaphorins may therefore affect the ability of a tumor to invade or stimulate angiogenesis. Today, compelling evidence suggests that semaphorin signaling as well as plexin activation inhibit integrin-dependent adhesion and migration of different cell types (see Fig. 5). Notably, in 2004, it was shown that SEMA3F expression in melanoma cells induces a poorly vascularized, nonmetastatic phenotype, which was partly attributed to a decrease in β_1-integrin-mediated migration of the cancer cells. This highlights an alternative way by which class 3 semaphorins may act as tumor suppressors.

Model IV: Semaphorins May Act as Regulators of the Immune Response

From the very establishment of the semaphorin family in 1993, pox viruses have been known to encode semaphorin genes; and this has sponsored ideas concerning possible immunosuppressive functions of semaphorins. Later, the Vaccinia virus encoded semaphorin called A39R was indeed shown to negatively affect the migration of monocytes and phagocytosis by dendritic cells. Today, it is clear that semaphorins and plexins fulfill part of their normal functions within the immune system with at least five semaphorins (SEMA3A, SEMA4A, SEMA4D, SEMA6D, and SEMA7A) and at least five plexins (Plexin-A1, -A4, -B1, -C1, and D1) expressed by dendritic cells or lymphocytes. Among the semaphorins, SEMA3A and SEMA4D have been shown to inhibit aspects of immune functions, such as monocyte migration; and suppression of the immune response is clearly one way in which these cancer-associated semaphorins may contribute to tumor progression. However, recent advances in cancer research have shown that the immune response may act as a double-edged sword during tumorigenesis, which advances the spread of



Semaphorin. Fig. 5 Model for how semaphorin signaling may influence the adhesion and migration of tumor cells. When semaphorins do not bind plexins, R-Ras is active (binding GTP), resulting in integrin-mediated binding to the extracellular matrix leading to higher adhesive and migratory behavior of the tumor cells. When semaphorins bind and activate plexin receptors, the GAP (GTPase-activating protein) activity of plexins causes the conversion of R-Ras from a GTP-bound state (active) to a GDP-bound state (inactive). This leads to a decrease in integrin-mediated attachment and hence diminished cellular adhesion and migration. The activation of the plexin GAP activity requires the intracellular binding of the small GTPase RND1 to the plexins (not shown)

cancer cells in the attempt to reduce the primary tumor mass. Tumor-associated macrophages (TAMs) originate from circulating monocytes and once recruited to the tumor they orchestrate an inflammatory process that facilitates angiogenesis, local invasion, and distant metastasis. Interestingly, SEMA3B was recently shown to stimulate the recruitment of TAMs through induction of interleukin-8; and although SEMA3B suppressed the growth of primary tumors, it promoted lung metastasis. Also, TAMs are one of the main producers of SEMA4D, which has been shown to exhibit pro-angiogenic and proinvasive activity, and SEMA4D itself induces the production of pro-inflammatory cytokines.

These examples indicate that semaphorins may contribute to tumor progression, not by inhibiting monocytes, but instead by creating or amplifying cancer-associated inflammation.

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Semaphorin Receptors

► Plexins

Seminoma

► Seminomatous Germ Cell Tumor

Seminomatous Germ Cell Tumor

► Testicular Cancer

SENCAR Mice

Definition

The SENCAR mouse stock was selectively bred for eight generations for sensitivity to skin tumor induction by the two-stage tumorigenesis protocol using 7,12-dimethylbenz(a)anthracene (► DMBA) as the initiator and 12-O-tetradecanoylphorbol-13-acetate (► TPA) as the promoter. The SENCAR mouse was derived by crossing Charles River CD-1 mice with skin-tumor-sensitive mice (STS). The SENCAR mice are much more sensitive to both DMBA tumor initiation and TPA tumor promotion than CD-1, BALB/c, and DBA/2 mice. An even greater difference in the sensitivity to two-stage skin tumorigenesis is apparent between SENCAR and C57BL/6 mice when using DMBA-TPA treatment. However, the SENCAR and C57BL/6 mice have a similar tumor response to DMBA-benzoyl peroxide treatment, suggesting that TPA is not an effective promoter in C57BL/6 mice. The DBA/2 mice respond in a similar manner to the SENCAR mice when using *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG)-TPA treatment. The SENCAR mouse model provides a good dose–response relationship for many ► carcinogens used as tumor initiators and for many compounds used as tumor promoter. When compared to other stocks and strains of mice, the SENCAR mouse has one of the largest data bases for carcinogens and promoters [1].

Sendai Virus

Definition

► Hemagglutinating Virus of Japan.

Senescence

Definition

Is the limited capacity of cells to divide, an irreversible growth arrest state that depends on the age or cell doublings of a cell, a stage in the life cycle of a cell at which it can no longer divide. This state is dependent on the number of cell divisions and is generally brought about through the gradual shortening of the telomeres (repeat sequences at the ends of chromosomes) with each successive population doubling. Senescence is a permanent growth arrest that occurs after cells have exhausted their proliferative capacity. In normal human fibroblast, senescence takes place after approximately 60 population doublings in culture.

► Aging
► Senescence and Immortalization
► Telomerase

Senescence Accelerated

Definition

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

► Mitotic Catastrophe
► Senescence and Immortalization

Senescence and Immortalization

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Definition

Senescence is the permanent exit of a cell from the cell division cycle, accompanied by morphological and biochemical changes characteristic of aging.

Immortalization is the ability of cell populations to undergo an unlimited number of cell divisions.

Characteristics

Senescence

Normal mammalian somatic cells can proliferate only a limited number of times in vitro, and the maximum number is often referred to as the "Hayflick limit." When this limit is reached, the cells undergo various morphological and biochemical changes suggestive of aging, so the process is referred to as senescence. Senescent cells can remain metabolically active for a long period of time, even though they have permanently exited from the cell cycle. Senescence is thus distinct from cell death (including ► apoptosis and ► autophagy). It is also distinct from terminal differentiation, where cells also exit permanently from the cell cycle and undergo changes that allow them to perform specialized normal functions. Senescent cells have been extensively studied as an in vitro model of aging. In humans, cellular senescence appears to be a major barrier to the development of cancer.

Immortalization

It is not practicable to test whether cells are truly capable of continuing to divide forever, so cells are usually regarded as being immortalized if they have undergone many cell divisions (typically 100) beyond the Hayflick limit. Many cancers contain immortalized cells and some cancer-derived cell lines have been proliferating in vitro for many decades.

Relevance of Senescence and Immortalization to Cancer

Although the Hayflick number may be quite large (fibroblasts from an adult, for example, may divide up to 40 times before they become senescent), in most situations it is not large enough to permit tumor formation. A tumor containing 2^{40} cells would be big enough to be lethal, but there are two major reasons why 40 cell divisions do not result in a tumor of this size. The first is that cell death occurs at a very substantial rate within tumors, for reasons that include genetic instability (see chapters on ► chromosomal instability and ► microsatellite instability) and difficulties with blood supply (see chapter on ► angiogenesis) that result in cell death. The second is that the genesis of a fully malignant tumor cell requires the accumulation of a number of critically important genetic changes. Most of these changes occur at random and provide a growth advantage to the nascent tumor cell. This process consumes many more cell divisions than a normal cell is able to undergo before it becomes senescent.

Consequently, senescence forms a major barrier to carcinogenesis in humans. A cell containing some of the genetic changes required for carcinogenesis will not usually be able to proliferate sufficiently to form a clinically significant tumor while the senescence barrier is intact. Human cells become immortalized at a very low frequency (so low, that no clear example has yet been found of a normal human cell undergoing immortalization spontaneously in cell culture), so immortalization is a rate-limiting step in human carcinogenesis. In contrast, mouse cells become immortalized spontaneously at a measurable frequency and, correspondingly, the probability of a mouse cell becoming malignant is many orders of magnitude higher than for human cells. The ability to suppress tumor formation is a major selective advantage for a long lived species such as *Homo sapiens*.

A Cell Division Counting Mechanism

The existence of a limit to the number of times a cell can divide implies that there must be a cell division counting mechanism. According to the telomere hypothesis of senescence, the counting mechanism is based on the progressive shortening of the ends of chromosomes (► Telomeres) that occurs with cell division. Telomeres form protective caps that prevent the cell recognizing the ends of chromosomes as

double strand breaks and repairing them, for example, by fusing the ends to each other. They contain repetitive DNA (in all vertebrates the repeat unit is a hexanucleotide, TTAGGG), which ends in a single-stranded G-rich tail. Telomeres are able to fold back on themselves and form a loop structure (referred to as a "t-loop") when the single-stranded telomere invades duplex telomeric DNA and anneals to the complementary strand, thus hiding the free single-stranded telomere end. Telomeric DNA is recognized by specific binding proteins, including TRF1 and TRF2 which bind to double-stranded telomeric DNA and POT1 which binds to single-stranded telomeric DNA. The reasons for telomere shortening include the following. First, DNA replication depends on small RNA primers, which get degraded and replaced by DNA. However, there is no mechanism for replacing the terminal RNA primer required for lagging strand DNA synthesis, which results in the template for the next round of DNA synthesis being shorter. This is known as the "► end replication problem." Second, there appears to be a 5′-3′ exonuclease that shortens the C-rich strand, which creates or increases the length of a single-stranded G-rich telomeric tail.

Regardless of the exact mechanism of telomere shortening, eventually telomeres become so short that they trigger the cell to exit permanently from the cell cycle. In order for a cell to become immortalized, it must somehow prevent telomere shortening. In most cancers this is achieved by the activity of an enzyme, ► telomerase, and in a minority it is achieved by another mechanism referred to as ► alternative lengthening of telomeres (ALT). Every immortalized cell line examined to date has either telomerase or ALT activity.

Telomerase

The telomerase holoenzyme complex is normally expressed in cells of the germ-line. It is also found in some normal somatic cells, especially those that are required to undergo extensive proliferation, but at levels that are insufficient to prevent telomere shortening. Telomerase synthesizes telomeric DNA to replace the DNA lost during cell division. The essential subunits include an RNA molecule (TElomerase RNA; TER; encoded by a gene designated TERC, which is an abbreviation of TElomerase RNA Component) that acts as the template for synthesis of telomeric DNA, the reverse transcriptase catalytic subunit (TElomerase Reverse Transcriptase; TERT) that carries out the synthesis, and dyskerin (encoded by the DKC1 gene), a protein that binds to TER. Telomerase activity can be detected in some normal human somatic cells, especially cells in highly proliferative tissue compartments such as the bone marrow, skin, mucous membranes, and epithelia of the gastrointestinal tract (GIT), but not at sufficient levels to completely prevent telomere shortening. Telomerase has an important role in these tissues, because inherited mutations in any of the genes that encode one of the three telomerase components (TERT, TERC, or DKC1) result in a condition called dyskeratosis congenita which is characterized by premature failure of proliferative capacity in tissues such as the bone marrow, skin, and GIT. In contrast, at least 85% of all cancers contain sufficient levels of telomerase to prevent telomere shortening and the percentage is even higher in most types of carcinomas. The factors controlling hTERT expression are not well understood, but it is known that hTERT can be upregulated by ► MYC. If TERT expression is artificially switched on by genetic manipulation in normal cells, it is usually able to induce telomerase enzyme activity, because there is usually expression of the other telomerase subunits. This prevents telomere shortening and in some types of human cells this results in immortalization. Inhibiting telomerase activity in cancer cells may cause cellular senescence or cell death, so telomerase is an attractive target for the development of new anticancer treatments.

Alternative Lengthening of Telomeres (ALT)

Some immortalized cell lines and cancers have no detectable telomerase activity and maintain their telomeres by an alternative mechanism, referred to as Alternative Lengthening of Telomeres (ALT). Overall, about 8–10% of human tumors utilize ALT to prevent telomere shortening. Although the details are not fully understood, ALT is likely to be a recombinational mechanism in which one telomere uses another telomere (or itself via looping back) as a template for synthesis of new telomeric DNA. The ALT mechanism depends on the activity of the MRN complex which is known to be involved in ► homologous recombination. Cells that maintain their telomeres by ALT characteristically have very heterogeneous telomere lengths, ranging from undetectably short to extremely long telomere lengths. They also have substantial quantities of extrachromosomal telomeric repeat

DNA, that may be either linear or circular, some of which is sequestered within ▶ PML nuclear bodies. The presence of telomeric DNA and telomere binding proteins within PML bodies is highly characteristic of ALT-positive cells, and may be used to determine whether a tumor utilizes the ALT mechanism. Types of tumors where ALT is common include ▶ glioblastoma multiforme (the most common primary brain tumor in adults), ▶ osteosarcomas, and some types of soft tissue sarcomas such as malignant fibrous histiocytomas and ▶ liposarcomas.

Tumor Suppressor Genes

Immortalization is facilitated by loss-of-function of the ▶ p16INK4a or ▶ RB1 genes, and the ▶ p53 gene. Loss of the normal function of these genes results in a significant, but finite, increase in cellular proliferative potential. This permits the accumulation of additional genetic changes and increases the probability that activation of a telomere maintenance mechanism will occur. Cells containing an inherited p53 mutation from individuals with ▶ Li-Fraumeni syndrome are the only type of human cells known to undergo immortalization spontaneously.

Clinical Relevance

Treatments that reverse the immortal phenotype may be a useful form of cancer therapy. An attractive target is telomerase, but inhibitors of telomerase may need to be combined with inhibitors of ALT to prevent the emergence of drug resistance.

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Senescence-associated Chronic Inflammation

▶ Aging-Associated Inflammation

Senile Involution

▶ Lobular Involution

Sensorineural

Definition

Associated with the inner ear or nerves involved in hearing.

▶ Connexins

Sentinel Lymph Node

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Synonyms

First-echelon node; First-tier node

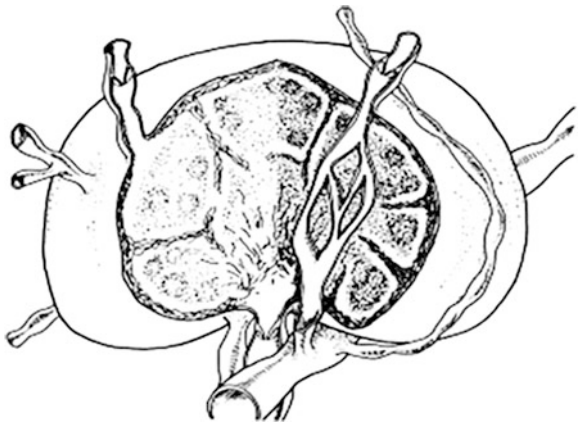
Definition

▶ A sentinel lymph node is a lymph node upon which the primary tumor drains directly.

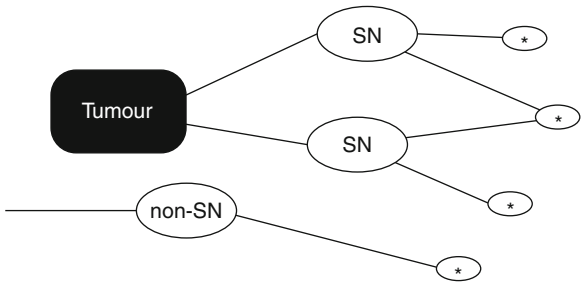
Characteristics

General Anatomy and Physiology of the Lymphatic System

Lymphatic capillaries are 10–50 μm in diameter, consist of a single endothelial layer with a discontinuous membrane and are supported by collagen filaments. They are filled with lymph fluid originating from the interstitial space due to an osmotic pressure gradient and fluctuating intraluminal pressures. These intraluminal pressures are caused by lymphatic flow



Sentinel Lymph Node. Fig. 1 The different relations between lymphatic vessels and lymph nodes. Afferent lymphatic ducts on the left discharge their contents into the marginal sinus. One lymphatic duct runs through the node on the right and another over its surface, bypassing the germinal centers. (Illustration made by Tanis PJ; Reprinted from [2]. With permission from "The American College of Surgeons")



Sentinel Lymph Node. Fig. 2 A sentinel lymph node (SN) is the lymph node upon which the primary tumor drains directly. Two lymphatic channels originating in the primary tumor can run to two different sentinel lymph nodes. Lymph fluid moves subsequently to second-tier (*) and third-tier nodes. The SN is not always the node nearest to the primary tumor (Non-SN)

that is generated by lymph formation, contractions of the vessel wall, and external pressure. Lymph fluid absorbed by lymphatic capillaries drains into larger collecting ▶ lymphatic vessels. Such lymphatic vessels drain into marginal and medullar sinuses located between germinal centers within a lymph node. These centers contain large numbers of phagocytic cells that accumulate protein colloids. Then, a plexus within the lymph node drains to the efferent lymphatic vessel that joins the artery and vein in the hilum. Direct drainage of the marginal sinus into the efferent vessel also exists (Fig. 1).

The Sentinel Node

The ▶ sentinel node is the lymph node upon which the primary tumor drains directly. Lymph fluid moves subsequently to ▶ second-tier and third-tier lymph nodes. Lymph from the primary tumor region does not necessarily travel to the nearest node. Two lymphatic channels originating in the primary tumor can run to two different sentinel lymph nodes (Fig. 2).

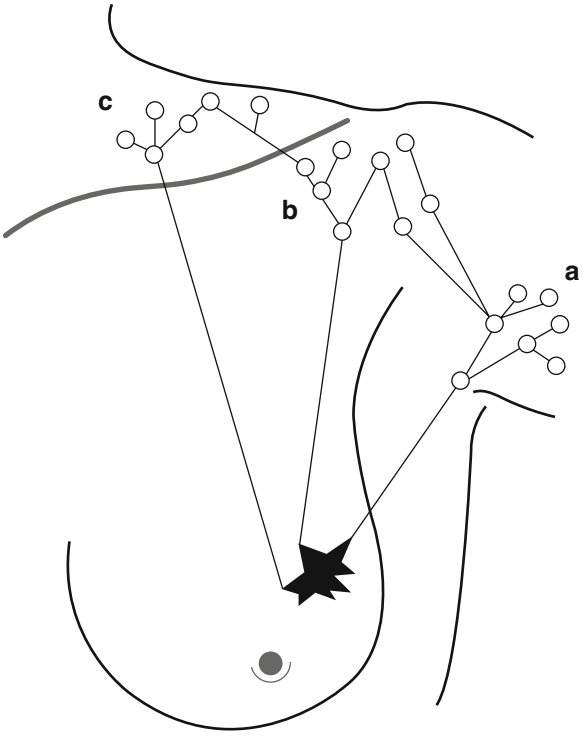
The sentinel node hypothesis implies orderly progression of ▶ metastases from a primary lesion through the ▶ lymphatic system. The concept is only relevant in tumors (▶ Cancer) with predominant lymphatic dissemination, such as melanoma and cancer of the breast, penis, or colon. If the first node contains a metastasis, there is a chance of tumor spreading downstream. In case of a tumor-negative sentinel node, second-tier and third-tier nodes are generally without metastases.

Lymphatic Mapping

The lymphatic drainage pattern can be visualized by ▶ lymphoscintigraphy after injection of a ▶ radiolabeled tracer in or near the site of the tumor. The radiolabeled tracer is cleared from the lymphatic channels and accumulated by the phagocytic cells in the lymph node. ▶ Lymphoscintigraphic images depict the lymph channels and the lymph node or nodes that contain the injected tracer. Dynamic scintigraphy and intraoperative ▶ blue dye mapping give insight in the lymphatic drainage pattern, which enables the surgeon to find the sentinel node(s).

The earliest sentinel lymph node identification techniques involved the injection of a vital blue dye, usually isosulfan blue. It was a key point in the general acceptance of ▶ sentinel node biopsy. The blue dye was injected intradermally at the primary tumor site in melanoma patients. An incision was made over the expected lymph node region and the lymphatic channel was visually identified. This channel was dissected and followed to the first draining lymph node.

Subsequent reports described the use of radiolabeled tracers, such as technetium-99 m-bound colloids. Colloids with a small particle size can rapidly pass the openings of interendothelial junctions and allow visualization of the lymphatic channels leading directly to the sentinel node. A disadvantage of small-sized particles is that some of the tracer moves on to



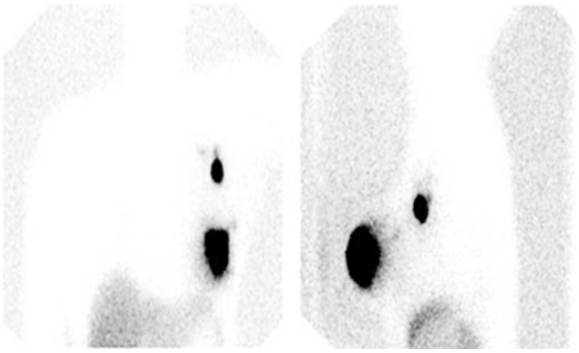
Sentinel Lymph Node. Fig. 3 A tumor (*black area*) with lymphatic channels to nodes in the axilla (**a**) and to lymph nodes *below* (**b**) and *above* (**c**) the clavicula. A metastasis may be found along this lymphatic pathway

nodes further downstream, because phagocytic cells in the first node cannot trap them all. Larger colloid particles enter lymphatic channels more slowly. The tracer almost never moves on to subsequent nodes, but the channels are visualized less often.

Nowadays, the ▶ lymphatic mapping technique mostly used involves administration of a radiolabeled tracer into or near the primary lesion in combination with blue dye. During surgery, the sentinel node is found with the assistance of both blue dye and a ▶ gamma-ray detection probe. Preoperative lymphoscintigraphy is added for better specification of the location and number of sentinel nodes. Different methodologies based on these lymphatic mapping techniques are nowadays applied all over the world.

Sentinel Node Biopsy

A sentinel node biopsy is a minimally invasive technique that was initially developed as an alternative to complete lymph node dissection in patients with melanoma. The majority of patients are spared a more



Sentinel Lymph Node. Fig. 4 Anterior and lateral lymphoscintigraphic images of a woman with *left-sided* breast cancer. A sentinel node and several second-tier nodes are visualized in the axilla

complex surgical procedure with a higher morbidity rate while the same staging information is obtained.

All nodes of a complete node dissection are used to be bisected and evaluated by hematoxylin-eosin staining. This way, metastases larger than 2 mm were usually identified. With the selective sentinel node biopsy, the pathologist can focus on the one or few nodes that are most likely to contain metastatic disease. The sentinel nodes are evaluated by both hematoxylin-eosin and immunohistochemistry staining, which occasionally distinguish metastases with a size of one tumor cell.

The combined procedure of lymphatic mapping and sentinel node biopsy provides prognostic information, identifies patients who may benefit from early regional therapy (▶ Locoregional Therapy) and, depending on the situation, from adjuvant systemic treatment (▶ Adjuvant Therapy). By this way optimal survival rates may be realized.

Breast Cancer

The predominant lymphatic drainage pathway from the breast is toward the axilla. Metastases initially remain localized in the lower axilla, and then may travel higher up the chain to the subclavicular and the supraclavicular basins (Figs. 3 and 4).

Axillary lymph node dissection used to be performed in almost every breast cancer patient. This operation has several side effects, such as lymph edema, pain, and decreased mobility of the arm, and often no metastases were found. With the introduction of sentinel node biopsy, axillary lymph node dissection

is only indicated if this node is involved. As a result, many patients are spared an unnecessary operation.

Whether the omission of routine axillary node dissection jeopardizes regional tumor control and survival is still a subject of research. Large observational studies revealed excellent results in patients who did not receive axillary node dissection because of a tumor-negative sentinel node. Recurrence rates vary between 0.12% and 0.6%, and these numbers do not exceed the known recurrence rates after routine axillary clearance.

Melanoma

There is consensus on the way lymphatic mapping should be carried out in melanoma patients. Preoperative lymphoscintigraphy and intraoperative use of blue dye and a gamma-ray detection probe are standard. The first large studies on sentinel node biopsy in melanoma showed a 95% sensitivity. Recent studies show false-negative rates of around 10%. False negative means that the sentinel node is disease free, while there are metastases in the lymph node basin.

Patients with an involved sentinel node have a 5-year survival rate of around 65%, and in patients with a tumor-negative sentinel node this is 90%. A large randomized study showed that early regional node dissection based on a positive sentinel node improves survival in patients with an intermediate-thickness ▶ melanoma.

Concluding Remarks

The development of the sentinel node concept is a milestone in the understanding of dissemination of solid malignancies. The introduction of lymphatic mapping in 1989 initiated the widespread use and general acceptance of this approach. Now that the technique has been validated, many patients are spared unnecessary surgery without compromising regional control and the accuracy of staging.

Lymphatic mapping with ▶ sentinel lymph node biopsy has become a standard component in the management of patients with breast cancer or melanoma. This suggests potential in other tumors that spread primarily through lymphatic channels.

In the future, studies need to focus on more peripheral issues such as the prognostic significance of ▶ micrometastases and techniques such as molecular assays or markers. These may provide more information to optimize the staging of tumor dissemination and will enable the fine-tuning of therapy.

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Sentinel Node

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Synonyms

Draining lymph node; Sentinel lymph node

Definition

Sentinel means a lookout, and the sentinel node is defined as the first ▶ lymph node or nodes that are located on the direct draining lymphatic route from the area of a primary tumor.

Characteristics

The ▶ lymphatic system was first described in the seventeenth century by Olof Rudbeck who systematically studied the lymphatic vessels collecting extra

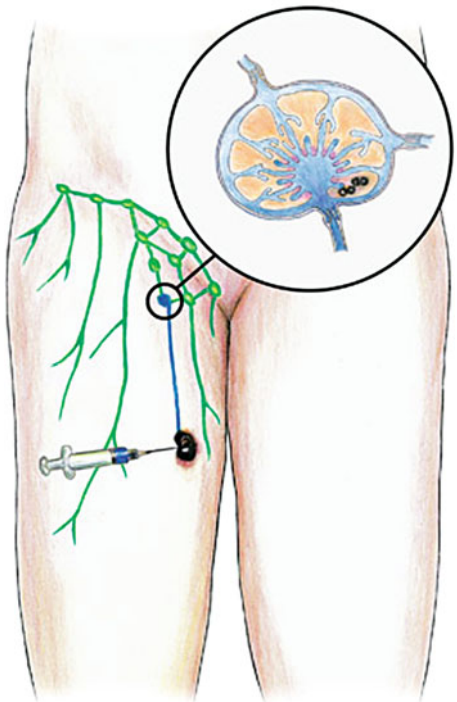
cellular fluids from tissues emptying into ▶ lymph nodes. The lymphatic system is more variable than the blood system and is anatomically less well defined. The drainage from tumors seems to vary considerably, making prediction of the draining lymph node difficult without guidance. Tumor cells become metastatic either by invasive growth through basal membranes or by entering into capillaries. The endothelial cells comprising the lymph capillaries are widely fenestrated (▶ Fenestration) making easy access to the lymph vessel. There is also an active transport of primarily white blood cells into the lymphatic vessels, but tumor cells may also use this mechanism. After entering into the capillaries of the lymphatic vessel, the metastatic cell may then enter into the draining lymph node. Thus, the tumor draining lymph node is the primary location to find lymph node metastases. If tumor cells are present in the sentinel node, the risk of systemic dissemination of the disease is high, since about half of the lymphatic fluid entering a lymph node continues directly to the blood circulation via ▶ lymphovenous shunts.

In 1960, Gould first described a sentinel node draining a cancer of the parotid gland. Cabanas introduced the technique in 1977 for guidance of the surgical procedure for inguinofemoroiliac lymph node dissection of patients with penile carcinoma. This first study demonstrated two important principles regarding sentinel node investigations:(1) the sentinel node is the first site of metastases and may be the only lymph node involved containing metastatic cells and (2) sentinel nodes negative for metastatic cells suggest that no spreading of the disease has occurred and this finding is coupled to increased survival. Studies of prognostic factors in colorectal cancer point out the histological status of the regional lymph nodes as the most important predictor of survival, and the presence of regional lymph node metastases implies a 50% reduction in 5-year survival rates. Similar observations have been reported in most solid tumors. However, the quality of the staging process of lymph node investigations is dependent on the total number of locoregional lymph nodes identified and the extent of the following histopathology investigation.

Malignant tumor often induce a peritumoral upregulation of lymphatic vessels and blood vessels by producing ▶ angiogenic factors such as the vascular endothelial growth factor (▶ VEGF), making the pathways for lymphatic drainage difficult to predict. Studies have shown that elevated levels of the cytokine cascade of VEGF-C and VEGF-D promote tumoral ▶ lymphangiogenesis and that inhibition of their joint receptor, VEGFR-3, suppresses the effects. Further, elevated levels of VEGF-C and VEGFR-3 correlate with the presence of lymph node metastases and lymphatic invasion in human colorectal cancer. A large study of patients with breast cancer demonstrated an increased presence of lymphatic vessels in metastatic axillary lymph nodes (85%) compared to nonmetastatic lymph nodes (25%) and that intra- and perinodal lymphatic endothelial cell proliferation fractions were higher in metastatically involved lymph nodes. These discoveries underline the association of lymphangiogenesis with lymph node metastases.

Sentinel Node Procedure

The sentinel node is either located before surgery through lymphoscintigraphy or during surgery by the superficial injection of a tracer substance around the tumor (Fig. 1). When the tumor is located in the skin, the injection is usually placed around it in four places subcutaneously or intradermally. In visceral tumors (colorectal and gastric tumors) the injections should be superficial, precisely beneath the serosa whereas in parenchymatous organs (liver) the injections are preferably placed under the capsule into the parenchyma around the lesion. When approaching the tumor from the mucosal side, the injections are made through the mucosa and into the muscular wall (urinary bladder tumors). Within a few minutes the tracer has entered the ▶ fenestrated lymph capillaries. The tracer is then transported inside lymph vessels and accumulates through phagocytosis by ▶ dendritic cells in the sentinel node(s). Coal particles, dyes such as patent blue, isosulfan blue, and/or radioactive ^{99}Technetium colloid markers have been used as tracers for intraoperative ocular identification or detection using a hand held γ-counter respectively. A preoperative lymphoscintigraphy can facilitate the location of the regional lymph node basin draining the tumor and may be combined with computerized tomography for precise anatomical information in certain cases. The procedure is dependent on the surgeon's experience with the method, and initial procedures are more likely to give rise to false-negative sentinel lymph node, that is, sentinel node does not contain metastatic cells whereas other regional lymph nodes are positive for tumor cells. The false-negative



Sentinel Node. Fig. 1 The sentinel node concept. Identification of the tumor draining (sentinel) node is accomplished by peritumoral injections of patent blue dye and/or a radioactive tracer ^{99}Technetium. The tracers enter into the lymph capillaries, which follows the lymphatic drainage and accumulation in a lymph node. During surgery, the area of drainage is explored after the guidance of a γ-counter and the sentinel node is visualized by dissection. Since the tumor is in contact with the sentinel node, the sentinel node is the preferred site for metastases. When the sentinel node is investigated microscopically, it can be considered diagnostic for the whole lymph node region, guiding prognosis and therapy. In a progressively growing tumor, there is a rapid turnover of cells and disruption of normal tissue architecture, causing release of inflammatory mediators and attraction of macrophages and dendritic cells. Tumor cell debris is phagocytosed by these antigen presenting cells, which after the encounter migrate to the sentinel node, presenting peptides derived from tumor antigens to naïve T lymphocytes. Sentinel node lymphocytes mount proliferative responses and become clonally expanded against tumor antigen in an attempt to fight cancer

rates in large series performed by experienced surgeons are usually as low as 3–5%.

Clinical Relevance

During recent years, the importance of correct staging (▶ Staging of Tumors) regarding lymph node metastases including presence of micro metastases has become evident for most types of human solid tumors. However, the search for metastases or micro metastases among a large number of lymph nodes in a pathology specimen is time consuming, labor intensive, and expensive. Therefore, the use of the sentinel node technique has received increasing interest, since it entails detailed investigation of (in most cases) only one to three lymph nodes. Thus, an increased awareness regarding an extended histopathological investigation of lymph nodes for staging and guidance for therapy has become apparent. The sentinel node concept entails the possibility of multiple drainage pathways from different parts of the primary tumor, thus a patient may have more than one sentinel node. The tumor status of this node reflects the status of the regional lymphatic field and has a strong impact on prognosis. Thus, the sentinel node procedure is increasingly used in malignant melanoma and breast cancer where it has become part of the standard staging (Staging of tumors) procedure and an important factor to consider when deciding about postoperative adjuvant therapy. In fact, even insurance companies have taken the status of the sentinel node into account when determining life insurances. In a large randomized study of patients with malignant melanoma, sentinel node biopsy was compared with nodal observation demonstrating that sentinel node biopsy-guided staging provides important prognostic information and furthermore identifies patients that may benefit from immediate lymphadenectomy. In breast cancer, the introduction of sentinel node biopsy has limited the number of axillary lymph node dissections. Thus, the removal of the tumor draining sentinel node permits a smaller surgical axillary procedure and decreases the risk for different side effects including lymphedema and nerve damage. The 5-year survival rate in patients with negative sentinel node biopsies is not different from women with breast cancer having undergone axillary lymph node dissection without the presence of metastases. Thus, sentinel node biopsy is a safe and accurate metastases screening method for patients with breast cancer. The sentinel node procedure has recently been shown to be applicable in many solid tumors including colorectal cancer, urinary bladder cancer, vulvae cancer, and gastric cancer, and the procedure may be valid for staging the majority of solid tumors.

Immunology of the Sentinel Node

According to the immune surveillance hypothesis, the immune system is continuously sensitized against

developing tumors, most of which are eliminated at an early stage. Not only metastatic cells from the tumor enter into the lymphatic vessels but also antigen presenting cells, dendritic cells, which have endocytosed dying tumor cells, or debris from tumor cells containing ▶ tumor antigens, accumulate in the sentinel node. Since experimental evidence indicates that activation of naïve T cells (▶ T-cell Response) occurs within the highly specialized microenvironment of secondary lymphoid organs, that is, lymph nodes, the sentinel node may be regarded as the primary site for the immune system to encounter tumor antigens. Thus, sentinel node acquired lymphocytes are clonally expanded (Clonal Expansion) T cells recognizing tumor antigens and may therefore serve as a useful source for immunotherapy.

▶ Adoptive Immunotherapy
▶ Immunotherapy
▶ Sentinel Lymph Node

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Sentinel Node Biopsy

Definition

A minimally invasive technique to remove the first node in the draining lymphatic basin. The sentinel node is examined by the pathologist for cancer cells.

▶ Sentinel Lymph Nodes

Sentinel Vessels

Definition

Dilated episcleral vessels that provide nourishment to an underlying ciliary body tumor.

▶ Uveal Melanoma

SEP

Definition

Solitary extraosseous or extramedullary plasmacytoma.

▶ Plasmacytoma

Separin

Definition

A protease, also called separase, which cleaves the cohesin subunit Scc1/Mcd1 to induce sister-chromatid segregation. Its C-terminus is conserved; it is inhibited by association with securin at the non-conserved N-terminus.

Sequence Identification

Definition

Determination of the primary structure of a biomolecule (e.g., amino acids; DNA).

▶ Oncopeptidomics

Sequestered

Definition

In biochemistry a term, for example, for a protein, synonymous to covered up, shielded, locked away

resulting in biologicaly not available orphan receptor. It is the collective term for receptors with unidentified ligands/without known ligands.

► Arachidonic Acid Pathway

Ser/Thr-Phosphorylation

Definition

Protein phosphorylation is a post-translational modification carried out by enzymatic transfer of a phosphate group from a donor molecule to a polypeptide backbone. The enzymes that catalyze this type of reactions are known as kinases. They transfer phosphate groups either to tyrosine residues (Tyr-kinases) or to serine or threonine residues (Ser/Thr-kinases) according to defined motifs within the polypeptide.

► Cystatins

SERCA

Definition

A pump situated in the membrane of the sarcoplasmic/endoplasmic reticulum that couples ATP hydrolysis to the import of Ca^{2+} from the cytosol to the endoplasmic reticulum lumen.

► Endoplasmic Reticulum Stress

SEREX

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Definition

A method for identification and molecular analysis of antigens by recombinant expression cloning; acronym for *se*rological analysis of antigens by *re*combinant *ex*pression cloning.

Characteristics

For SEREX, a cDNA library is constructed from fresh tumor specimens, cloned into λ phage expression vectors and phages are used to transfect *Escherichia coli*. Recombinant proteins which are expressed during the lytic infection of the bacteria are transferred onto a nitrocellulose membrane. These membranes are incubated with the diluted serum of the autologous patients and screened for clones reactive with high-titered IgG antibodies. Positive clones are visualized by staining after incubation with an enzyme-conjugated secondary antibody specific for human IgG. Positive clones are subcloned to monoclonality, and the nucleotide sequence of the inserted cDNA is then determined.

The SEREX approach is technically characterized by several features:

- The use of fresh tissue obviates the need for culturing cells in vitro and therefore circumvents in vitro artifacts.
- The analysis is restricted to genes that are expressed by the tissue in vivo.
- The use of high-titered IgG antibodies in the initial screening procedure limits the analysis to such antigens which elicit a strong immune response in the host and imply a cognate T-cell help.
- The serological analysis covers the whole repertoire of proteins expressed by the respective tissue.
- SEREX uses polyspecific sera to scrutinize monoclonal antigens which are highly enriched in lytic plaques. This allows for a direct molecular definition of antigens, since the cDNA sequence of the antigen can be determined instantaneously.
- The specificity of the antigen, that is, its expression spectrum is determined by the analysis of the mRNA expression pattern by northern blots and RT PCR.
- If defined types of antigens are to be preselected for, the original SEREX approach can be modified appropriately by using biased cDNA libraries (e.g., normal testis) or modified detection systems (e.g., for IgA, IgM).

To overcome the problem of incorrect folding and the lack of posttranslational modifications, which are

both inherent problems of bacterial expression systems, a eukaryotic expression system in yeast, designated as "recombinant antigen expression on yeast surface" (RAYS), has been established. For RAYS, a cDNA library is cloned for expression as Aga2 fusion proteins on the yeast surface. After incubation with patient's sera, FACS-sorted positive clones are spotted onto 96-well-plates, re-analyzed for specific detection, and the nucleotide sequence for the cDNA insert is then determined.

Cellular and Molecular Regulation

High-titered IgG responses imply a cognate T-cell help. Therefore, in an approach of "reverse T-cell immunology" antigens detected by SEREX can be used for the definition of epitopes that are presented in the context of MHC I and MHC II. Preferably, specific T-cell reactivities are looked for in patients with high serum antibody reactivity to the respective antigen, and for many SEREX antigens both CD4 and CD8 stimulating epitopes have been identified.

Clinical Relevance

SEREX allows for an unbiased search and the direct molecular definition of immunogenic proteins based on their reactivity with autologous and allogeneic patient sera. Hence, while SEREX was originally developed for the serological analysis of human ► tumor antigens, it can be used whenever antibody reactivities against tissue antigens are suspected and neither the antibody nor the antigen is known, for example, for the identification and molecular characterization of ► autoantigens in ► autoimmune diseases. Using the RAYS approach might enable us to identify antigens that have escaped detection to date, because they elicit immune responses against post-translational modified or conformational epitopes not detectable by conventional SEREX. An international effort led by the Ludwig Institute for Cancer Research aims at defining the entire spectrum of antigens expressed by human tumors. All SEREX data are entered into the Cancer Immunome Database, which is accessible to the public (http://www2.licr.org/CancerImmunomeDB/).

► Autoantibodies

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Serine Elastase

► Neutrophil Elastase

Serine Protease

Definition

A class of enzymes that cleave peptide bonds in proteins that contain a serine residue in the active site of the enzyme. They play an important role in digestion, blood clotting, and the complement system.

► Protease Activated Receptor Family

Serine Protease Inhibitor

Definition

Large group of proteins that inhibit or antagonize the biosynthesis or actions of serine-type proteases. Serine proteases share a common reaction mechanism based on the formation of an acyl–enzyme intermediate on a specific active serine residue.

► Class II Tumor Suppressor Genes

Serine Protease Inhibitor-like Domain

▶ RECK Glycoprotein

Serine Proteases (Type II) Spanning the Plasma Membrane

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Synonyms

Matriptase: Epithin, MT-SP1, suppression of tumorigenicity 14; Transmembrane protease serine 1–13: hepsin (TMPRSS 1); Epitheliasin (TMPRSS 2); TMPRSS2 = TMPRSS4; Spinesin (TMPRSS5), matriptase-2 (TMPRSS6), matriptase-3 (TMPRSS7), distal intestinal serine protease (mouse only, TMPRSS8), polyserase (TMPRSS9), Corin (TMPRSS10), TMPRSS11–13 (human genes)

Definition

The family of type II transmembrane serine proteases consists of 14 members identified to date. They share a transmembrane orientation with an intracellular N-terminus and a basic, multidomain structure, which contains several individually folded protein modules in the extracellular domain (Fig. 1). Members of the family possess specific tissue and cell type distributions. For example, ▶ matriptase and ▶ hepsin are epithelial, while ▶ corin (TMPRSS10) is expressed in cardiac muscle cells. Matriptase, TMPRSS2, and hepsin are associated with cancer development or progression.

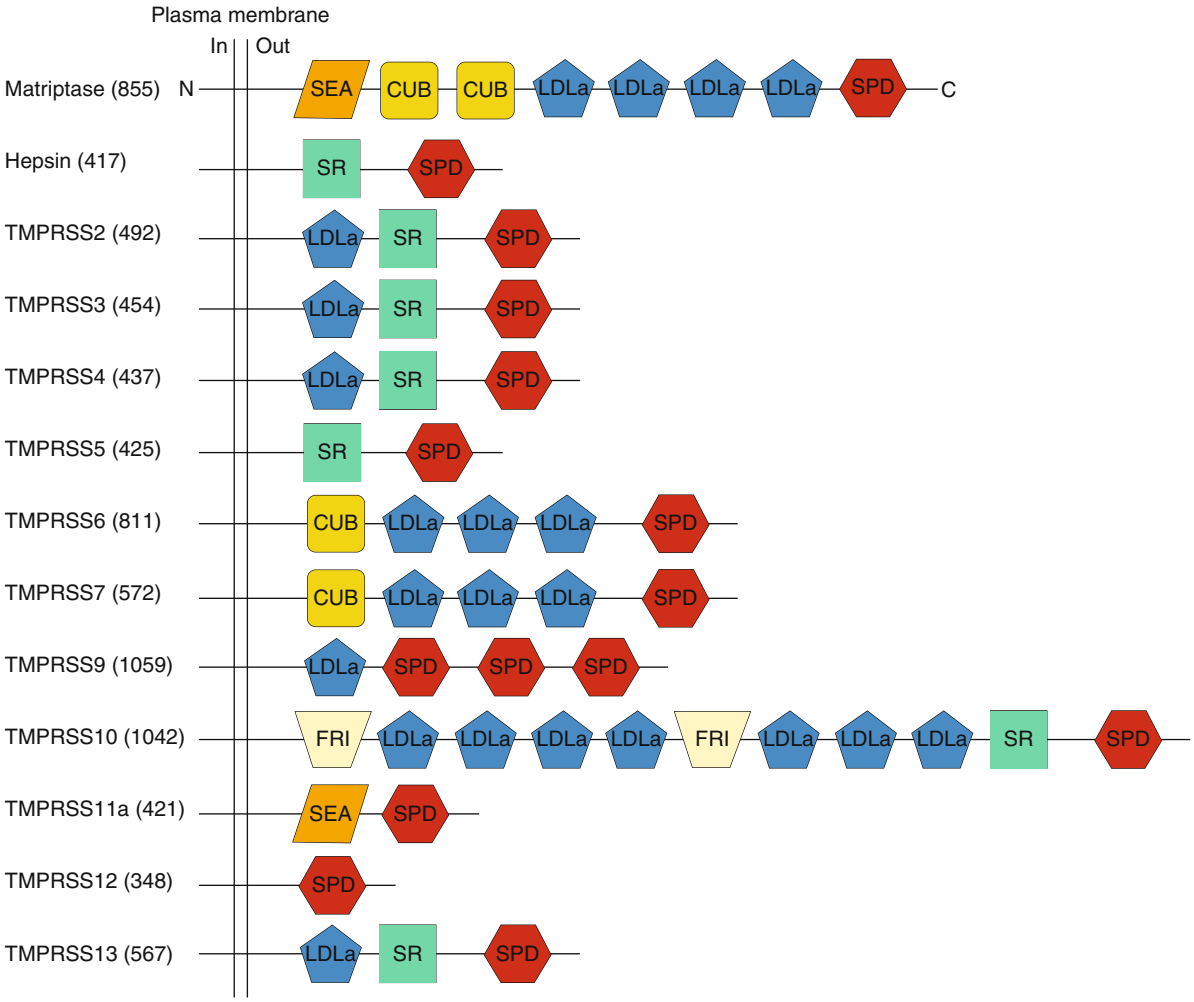
Characteristics

The serine protease domain, which is located at the C-terminal end of all family members, encodes the central function of the extracellular domain. The mechanism of activation, substrate specificity, and physiological function of the serine protease domain are best understood for matriptase and are described below. Hepsin is functionally related to matriptase in its ability to autoactivate, digest epithelial basement membrane proteins, and activate hepatocyte growth factor/▶ scatter factor. Hepsin was discovered in liver and reported as overexpressed in metastatic prostate cancer cells. TMPRSS2 is preferentially expressed in the prostate under the regulation of androgen, and the fusion of its promoter with genes from the family of ▶ ETS transcription factor plays an important role in prostate cancer development and progression (▶ TMPRSS2/ERG Fusions). TMPRSS2 is, itself, overexpressed in the majority of advanced prostate cancers where it often becomes mislocalized away from the plasma membrane.

Regulation of Activity

The activity of matriptase is regulated through autoactivation and is facilitated by lysophospholipids, steroid hormones, and the polyanionic compound, ▶ suramin. For complete activation, matriptase requires glycosylation and serine protease domains to increase protein stability, an intact ▶ LDL receptor domain, and an initial cleavage at Gly149 in the juxtamembrane ▶ SEA domain. In addition, the interaction of the LDL receptor domain with the serine protease inhibitor, hepatocyte activator inhibitor-1 (HAI-1), is critical for autoactivation. Active matriptase has a limited range of known substrates. These are ▶ HGF, urokinase plasminogen activator (▶ Plasminogen Activating System), ▶ stromelysin (Matrix metalloproteinases), ▶ protease activated receptor (PAR-2), and ▶ extracellular matrix and cell ▶ adhesion proteins.

The enzymatic activities of matriptase and hepsin are inhibited by HAI-1, a membrane-associated Kunitz type-1 serine protease inhibitor. HAI-1 shares amino acid sequence homology and a similar domain structure with HAI-2 and consists of two Kunitz domains (N-terminal KD-1 and C-terminal KD-2) intersected by a low-density lipoprotein receptor (LDLR)-like domain. HAI-1 has a remarkable and unique specificity for a serine protease inhibitor and only inhibits the serine proteases matriptase, hepsin, prostasin, hepatocyte growth factor activator (HGFA), and a phosphatidylinositol (GPI)-anchored epithelial serine protease. Through regulation of matriptase, hepsin, or HGFA



Serine Proteases (Type II) Spanning the Plasma Membrane. Fig. 1 Domain structures of the known type II transmembrane serine proteases. All are oriented with their amino terminal in the cytoplasm, a single transmembrane spanning domain, and their carboxy terminal exposed extracellularlly. They are not drawn to scale, but the total amino acid residues for each are indicated in parentheses. TMPRSS11 has known isoforms (a, b, d, e, and f) with similar domain structures and vary by only a few amino acids in length. Domains are abbreviated as: *SEA* sea urchin sperm protein, enterokinase and agrin, *CUB* complement C1r/C1s, Uegf and BMP-1, *LDLa* low-density lipoprotein receptor class A, *SPD* trypsin-like serine protease, *SR* scavenger receptor cysteine-rich, *FRI* frizzled

activity, HAI-1 controls the amount of biologically active two-chain HGF on the cell surface and the activation of the Met receptor (▶ MET).

Biological Responses

Matriptase and hepsin have been shown to facilitate the invasion and metastasis of cancer cells through proteolysis of extracellular matrix, activation of uPA and MMP-3, and increasing in ▶ angiogenesis. Transgenic mice overexpressing hepsin show a defect in the epithelial basement membrane, characterized by loss and disorganization of collagen-IV and laminin-5 (▶ Adhesion; ▶ Laminin Signaling).

Several proteases and proteolytic cascades mediate the activation of HGF (▶ Scatter Factor). Matriptase and hepsin are central proteases for HGF activation, since pro-HGF is their direct substrate. In addition, matriptase also activates uPA, and thereby magnifies the activity for HGF activation on the cell surface.

Studies in genetic mouse models reveal that matriptase is required for postnatal survival by maintaining the barrier function of the epidermis.

Matriptase deficiency phenocopies the loss of its proteolytic target, the serine protease, prostasin/CAP1/Prss8. Matriptase also plays an important role in hair follicle development, thymic homeostasis, and keratinocyte differentiation. In humans, matriptase is shed from the cell surface and is found complexed with HAI-1 in human milk.

Expression in Cancer

Matriptase is expressed on cancer cells that originate from a variety of epithelial origins and has been implicated in cancer development, progression, and ▶ angiogenesis. Matriptase translocates from a perinuclear reservoir to activation foci in cell–cell junctions and later moves to membrane protrusions in response to growth factor stimulation. On the cell surface, the balance between matriptase and hepatocyte activator inhibitor-1 (HAI-1) and HAI-2 regulates matriptase activity. The ratio of matriptase to HAI-1/HAI-2 expression increased with tumor grade and progression in ovarian, cervical, prostate, and colon cancer. The resulting increase in active matriptase on the cell surface facilitates invasion and metastasis of cancer cells.

Matriptase and HAI-1 are indirectly regulated by ▶ androgens. In a ▶ prostate cancer cell line, treatment with ▶ testosterone transiently increased matriptase expression and activity and increased shedding of proteolytically cleaved HAI-1. As a result, an increase in HAI-1–matriptase complexes occurred in the extracellular space. In normal human prostate glands, HAI-1 protein expression decreased after androgen suppressive therapy; however, in cancerous glands, intracellular protein expression was not affected by androgen.

Clinical Relevance

Pathologic deregulation of matriptase, hepsin, or TMPRSS2 can result from increased expression, activation, and from an imbalance relative to their cognate inhibitors, for example, HAI-1. Elevated expressions of matriptase and HAI-1 are associated with poor outcome in several human cancers, including node-negative breast, prostate, and ovarian cancers. In a transgenic mouse model, modest expression of matriptase in epidermis is sufficient to cause ▶ squamous cell carcinoma, and matriptase expression enhances ▶ gastric cancer metastasis in ▶ nude mice. Forced expression of hepsin in prostate cancer stimulates metastasis while inhibiting local growth. Consistent with matriptase's role in cancer, inhibition of matriptase expression or activity by matriptase-specific small molecular inhibitors, matriptase antisense, or ▶ siRNA, suppresses cancer growth in cell culture and ▶ xenograft models.

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Serine/Threonine Kinase

Definition

Enzyme that adds phosphate group to serine or threonine amino acid residue in substrate protein.

▶ Herceptin
▶ Protein Kinase C Family

Serine-Threonine Kinase Receptor-Associated Protein

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Synonyms

hMAWD: Human MAPK activator with WD repeats; STRAP; Unrip: unr-interacting protein

Definition

STRAP is a 39 kDa protein of WD-40 family involved in probable chaperoning function during the formation of multiprotein complexes shown to be active in the ▶ transforming growth factor-β (TGF-β) receptor signaling network and U-rich small nuclear ribonucleoprotein (U snRNP) assembly. ▶ Oncogenic STRAP is upregulated in human ▶ cancers and may be involved in tumor progression.

Characteristics

STRAP was first cloned from mouse embryonic cDNA library while searching for novel proteins that bind the cytoplasmic region of TGF-β type I receptor (TβRI) using yeast two-hybrid screening. Later, it was found that STRAP could associate with both type I and type II (TβRII) serine-threonine kinase receptors. Southern blot analyses demonstrate that STRAP is evolutionarily conserved from yeast to mammals. The importance of this conservation was revealed when STRAP knockout mice were generated using the gene trap mutagenesis technology coupled with ▶ microarray in the process to identify the probable targets of ▶ platelet-derived growth factor (PDGF) signaling. *STRAP* was found to be a PDGF-BB-inducible gene. The gene trap insertion results in embryonic lethality between embryonic day (E) 10.5 and E12.5. Homozygous mutant embryos had defects in ▶ angiogenesis, cardiogenesis, somitogenesis, neural tube closure, and embryonic turning. This highlights an indispensable function for STRAP during early development. Later, STRAP was also identified in humans as an interacting protein with upstream of N-ras (unr) that is involved in the internal initiation of translation of human rhinovirus RNA and is implicated in cap-independent translation.

STRAP belongs to the family of WD repeat proteins that are known to have four or more repeating units containing a conserved core of approximately 40 amino acids that mostly end with tryptophan-aspartic acid (WD). Most of them are thought to form a circularized β propeller structure. Though the underlying common function is coordinating multiprotein complex assemblies, these proteins are also involved in various cellular processes like signal transduction, transcriptional regulation, programmed cell death, RNA synthesis/processing, chromatin assembly, cell cycle progression, and vesicular trafficking. Other common examples of WD repeat proteins are the β subunit of the ▶ G proteins, TATA box binding protein–associated factor II (TAFII), ▶ apoptotic protease-activating factor 1 (APAF-1), retinoblastoma-binding protein p48 (RbAp-48), receptor-activated protein kinase C 1 (RACK1), and phospholipase A2-activating protein (PLAP), or TGF-beta receptor-interacting protein 1 (TRIP-1), which is also known to interact with TβRII.

The human *STRAP* gene is placed in chromosome 12p12.3 near the marker D12S1593. Northern blot analysis, using different tissues in mice, indicated a major transcript of 1.8 kb, though in some tissues a larger transcript was detectable. This may suggest alternative splicing of the STRAP RNA at least in some tissues. It is ubiquitously expressed in all mouse tissues with highest levels in liver and testes and less abundantly in spleen. In humans, STRAP expression has also been shown to be ubiquitous and it forms a 2 kb transcript. Both human and mouse *STRAP* genes contain 10 exons, which finally form a 350 amino acid protein migrating with an apparent mass of 39 kDa on SDS-PAGE. Murine STRAP has more than 97% amino acid identity over the entire sequence with its human version. Sequence analysis indicates that STRAP contains six WD40 repeats. STRAP shows a 55% similarity in base pairs and a 19% similarity in amino acid sequence to another known WD40 protein, TRIP-1. Some of these similarities are among the conserved amino acid residues within the WD repeats. STRAP is localized predominantly in the cytoplasm, but a good level is also present in the nucleus. It forms homo-oligomers probably through the WD repeats and this may be important for the multiprotein complex assembly. The physical interaction of STRAP with the TGF-β receptor complex raises the possibility that STRAP is a substrate of the receptors. Our findings showed that an increase in the phosphorylation of STRAP requires the kinase activity of receptors in vivo, but STRAP does not appear to be a direct substrate of the receptors during in vitro kinase assays. The C-terminal 57 amino acids are important for this phosphorylation. Eukaryotic linear motif resource (ELM) search indicates the presence of putative phosphorylation sites in the C-terminal region for the casein kinases I and II. Multiple phosphorylation sites also seem to be present inside the

different WD repeat domains for different kinases and may serve to modify the function of STRAP.

Apart from TβRI and TβRII, STRAP also binds with Smad2, Smad3, Smad6, Smad7, 3-phosphoinositide-dependent protein kinase 1 (PDK1), Ewing's sarcoma protein (EWS), hMAWD-binding protein (MAWBP), unr, microtubule-associated protein 1B (MAP1B), nuclear export factor (NXF) proteins, Gemin6, Gemin7, and 3 small nuclear ribonucleoproteins SmB; SmD2; and SmD3. Additionally, using ELM motif search, it also shows putative binding sites for other proteins like C-terminal-binding protein (CtBP), protein phosphatase 1, ▶ retinoblastoma protein (pRb), TNF receptor–associated factor 2 (TRAF-2), and glycosaminoglycans and also has binding sites for domains like class II PDZ domain, ▶ SH2 domain, and class IV WW domain. STRAP also shows the presence of multiple potential membrane targeting N-myristoylation sites, at least one of which is outside the WD repeat domains in the C-terminal region. Structural analysis of the WD repeat proteins in general shows that they act as a very rigid platform or scaffold, irrespective of the proteins with which they interact.

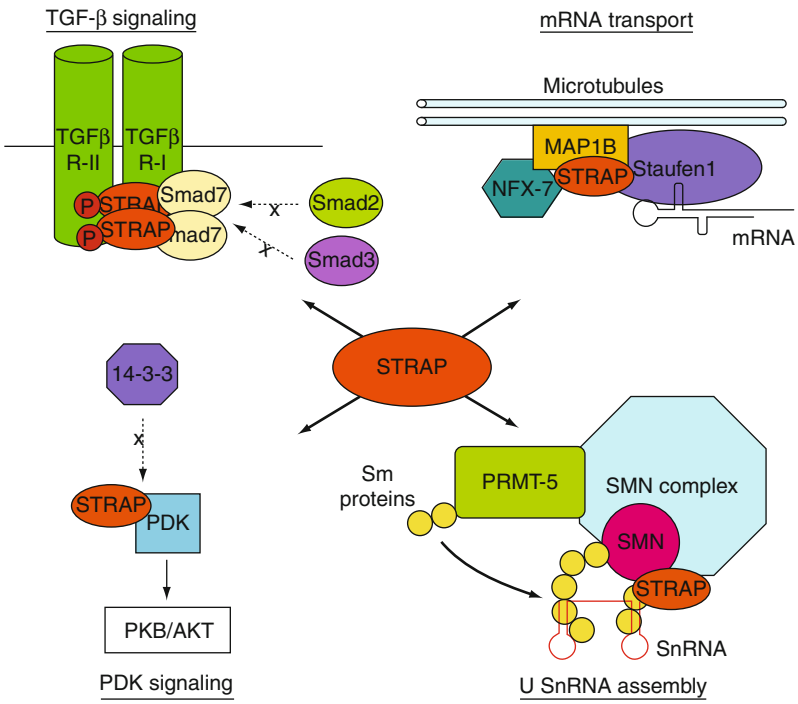
Mechanisms

STRAP binds with both TβRI and TβRII in a ligand-independent manner. It synergizes specifically with Smad7, but not with another inhibitor Smad6, in the inhibition of TGF-β signaling. STRAP stabilizes the association between Smad7 and activated receptor complex, thus assisting Smad7 in preventing phosphorylation and activation of Smad2 and Smad3 by the receptor complex. Though Smad6 is also shown to interact with STRAP, this association does not seem to interfere with bone morphogenic protein (BMP) signaling in which Smad6 acts as an inhibitor. STRAP inhibits TGF-β-induced nuclear translocation of Smad2/3 and Smad4 and as a result, activation of TGF-β responsive reporter genes including *plasminogen activator inhibitor 1 (PAI-1)* and ▶ p21 is abrogated. Downregulation of $p21^{Cip1}$ by STRAP leads to hyperphosphorylation of ▶ retinoblastoma protein (pRb). In vitro kinase assay demonstrated that overexpression of STRAP can induce extracellular signal-regulated kinase (MEK/ERK) activity in a TGF-β-independent manner. Activation of MEK/ERK pathway by endogenous STRAP was further confirmed by knocking it down using ▶ small interfering RNA (siRNA). Although STRAP is not a kinase, it may facilitate the activation of MAPK pathway by functioning as a chaperone for upstream kinases. Therefore, STRAP may inhibit activation and nuclear translocation of Smad2 and Smad3 by interacting with receptors and Smad7 and/or by activating MAPK/ERK pathway (Figs. 1 and 2).

3-phosphoinositide-dependent protein kinase-1 (PDK1) has been shown to phosphorylate and activate many members of the protein kinase A, G, and C subfamily that include protein kinase B (PKB), p70 S6 kinase, protein kinase A, and serum/glucocorticoid-regulated kinase (SGK). STRAP interacts with the catalytic domain of PDK1, and this interaction is important for the modulation of PDK1 activity. The interaction of STRAP and PDK1 is inhibited by TGF-β and induced by insulin. This induction in binding by insulin is abrogated by wortmannin, a PI3K inhibitor. The mechanism behind PDK1 activation by STRAP is thought to be due to the displacement of the 14-3-3 protein from PDK1 complex, which negatively regulates it (Fig. 1). The binding of PDK1 with STRAP also potentiates negative regulation of TGF-β-mediated transcription by STRAP. This repression occurs through increased association of Smad7 with both STRAP and TβRI. Cell survival is induced by this interaction between STRAP and PDK1 probably through phosphorylation of Bad and attenuation of ▶ tumor necrosis factor-alpha (TNF-α) induced ▶ apoptosis.

STRAP is localized in both cytoplasm and nucleus. It colocalizes and associates with the oncogenic EWS protein in the nucleus through its NH_2 and COOH termini. STRAP inhibits the interaction between EWS and p300, a protein that is a transcriptional coactivator of EWS. This results in downregulation of EWS target genes like *ApoCIII* and *c-fos*. Although TGF-β has no effect on the interaction between STRAP and EWS, TGF-β-dependent transcription is inhibited by EWS.

Unr is an RNA-binding protein that plays an important role in the initiation of HRV-IRES dependent translation of the animal picornavirus RNA, like the rhinoviral RNA. STRAP interacts with unr and is thought to be important during the translation stimulation activity. The macromolecular survival motor neuron (SMN) complex that helps in the assembly of



Serine-Threonine Kinase Receptor-Associated Protein. Fig. 1 STRAP in diverse biological functions through a role in protein complex assemblies in different signaling pathways

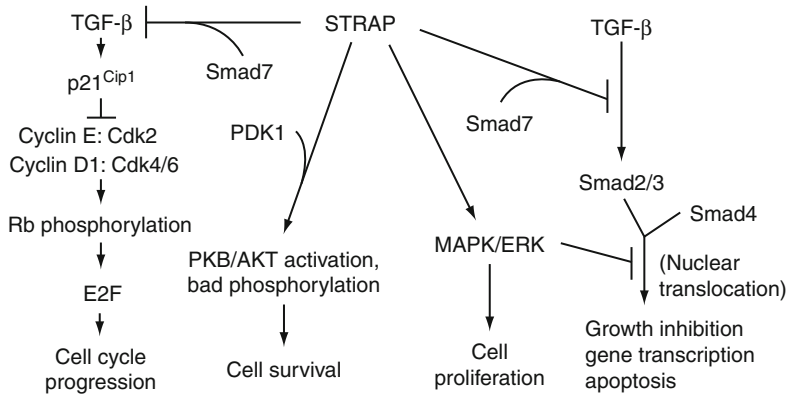
spliceosomal Uridine-rich small ribonucleoprotein (U snRNP), contains the SMN protein and six additional proteins, named Gemin2-7, according to their localization (Fig. 1). STRAP seems to be involved in this assembly through its interaction with Gemin6 and Gemin7 as well as SmB, SmD2, and SmD3 components of the SMN complex. Although STRAP is localized in both cytoplasm and nucleus, it is present predominantly in the cytoplasm and may help in the nuclear-cytoplasmic distribution of the SMN complex. The presence of STRAP in the SMN complexes was shown to be essential for U snRNP assembly. In contrast, STRAP was also shown not to be essential for this assembly by other groups.

STRAP along with EWS was shown to be present in the kinesin driven mRNA transport granules in the dendrites of murine neurons. In eukaryotes, the nuclear export of mRNA is mediated by nuclear export factor 1 (NXF1) receptors. As shown in mouse neuroblastoma N2a cells, NXF proteins bind to brain-specific-microtubule-associated proteins (MAP) such as MAP1B and also STRAP. Additionally, MAP1B also binds with STRAP. This assembly helps in the nuclear export of mRNA. In an independent setting, NXF-7 binds with MAP1B and STRAP only in the cytoplasm and colocalizes with Staufen1 containing mRNA transport granules in the neurites of these cells (Fig. 1). As in other cases, STRAP seems to play a role in the multiprotein complex assembly required for both nuclear export of mRNA and the cytoplasmic transport of mRNA containing granules along microtubules.

STRAP is a substrate for SUMO4 (a novel member of the *SUMO* gene family) sumoylation. Although the significance of this is not yet known, SUMO4 sumoylation is predicted to have a role in the regulation of intracellular stress. It will be interesting to determine whether sumoylation of STRAP has any effect on its biological functions.

Clinical Aspects

Although STRAP seems to be involved in mutually independent biological functions, there is increasing clinical and experimental evidence suggesting that STRAP acts as an oncogene. The level of STRAP is found to be altered in different ▶ cancers. The protein level is elevated in 60% of ▶ colorectal, 78% of ▶ lung, and 46% of ▶ breast carcinomas. Several lines of evidence suggest that carcinoma cells frequently lose the tumor suppressor function of TGF-β.



Serine-Threonine Kinase Receptor-Associated Protein. Fig. 2 STRAP may be involved in the progression of human cancers by activating multiple oncogenic pathways

Upregulation of TGF-β signaling inhibitors like STRAP and Smad7 and their synergistic function present a novel intracellular mechanism by which a portion of human tumors become refractory to antitumor effects of TGF-β. STRAP also exerts several other biological functions in a TGF-β independent manner that contributes to cell proliferation and inhibition of apoptosis (Fig. 2).

Ectopic expression of STRAP in different cell lines promotes cellular proliferation, induces anchorage-independent growth, and increases tumorigenicity during in vitro and in vivo experiments. Downregulation of $p21^{Cip1}$, which results in hyperphosphorylation of pRb as well as activation of MAPK/ERK pathway, may contribute to the tumorigenic effects of STRAP during tumor formation and progression (Fig. 2). As noted earlier, STRAP also has an anti-apoptotic role probably through Bad phosphorylation and inhibition of TNF-α induced apoptosis. STRAP interacts with Ewing sarcoma protein (EWS), an oncoprotein known to be involved in 80% of Ewing tumors after chromosomal translocations. Normal EWS protein is also upregulated in human cancers, which correlates with the upregulation of STRAP in 71% of colorectal cancers and 54% of lung cancers, suggesting a cooperative role of these two proteins in human cancers. In an attempt to determine whether STRAP is of prognostic value or predictive of ► chemotherapy benefit, *STRAP* gene was found to be amplified in 23% of colorectal tumors, and amplification of STRAP in patients without adjuvant chemotherapy was found to exhibit better prognosis. These patients had a worse survival when treated with ► adjuvant therapy when compared with patients without chemotherapy. In contrast, patients carrying tumors with diploidy or deletion of STRAP benefited from the treatment. These results suggest that STRAP is an unfavorable prognostic marker for 5-FU-based adjuvant chemotherapy.

Taken together, STRAP appears to facilitate multiple steps in the process of tumorigenesis and possibly during ► metastasis, and it could be a potentially important drug target for therapeutic intervention in human cancers.

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SERM

Definition

► Selective Estrogen Receptor Modulator.

SERMs

Definition

► Selective Estrogen Receptor Modulators.

Serological Analysis of cDNA Expression Libraries

Synonyms

SEREX

Definition

A method to identify cancer-specific antigens based on screening of cDNA expression libraries with serum from cancer patients.

► Cancer-Germline (CG) Antigens

Seropositive

Definition

Indicative of the presence of an antibody to a particular antigen in a patient's blood.

Serotonin

Definition

5-hydroxytryptamine, 5-HT, is a neurotransmitter synthesized in specific nerve cells (serotonergic) in the central nervous system (CNS) and is believed to play an important role in the regulation of mood, sleep, emesis (vomiting), sexuality, and appetite.

► Fluoxetine

Serotypes

Definition

A taxonomic subgroup of a microorganism or virus determined by the antigens expressed.

► Oncolytic Adenovirus

Serous

Definition

Related to an epithelial surface that produces a proteinaceous fluid. Example: The mesothelial surfaces of the pleura and peritoneum, as well as the epithelium of the fallopian tubes, are serous epithelia. In serous ovarian tumors, the neoplastic epithelium is thought to arise from the serous surface epithelium.

► Ovarian Cancer Pathology

Serpin

Definition

Serine protease inhibitor – a group of structurally related proteins, many of which inhibit proteases.

► Maspin

Sertoli Cells

Definition

Tall columnar cells found in the mammalian testis closely associated with developing spermatocytes and spermatids. Probably provide appropriate microenvironment for sperm differentiation. Its main function is to nurture the developing sperm cells through the stages of spermatogenesis. Because of this, it has also been called the "mother cell." It provides both secretory and structural support.

Sustentacular cell of the testicular seminiferous tubule. Produces various hormones, including

anti-Müllerian hormone, inhibin, and estradiol-aromatase. Rarely, similar cells can form ovarian tumors (Sertoli cell tumor, ► Sertoli-Leydig cell tumor).

Sertoli-Leydig Cell Tumor

Synonyms

Arrhenoblastoma

Definition

Is a rare type of ► ovarian cancer. The cancer cells produce and release high level of a male sex hormone testosterone, which may cause the women to develop male physical characteristics, including facial hair and a deep voice. While the tumor can occur at any age, it develops most often in young adults.

► Ovarian Tumors During Childhood and Adolescence

Serum

Definition

The clear liquid that separates from the blood when it is allowed to clot. This fluid retains any ► antibodies that were present in the whole blood.

Serum Biomarkers

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Definition

Cancer ► biomarkers are usually proteins detected in the blood, urine, or other body fluids that are either produced by the tumor itself or in response to the presence of cancer. Ideally, biomarkers should allow at least one of the following:

- ► Early detection of cancer by screening a healthy or high-risk population
- Help to confirm the diagnosis of cancer or of a specific type of cancer
- Predict prognosis
- Monitor treatment response
- Detect early recurrence

Characteristics

Most biomarkers are not specific for tumors or organs, and their levels may rise in other diseases. The diagnostic value of a tumor marker will depend on the prevalence of a disease in a population group and on its specificity (percentage of normal individuals without disease for whom a negative result is obtained) and sensitivity (percentage of tests that are correctly positive in the presence of a tumor). A cancer biomarker should be measured at a low cost, by a widely available assay with reproducible results in a specimen that is easy to access.

Alpha-Fetoprotein (AFP)

► Alpha-fetoprotein (AFP) is a fetal serum protein that shares sequence homology with albumin. It is normally synthesized during gestation by liver, yolk sac, and gastrointestinal tract. Following birth, AFP rapidly clears from the circulation. It may be elevated in patients with ► cirrhosis, viral hepatitis, drug or alcohol abuse, as well as pregnancy, and may be used for screening of fetal spinal cord defects and placental disease.

Serum levels of AFP are elevated in up to 80% of patients with ► hepatocellular carcinoma (HCC). Changes in serum AFP levels reflect the course of disease. In patients with ► cirrhosis, elevation of serum AFP may be increased for up to 18 months before symptoms of HCC manifest and may be used to assist in the diagnosis. An elevation of AFP greater than 400 ng/mL is predictive for HCC with a specificity >95%. In the setting of cirrhosis and a growing liver mass, many centers use a level greater than 1,000 ng/mL as presumptive evidence of HCC and do not require a ► biopsy.

Serum Biomarkers. Table 1 Tumor markers

Tumor marker	Primary tumor and other cancers	Other conditions with elevated levels	Use of tumor marker	
			Diagnosis	Monitoring of treatment response
AFP	*Hepatocellular carcinoma,* nonseminomatous germ cell tumor, ▶ gastric, biliary, and pancreatic cancer	Cirrhosis, viral hepatitis, pregnancy	Yes (poorly differentiated cancer of unknown origin, patients with cirrhosis and a liver mass)	Yes
β-hCG	*Nonseminomatous germ cell tumors,* gestational trophoblastic disease, gastrointestinal cancer (rare)	Hypogonadal states, marijuana	Yes (poorly differentiated cancer of unknown origin, gestational trophoblastic disease)	Yes (nonseminomatous germ cell tumor and gestational trophoblastic disease)
CA15-3	*Breast*, ▶ ovarian, ▶ lung, or prostate cancer	Benign breast disease, benign ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis	No	No (except in selected situations, i.e., follow-up by conventional clinical procedures not possible)
CA19-9	*Pancreatic and biliary tract*, ▶ colon, eosophagal, and pancreatic cancer	Pancreatitis, biliary disease, cirrhosis	Yes (selected pancreatic masses)	No
CA27-29	*Breast*, ▶ stomach, ovary, lung, or prostate cancer	Benign breast or ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis	No	No (except in selected situations, i.e., follow-up by conventional clinical procedures not possible)
▶ CA125	*Ovarian*, endometrium, fallopian tubes, pancreas, breast, colon, and lung cancer	Menstruation, pregnancy, endometriosis, pelvic inflammatory disease, liver disease, pancreatitis, peritonitis, and inflammation of the pleura	Yes	Yes
▶ CEA	*Colorectal*, breast, lung, stomach, pancreas, bladder, medullary thyroid, head and neck and liver cancer, lymphoma, melanoma	Tobacco, peptic ulcer, inflammatory bowel disease, pancreatitis, hypothyroidism, cirrhosis, biliary obstruction	No	Yes
▶ PSA	Prostate cancer	Prostatitis, benign prostate hypertrophy, prostate trauma, after ejaculation	Yes	Yes
YKL-40	Glioblastoma multiforme, ovarian, and endometrial cancer	Inflammatory disease	No	No

In men with nonseminomatous ▶ germ cell tumors (NSGCT), AFP is produced by yolk sac (endodermal sinus) tumors (▶ Yolk Sac Tumor) and, less often, embryonal carcinomas. In addition, up to 80% of mediastinal NSGCTs are associated with elevated serum AFP, regardless of histologic subtype. Whereas, only 10–20% of stage I tumors express elevated levels of AFP, 40–60% of disseminated NSGCT express abnormal levels of AFP. Although these markers may be helpful for the initial diagnosis of a ▶ testicular cancer and for prognostication, their main utility is for follow-up of disease status (Table 1).

Beta Subunit of Human Chorionic Gonadotropin (β-hCG)

▶ Beta subunit of human chorionic gonadotropin (β-hCG) is normally produced by the placenta and human fetal tissue. Elevated serum β-hCG is most commonly associated with pregnancy, gestational trophoblastic disease, and germ cell tumors. It can also be found in hypogonadal states and with marijuana use. β-hCG and AFP are elevated in up to 85% of patients with germ cell tumors, but only in about 20% of those with stage I disease. In patients with extragonadal or metastatic disease, highly elevated

serum levels of β-hCG or AFP can establish a diagnosis of nonseminomatous germ cell tumor instead of biopsy. Serial measurements of β-hCG every 2–3 months for 1 year following treatment is important as elevation is often the first sign of recurrence, indicating the need to restart treatment. β-hCG is also used to diagnose and monitor response to treatment of gestational trophoblastic disease (Table 1).

Cancer-Associated Antigen 15-3

Elevated ▶ cancer-associated antigen 15-3 (CA15-3) levels may be found in patients with ▶ breast cancers, and with much less specificity and sensitivity in patients with ▶ ovarian cancer, ▶ lung cancer, or ▶ prostate cancer. Increased levels may be associated with pregnancy and lactation, benign breast or ovarian disease, ▶ endometriosis, pelvic inflammatory disease, and hepatitis.

Because of a lack of sensitivity for ▶ early detection and lack of specificity, it has not been approved for screening of early breast cancer. However, high preoperative serum levels of CA15-3 are associated with adverse patient outcome. CA15-3, like ▶ carcinoembryonic antigen (CEA) and cancer-associated antigen 27-29 (CA27-29), is most useful for monitoring treatment response in women with breast cancer, especially in advanced disease.

Serial determinations of tumor markers after primary treatment can detect recurrent/metastatic disease with lead times of 2–9 months over clinical symptoms. However, the clinical value of this lead time remains to be determined. The ASCO guidelines do not recommend serial monitoring of CEA, CA27-29, or CA15-3 after primary therapy, except in selected situations, where patients cannot be followed by conventional diagnostic techniques.

Cancer-Associated Antigen 19-9

▶ Cancer-associated antigen 19-9 (CA19-9), an intercellular ▶ adhesion molecule, is an epitope of sialyated Lewis A blood group antigen. Elevated serum levels of CA19-9 are found typically in patients with ▶ pancreatic and biliary tract cancers, and less often in ▶ gastric cancer, ovarian cancer, ▶ colorectal cancer, ▶ lung cancer, ▶ breast cancer, and ▶ endometrial cancer. Elevated levels of CA19-9 are also found in acute cholangitis, ▶ cirrhosis, or other cholestatic diseases. Five percent of the population is genotypically Lewis-null blood type and will never produce CA19-9 antigen, even in the presence of tumoral disease. The sensitivity and specificity for pancreatic cancer has been estimated to be 80–90%. These values correlate with tumor size so that CA19-9 has limited value in identifying patients with small surgically resectable cancers (Table 1).

Cancer-Associated Antigen 27-29

Cancer antigen 27-29 (CA27-29; *synonym*: BR 27-29) is a normal epithelial cell ▶ mucin-1 (MUC1) apical surface glycoprotein. Elevated serum levels are highly associated with ▶ breast cancer. However, they can also be found in ▶ colorectal cancer, ▶ stomach cancer, ▶ renal cancer, ▶ lung cancer, ▶ ovarian cancer, ▶ pancreas, ▶ endometrial cancer, and ▶ liver cancer and in a number of noncancerous conditions, including first trimester pregnancy, ▶ endometriosis, ovarian cyst, benign kidney, liver, and breast disease. Therefore, it has no role in breast cancer screening. Serum CA27-29 levels are elevated in approximately one third of women with early-stage breast cancer (stage I or II) and in two thirds of women with stages III or IV. There is no agreement regarding the ability of CA27-29 to detect asymptomatic recurrence after curative treatment. Serial monitoring is currently not recommended by the ASCO guidelines (Table 1).

CA125

▶ CA125 is a glycoprotein that is expressed by celomic epithelium of ovaries, fallopian tubes, endometrium, and uterine cervix. Up to 90% of ▶ ovarian cancer are celomic ▶ epithelial ovarian cancer and overexpress CA125. This glycoprotein can be detected in most cases of ▶ endometrioid carcinoma, ▶ ovarian serous carcinoma, and ▶ ovarian clear cell carcinoma. Mucinous tumors, however, express this antigen less frequently. Serum levels of CA125 may also be elevated in cancers of endometrium, fallopian tubes, ▶ pancreas cancer, ▶ breast cancer, ▶ colorectal cancer, and ▶ lung cancer. Noncancerous conditions with elevated CA125 levels include menstruation, pregnancy, ▶ endometriosis, pelvic inflammatory disease, liver disease, pancreatitis, peritonitis, and inflammation of the pleura. CA125 is an important tumor marker for the diagnosis of ▶ epithelial ovarian cancer, although it is not perfectly sensitive or specific for ovarian cancer. CA125 can be used clinically to

determine response to treatment and predict relapse and survival (Table 1).

Carcinoembryonic Antigen (CEA)

CEA is a normal mucosal cell oncofetal glycoprotein involved in cell adhesion. It is overexpressed in gastric, pulmonary, breast, pancreatic, and predominantly colorectal adenocarcinomas. It may also be elevated in the serum of heavy smokers and patients with ▶ ulcerative colitis, pancreatitis, and ▶ cirrhosis. Its role as a screening tool remains uncertain because of poor sensitivity and specificity. In patients with established disease, the absolute serum level of CEA correlates with disease burden and has prognostic value. After complete resection, elevated preoperative levels of CEA return to baseline and persistently elevated levels should warrant a search for residual tumor. CEA serum levels should be checked every 3 months for at least 3 years in patients with stage II or III colon cancer if the patient is a candidate for re-resection because elevated levels give a 1.5–6 months lead time for detection of recurrence in comparison to other methods such as imaging. Early detection of asymptomatic recurrence increases the likelihood of a subsequent complete resection (Table 1).

Cytokeratin 19 Fragments

CYFRA 21-1 is a soluble fragment of cytokeratin 19 expressed in normal squamous cells. Elevated serum concentrations are found in tumors of squamous origin, including ▶ lung cancer and are associated with short patient survival. The sensitivity of ▶ cytokeratin 19 fragments (CYFRA 21-1) depends on the histological type, being lowest for ▶ small cell lung cancer (about 16–40%). It is not used in early detection or monitoring of disease and has not been incorporated in the ASCO guidelines for patients with lung cancer.

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is an ubiquitous enzyme that catalyzes the conversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+. Increased serum levels of LDH have been shown in a number of cancers, including ▶ melanoma, ▶ lymphoma, ▶ germ cell tumors, and ▶ small cell lung cancer. 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.

Matrix Metalloproteinase-9 (MMP-9)

▶ Matrix metalloproteinase-9 (MMP-9) is a member of the zinc-dependent homologous proteinase family that degrades extracellular matrix proteins, such as cell adhesion receptors, chemokines, growth factors and their receptors, and protease inhibitors. They are implicated in proliferation of endothelial and hematopoietic stem cells by inducing the release of ▶ KIT ligand.

MMP-9 expression is elevated in most tumor tissues, including ▶ ovarian cancer, ▶ thyroid carcinoma, ▶ hepatocellular carcinoma, and gastrointestinal carcinoma. Expression of MMP-9 has been detected in both ▶ glioma and its endothelial cells. Increasing levels of MMP-9 as measured by in situ hybridization and ▶ immunohistochemistry correlate with higher-grade gliomas. In ▶ glioblastoma patients, serum levels of MMP-9 are significantly higher in patients with radiologic evidence of disease compared to patients whose disease is inactive.

Placental Alkaline Phosphatase (PLAP)

Placental isoenzyme of PLAP is increased in 30% of ▶ ovarian cancer (especially serous cystadenocarcinoma), as well as ▶ endometrial cancer, ▶ lung cancer, ▶ breast cancer, and 40% of ▶ seminoma (75% in metastatic seminoma). Smokers may also have elevated PLAP levels.

Prostate-Specific Antigen (PSA)

▶ Prostate-specific antigen (PSA) is a glycoprotein that is expressed by normal prostate tissue and is overexpressed in ▶ prostate cancer. It is a sensitive and specific marker for this cancer. Levels of PSA depend upon the age and race of the patient. The predictive value for cancer is 20–30% if serum levels are greater than 4 ng/mL and 50% for values exceeding 10 ng/mL. However, 20–30% of patients with prostate cancer have levels within the normal range. The American Urological Association recommends that serial PSA measurements be obtained routinely to detect early recurrence in men who have undergone primary therapy for localized disease. PSA levels should decrease and remain undetectable after radical prostatectomy or at low levels following radiation therapy and cryotherapy. The nadir serum PSA and percent PSA decline at 3 and 6 months predict

progression-free survival in men with metastatic prostate cancer treated with ▶ androgen ablation therapy. The degree of PSA decline following second-line treatment of metastatic disease correlates with disease survival (Table 1).

YKL-40

YKL-40 is a member of the mammalian chitinase-like proteins that is highly conserved among different species. It has no enzymatic activity due to amino acid substitutions at the catalytic site. In vitro studies suggest that YKL-40 plays a role in proliferation and differentiation of malignant cells, by decreasing apoptosis of cancer cells, stimulating angiogenesis, and inducing proliferation of fibroblasts surrounding the tumor and remodeling of the extracellular matrix. Elevated serum values of YKL-40 have been reported in a variety of cancers, including breast, colon/rectum, ovary, lung, kidney, glioblastoma, and melanoma. YKL-40 levels may also be increased in other diseases with an inflammatory component.

YKL-40 is highly overexpressed in a subset of patients with ▶ glioblastoma multiforme (GBM) in comparison to low-grade ▶ glioma and detected in serum by ▶ ELISA. In a prospective study of 143 patients with high-grade gliomas, serum levels of YKL-40 were increased in patients with active disease compared to those with absence of radiographic disease. Elevated levels of YKL-40 also correlated with shorter survival in GBM patients.

In patients with ▶ endometrial cancer or ▶ ovarian cancer, elevated preoperative levels of YKL-40 may identify a subset of high-risk patients with worse clinical outcome.

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Serum-Ascites Albumin Concentration Gradient

Definition

Is a parameter of pressure reflecting presence or absence of portal hypertension. It is calculated by subtracting the albumin concentration of the ascitic fluid from the albumin concentration of a serum specimen obtained on the same day. If the gradient is less than 1.1 g/dL, portal hypertension can be ruled out.

Seven Transmembrane Receptors

Synonyms

GPCR

▶ G-protein Couple Receptor

Severe Combined Immunodeficiency

▶ Severe Combined Immunodeficiency Disease

Severe Combined Immunodeficiency Disease

Synonyms

SCID

Definition

► Severe Combined Immunodeficient Mice represent a model for the human disease.

Severe Combined Immunodeficient Mice

Synonyms

Scid mice

Definition

Spontaneous mutant mice that lack B- and T-cells. As a consequence, scid-mice are severely immunocompromised. Scid-mice do not reject implanted human cells as would normal mice. Hence, these mice allow investigators to study the growth and progression of human tumors in an in vivo model.

► Cystatins

Severe Hypoxia

► Anoxia

Sex Hormone

Definition

A chemical substance produced by a sex gland or other organ that has an effect on the sexual features of an organism. Like many other kinds of hormones, sex hormones may also be artificially synthesized.

► Androgens
► Estrogens

Sex Hormone Dependent Cancers

Definition

Prostate cancer is first dependent on androgens (e.g., testosterone) for growth and may become later hormone-independent. Breast cancer is mainly sex-hormone-dependent (estrogen and progesterone), but some breast tumors are hormone-independent and are more difficult to treat.

► Gonadotropin-Releasing Hormone

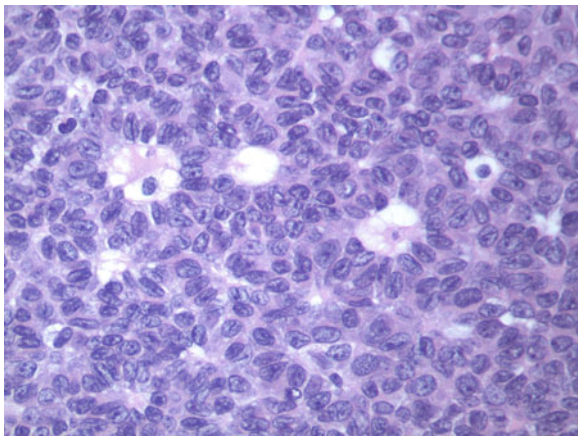
Sex-Cord Stromal Tumors

Definition

This category accounts for approximately 5% of all ► ovarian cancers. They include all tumors composed of gonadal stromal cells, granulosa cells, ► theca cells, steroid cells, and/or ► sertoli cells. Fibromas, (fibro) thecomas, sclerosing stromal tumors, ► Leydig cell tumors, and ► gynandroblastoma are usually benign. In contrast, granulosa cell tumor, ► sertoli-leydig cell tumor, steroid cell tumor "not otherwise specified" (NOS), and sex-cord tumor with annular tubules may behave in a malignant fashion.

Granulosa Cell Tumor

► Granulosa cell tumors account for 1–2% of all ovarian tumors. About 95% of them are classified as "adult type," because they most frequently occur in peri- and postmenopausal women. They may be hormonally active and typically produce ► estrogen. Grossly, granulosa cell tumors characteristically have a yellow/orange color, are solid/cystic, and often hemorrhagic. Microscopically, the tumor is composed of ovoid cells with rather scant cytoplasm. Nuclei are relatively uniform and often show prominent longitudinal grooves. The mitotic rate is low. The most characteristic architectural pattern is the microfollicular variant, which mimics ► Call–Exner bodies (small fluid-filled spaces surrounded by granulosa cells). Other patterns include solid or trabecular growth, macro-follicular, and "watered silk" patterns. The rare juvenile-type granulosa cell tumors occur in children and young women. In contrast to the adult type, they tend to form large follicles, cells have more abundant cytoplasm, and nuclei lack the longitudinal grooves. Unlike carcinomas, granulosa cell tumors may recur after a long latency interval, up to 30 years after the primary surgery (Fig.1).



Sex-Cord Stromal Tumors. Fig. 1 Granulosa cell tumor, microfollicular pattern with Call–Exner bodies. Note longitudinal nuclear grooves. Hematoxylin/eosin; original magnification x 400; ► ovarian cancer pathology

Sertoli-Leydig Cell Tumor

► Sertoli-Leydig cell tumor accounts for less than 0.5% of all ovarian tumors, about one third of patients present with signs of virilization, due to the capacity of tumor cells to produce androgens. Like granulosa cell tumors, they are yellow and solid/cystic on gross examination. Microscopically, they show an admixture of Sertoli cells and Leydig cells in variable proportions. Well differentiated, intermediate, and poorly differentiated forms are distinguished, depending upon the degree of tubule formation by the Sertoli cells. Only the intermediate and poorly differentiated variants have been reported to be potentially malignant.

Steroid Cell Tumors Not Otherwise Specified

These may produce a variety of steroid hormones, including androgens, estrogens, and corticosteroids. The typical color is yellow, but some can be brownish. Microscopically, they are composed of diffusely arranged polygonal cells with abundant esinophilic cytoplasm, distinct cell borders, and central nuclei with prominent nucleoli. Features that correlate with malignant behavior include size >7 cm, 2 or more mitoses per 10 high power fields, marked nuclear pleomorphism, hemorrhage, and ► necrosis.

Sex-Cord Tumor with Annular Tubules

SCTAT; this extremely rare tumor is associated with ► Peutz–Jeghers syndrome in 30% of cases. However, only cases not associated with this syndrome have been reported as malignant.

► Ovarian Tumors During Childhood and Adolescence

Sezary Syndrome

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Definition

Sezary syndrome (SS), named after the French dermatologist Albert Sezary (1880–1956), is a variant of cutaneous T-cell lymphoma (CTCL), a malignancy of mature T-helper cells involving the skin and blood. It is defined by the presence of an ► exfoliative erythroderma, lymphadenopathy, and evidence of neoplastic cells in the skin and blood. "Sezary" cells refer to enlarged mature CD4+ lymphocytes with hyperconvoluted nuclei. Historically, the presence of Sezary cells in the peripheral blood was a defining criterion for SS. However, an increased number of these cells can be found in several benign dermatologic conditions and is no longer the agreed upon standard. The presence of a clonally expanded population of CD4+ cells resulting in an increased CD4/CD8 >10 in the blood is considered a more accurate measure. Immunophenotypic abnormalities which support a diagnosis of SS include decreased/absent expression of T-cell antigens CD2, CD3, CD5, and CD7. The characteristic histologic features of involved skin seen in other forms of CTCL are often not present in SS and thus may not be a useful tool in its diagnosis. The etiology of SS is unknown.

Characteristics

SS is a rare disease making up less than 5% of all CTCLs. It occurs primarily in adults over the age of 60 with predominance in males.

Clinical features of SS include intractable, debilitating pruritus with an exfoliative erythroderma, edema, ▶ lichenification, thickening and fissuring of the palms and soles, nail dystrophy, hair loss, and ectropion. Malignant cell infiltrates of the skin can often lead to disfiguring features in the face (known as "leonine faces") and body.

Staging and Treatment

Staging of SS as CTCL stage III or IV depends on the degree of lymph node involvement and evidence of solid organ spread. With SS representing an advanced stage of the disease, the prognosis is usually poor with a median survival of 2–4 years. Immune compromise in these patients leads to increased bacterial infection and often to the demise of the patient.

Since SS involves both the skin and blood, systemic treatment is necessary to control the disease. The most common therapeutic tools used singly or in combination include: ▶ extracorporeal photochemotherapy (photopheresis), interferon alpha, bexarotene, denileukin diftitox, radiation with total skin electron beam therapy, leukeran, and gemcitabine.

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SFRP

Definition

Secreted frizzled related protein; family of proteins with a cysteine-rich domain (CRD) related to the Fz receptor CRD; in most cases inhibit Wnt signals by binding to Wnt proteins; often downregulated in cancer.

▶ Wnt Signaling

SH2 Domain

Definition

Src homology (SH) 2 domain is comprised of ~100 amino acid residues capable of binding specifically to a phosphorylated tyrosine residue in a signaling protein. Its binding specificity is determined through interactions between an SH2 sequence and a target sequence of a phosphorylated tyrosine and additional amino acid residues mainly from −1 to+3 positions surrounding the phosphotyrosine.

▶ SH2/SH3 Domains

SH2/SH3 Domains

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Synonyms

SH2/src homology 3; SH3 domains; Src homology 2

Definition

Proteins containing SH2/SH3 domains play critical roles in regulating the formation of intracellular signal transduction complexes. SH2 and SH3 domains recognize amino acid motifs containing phosphotyrosine and polyproline, respectively. Signaling pathways activated by SH2/SH3 domains subsequently lead to cellular responses including differentiation, proliferation, and migration.

Characteristics

SH2/SH3 domains were originally identified as conserved sequences found in the Src tyrosine kinase and a variety of other proteins. These domains are now

known to reside in hundreds of functionally diverse signaling molecules, ranging from tyrosine kinases and phosphatases to phospholipases and transcription factors.

- SH2 domains are composed of ~ 100 amino acids organized into a modular structure that recognize phosphotyrosine-containing sequences on protein tyrosine kinases and their substrates. The specificity of interaction is generally determined by the 3–4 amino acids C-terminal to the phosphorylated tyrosine position on the target protein. SH2 domain-mediated signaling events lead to cellular responses ranging from DNA synthesis to transcriptional activation.
- SH3 domains are about 50 amino acids in size and bind to target proteins containing the proline-rich consensus sequence PXXP (where P is proline and X is any amino acid). Unlike SH2 domains, SH3 domains generally remain constitutively associated with their cognate ligands, making their function less dependent on tyrosine phosphorylation. Protein interactions mediated by SH3 domains have been implicated in cytoskeletal alterations necessary for changes in cell morphology and motility.

In response to extracellular cues, SH2/SH3 domains act both intramolecularly and intermolecularly to regulate signal transduction. Their functions are well illustrated by two classical signaling cascades exemplified by the Src protein tyrosine kinase pathway and the Ras GTPase pathway.

Src is a membrane bound intracellular tyrosine kinase that contains one SH2 and one SH3 domain (the kinase domain was originally termed the SH1 domain). The post-translational phosphorylation of Src on tyrosine 527 negatively regulates kinase activity. This inhibition is the result of complex intramolecular interactions between phosphotyrosine 527 and the SH2 domain, as well the SH3 domain and a proline-rich central region (see Fig. 1). Concomitant dephosphorylation of tyrosine 527 and phosphorylation of tyrosine 416 relieves this steric block. This allows for the association of the SH2 and SH3 domains with target proteins and enhancement of Src catalytic activity. Thus, Src SH2/SH3 domains both positively and negatively regulate protein tyrosine kinase activity. Various protein tyrosine kinases utilize a similar mechanism to regulate their function.

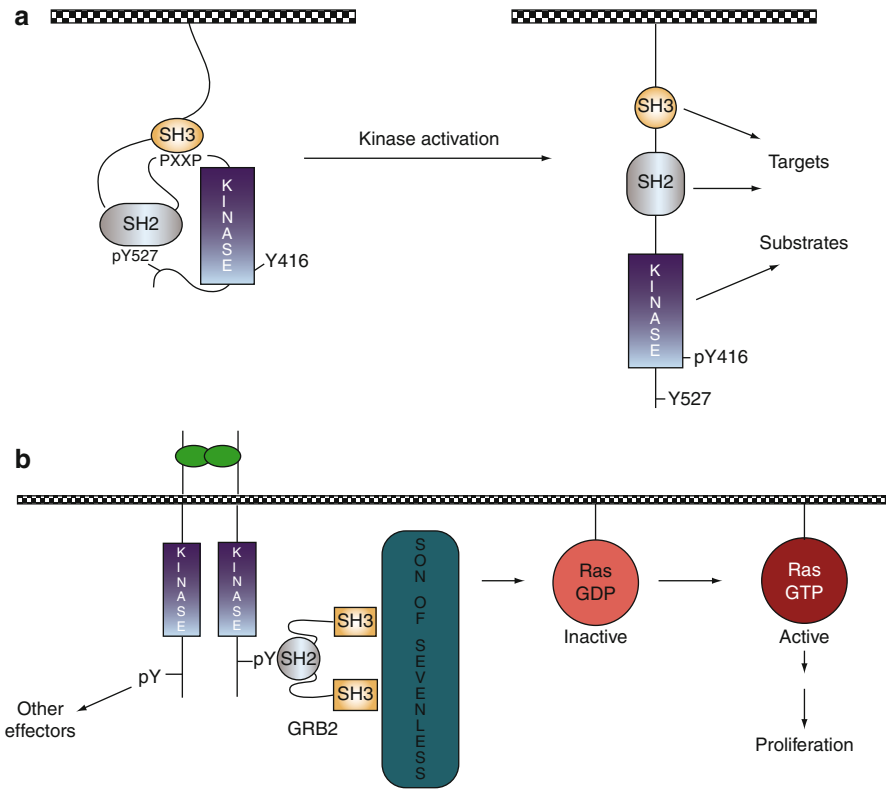
The Ras GTPase is a membrane associated growth regulator, and mutated forms are found in many human tumors. When in its GTP-bound state, Ras is considered "on" and activates multiple downstream signaling pathways. In many cases, growth factor stimulation of the Ras pathway occurs via the Grb2 SH2/SH3 protein (see Fig. 1). Grb2 is an SH2/SH3 "adaptor" protein, consisting of two SH3 domains flanking a central SH2 domain. The Grb2 SH3 domains constitutively associate with various proteins, including the son-of-sevenless (Sos) GTP exchange factor. Following growth factor stimulation, phosphotyrosine motifs on the activated receptors recruit the Grb2 SH2 domain. This event in part serves to relocalize the Grb2–Sos complex from the cytoplasm to the membrane, placing Sos in proximity to membrane bound Ras and allowing the exchange of Ras-GDP for -GTP. Based on similar observations made for other SH2/SH3 adaptor proteins, intracellular relocalization may be a common mechanism used to activate a variety of enzymatic pathways.

These examples illustrate important aspects of SH2/SH3 domain function in response to a specific stimulus. It is clear, however, that most signal transduction pathways are interconnected, and that a single extracellular cue can elicit a response involving hundreds of effector proteins. Identifying how various extracellular cues individually and combinatorally affect signaling pathways will be an important challenge for fully understanding SH2/SH3 domain functions.

Clinical Relevance

Many tumor cells possess amplifications and/or mutations in genes encoding components of the tyrosine kinase machinery. Therefore, it follows that proteins regulating cell proliferation have become central targets for drug discovery. The crystallographic structures of many SH2 and SH3 domains in complex with their various ligands have allowed for the rational design of highly specific peptidomimetic molecules. The challenge is to utilize drugs that affect only the pathological action of the specific targeted molecule, while enabling normal signal events to proceed unperturbed. For example, one can envision using SH2 and/or SH3 domain antagonists to inhibit the proliferative capacity of cells transformed due to hyperactivated or aberrantly expressed protein tyrosine kinases.

Importantly, ongoing genomic analyses will undoubtedly identify new SH2/SH3 domain-containing proteins. Characterizing the functions of these newly



SH2/SH3 Domains. Fig. 1 Pathways exemplifying SH2/SH3 domain functions. (**a**) Activation of the Src tyrosine kinase domain serves as an example of both intermolecular and intramolecular functions for SH2/SH3 domains. The Src SH2/SH3 domains serve to inhibit the kinase activity. Dephosphorylation of phosphotyrosine 527 and phosphorylation of tyrosine 416 results in a Src conformational change. The tyrosine kinase is activated and the protein associates with downstream substrates via its SH2 and SH3 domains. (**b**) The activation of the Ras GTPase by various receptor tyrosine kinases is a classic example of SH2/SH3 domains providing an intermolecular link between critical signal transduction components. Receptor autophosphorylation leads to relocalization of constitutively associated Grb2-Sos, leading to GTP exchange and Ras activation

identified effectors and linking them to the pathogenesis of specific cancers and other disease states will be a worthwhile goal. The use of novel proteomic techniques in combination with classical cell biology will greatly aid this process.

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SH2/src Homology 3

► SH2/SH3 Domains

SH3 Domain

Definition

Src-homology domain 3 is a protein domain of about 50 amino acid residues that binds to specific proline-rich sequences and is a modular protein interaction domain commonly found in signal transduction proteins. It binds amino acid recognition sequences that

include proline-X-X-proline in target proteins. It is seen often in adapter proteins that coordinate the function of macromolecular complexes with the actin cytoskeleton.

► SH2/SH3 Domains

SH3P9

► Bin1

SHANK

Definition

Refers to large adaptor proteins that can self-associate and bind a variety of different proteins. It is best characterized in neurons where they serve to organize receptor proteins at the synapse; shank proteins are also found in epithelia cells where their function is currently unclear.

► Cortactin

Shark Cartilage

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Definition

A dietary supplement derived from cartilage obtained from sharks, commonly hammerhead (*Sphyrna lewini*) and dogfish (*Squalus acanthias*) sharks.

Characteristics

Shark cartilage is a popular dietary supplement that traditionally has been proclaimed to be able to prevent and/or treat established cancer. In the early part of the twenty-first century, it has been one of the ten most common dietary supplements used among cancer patients. It has been suggested that the widespread popularity of this product has partly contributed to an increase in shark fishing, with a resultant sharp, ≥75%, decline in the world shark population in the past 15 years.

Shark cartilage is available as a capsule (taken orally), powder (for oral use when mixed with juice or as an enema), and also as an intravenous preparation. The recommended doses can vary from 500 mg to several grams per day. The adverse effects are predominantly gastrointestinal, such as nausea, abdominal pain, and diarrhea, and even hepatitis. Given its potential antiangiogenesis properties, it has been advised that pregnant females should not consume the product.

The popularity of shark cartilage soared in the early 1990s after a book (*Sharks Don't Get Cancer*) by William Lane postulated that sharks do not get cancer due to the high proportion of cartilage in their body, suggesting that shark cartilage has chemopreventive properties. The authors predicted that "As events and research continue to unfold, shark cartilage may prove to be the first momentous step toward preventing and conquering cancer." However, subsequent research has not substantiated this prediction. Moreover, sharks do develop cancers, although the rate of cancer incidence among sharks is unclear.

A number of studies have been conducted to evaluate the potential cancer treatment properties of shark cartilage. One of the first reports of a human trial was featured on the TV show "60 Minutes" wherein results from two trials were reported, receiving considerable media coverage. In one trial, researchers from Cuba claimed that 16 weeks of shark cartilage was effective in treating cancer in 3 of the 15 patients that received the treatment. In the other trial, researchers at Simone Protective Cancer Center (NJ) claimed 4 of the 20 cancer patients consuming shark cartilage showed partial or complete response with use of the product. However, these studies had serious methodological flaws (9%), the results suggested only a small response rate (20%), and the results were never reported completely in a peer-reviewed PubMed-indexed journal. In a well-designed phase II study, involving 60 patients with advanced cancer, 1 g/kg/day of shark cartilage was reported to be ineffective in reducing the progression of disease, to have no beneficial effect on quality of life, and to have substantial gastrointestinal toxicity in about 25% of the patients. Two other

phase II studies reported similar negative results with the use of shark cartilage among cancer patients.

Likewise, no suggestion of efficacy of shark cartilage was seen in a relatively small randomized placebo-controlled, double-blinded clinical trial. Among the 83 patients involved in this trial (42 receiving shark cartilage and 41 receiving placebo), there was no difference in overall survival or quality of life among the two groups. The shark cartilage and its identical smelling, tasting, and appearing placebo were not well tolerated by the study patients, with ~50% of subjects stopping the study medication within a month of initiation. Given the presently available evidence, shark cartilage has generally been considered to be an ineffective agent for cancer, and promotion of its use as an anticancer agent has been widely criticized.

While shark cartilage supplementation has been found to be ineffective as a chemoprevention or anticancer agent in clinical trials, laboratory research has identified a few compounds isolated from shark cartilage that could potentially have antiangiogenesis properties. In the late 1970s, Langer et al. first isolated a factor from shark cartilage that was found to strongly inhibit the growth of new blood vessels toward tumors and thereby restricted tumor growth. Similar findings have been reported independently by other authors. One of the standardized extract shark cartilage products obtained from dogfish (AE-941, or Neovastat) has been reported to be a promising agent. In a phase II trial among 80 patients with lung cancer, those receiving higher doses (>2.6 ml/kg/day) of AE-941 were reported to have better survival, compared to patients receiving lower doses (median, 6.1 months vs 4.6 months; $P = 0.026$). Neovastat is postulated to exert its antiangiogenesis effects by multiple effects, including inhibition of tissue metalloproteinases, modulation of VEGF (vascular endothelial growth factor), and enhancement of endothelial cell apoptosis. This product was tested in a large phase III lung cancer clinical trial funded by the United States National Cancer Institute. However, the early results of this trial did not support that this agent had any benefit.

Thus, in total, currently available study data do not support the efficacy of shark cartilage as a chemopreventive or anticancer agent. Given the lack of established efficacy, its toxicity, and the environmental impact related to harvesting sharks, the use of shark cartilage among patients with cancer should be discouraged.

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Shc

Definition

An adaptor protein that contains both phosphotyrosine-binding and Src homology 2 ▶ SH2 domain. It is a prominent effector of protein–tyrosine kinase signaling.

▶ Insulin Receptor

Shedding of Cells

▶ Exfoliation of Cells

Shimada System

Definition

Is the clinical classification of neuroblastoma.

Short Hairpin RNA

▶ RNA Interference

SHP-1

Definition

Src homology 2 domain-containing tyrosine phosphatase, non-receptor type 6, PTPN6.

► Erythropoietin
► SH2 Domain

SHP-2

Definition

The Src homology 2 (SH2) domain-containing tyrosine phosphatase which plays a positive role in transducing signals from receptor protein tyrosine kinases down to the MAPKs, Ras, ► PI3K, or the Src family kinases.

► Major Vault Protein
► SH2 Domain

SHR

► Steroid Hormone Receptors

ShRNA

► RNA Interference

SIADH

Definition

The syndrome of inappropriate antidiuretic hormone hypersecretion (SIADH) is a condition mostly found in patients diagnosed with ► small cell lung cancer, pneumonia, ► brain tumors, head trauma, strokes, meningitis, and encephalitis. This is a syndrome characterized by excessive release of antidiuretic hormone (ADH or ► vasopressin) from the posterior pituitary gland or another source. The result is hyponatremia, and sometimes fluid overload. The normal function of ADH on the kidneys is to control the amount of water reabsorbed by kidney nephrons. ADH acts in the distal portion of the renal tubule (Distal convoluted tubule) as well as on the collecting duct and causes the retention of water, but not solute. Hence, ADH activity effectively dilutes the blood (decreasing the concentrations of solutes such as sodium). As a background, serum sodium concentration is regulated by the balance of water intake, renal filtration, and reabsorption of sodium, and antidiuretic hormone (ADH) – mediated water conservation by the collecting duct. Water balance is normally mediated by thirst, the secretion of antidiuretic hormone (also known as vasopressin), the feedback mechanisms of the renin-angiotensin-aldosterone system, and renal handling of filtered sodium and water. Disorders in any one of these components of sodium balance can result in hyponatremia. ADH is secreted by supraoptic and paraventricular nuclei in the hypothalamus and transmitted via the neuronal axons to the posterior pituitary where it is secreted. It is released when a decrease in the effective circulatory volume is sensed by vascular baroreceptors primarily located in the large arterial vessels. The key action of ADH in the kidney is to trigger the insertion of aquaporin-2 into the principal cells of the collecting duct. Aquaporins' selective permeability allows water reabsorption and consequently urine concentration.

Sialoglycoconjugates

Definition

Are sugar chains terminally modified by an acidic sugar (sialic acid).

► Adhesion

Sialyl Lewis-a/x Determinants

Definition

Are blood group antigens that comprise type 1 (Lewis a) and type 2 (Lewis X) carbohydrates. Lewis a and Lewis x are regarded as tumor-associated markers, and these antigens and their derivatives interact with

E-selectin, mediating cancer cell-to-endothelial cell adhesion.

► E-selectin-Mediated Adhesion in Cancer

Sialyltransferases

Definition

Enzymes that catalyze addition of sialic acid (neuraminic acid).

► Lewis Antigens

Sicca Complex

Definition

Refers to dryness of the eyes and mouth and is a chronic, inflammatory disease that affects the exocrine glands. The primary targets appear to be the lacrimal and salivary gland duct epithelium.

► Sjögren Syndrome

Sicca Syndrome

► Sjögren Syndrome

Signal Sequence

Synonyms

Signal peptide

Definition

Is a *N*-terminal, short (18–26) amino acid sequence that acts like a zip code in nascent polypeptides and determines their subcellular targeting. A signal sequence is a protein region with which a protein can be directed to the appropriate cellular compartment within a cell; they initiate co-translational transfer through the membrane of the endoplasmic reticulum (ER). Proteins are often synthesized in an immature version (pre-protein) that is larger than the mature functional form. This is due to the presence of N-terminal amino acid stretches, referred to as leader sequences. The pre-protein is a transient precursor, since the leader sequence is cleaved off during protein processing. This signal sequence is a short stretch of 15–30 amino acids that mediate the transfer of any attached polypeptide to the endoplasmic reticulum. It provides the means for the ribosomes to attach to the ER membrane (ER regions with associated ribosomes are called "rough ER"). As soon as the first few amino acids of the protein have been synthesized, the nascent protein chain can be co-translationally transferred to the membrane.

The signal hypothesis proposes that the N-terminus of a secreted protein has a signal sequence whose presence marks it for membrane insertion. Once the protein chain is well inserted into the membrane, the signal sequence is cleaved off by a protease within the membrane and the protein can then enter or even pass through the membrane. This principle does not hold for nuclear proteins, which are synthesized in their mature form.

Signal Transducer and Activator of Transcription

► Signal Transducers and Activators of Transcription in Oncogenesis

Signal Transducers and Activators of Transcription in Oncogenesis

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Synonyms

Signal transducer and activator of transcription; STAT

Definition

Signal transducer and activator of transcription (STAT) proteins comprise a family of latent ▶ transcription factors that reside in the cytoplasm and have been shown to control normal ▶ cytokine and ▶ growth factor–induced responses. In response to extracellular signals, such as cytokines or growth factors, STATs are activated through ▶ phosphorylation by ▶ tyrosine kinases. Subsequently, these activated dimers translocate (▶ translocation) into the nucleus where they bind to specific DNA sequences and regulate the transcription of cellular genes. Thus, STATs perform dual roles in transmitting receptor-generated signals from the cytoplasm to the nucleus and regulating cellular genes necessary for ▶ ligand-induced biological responses.

Characteristics

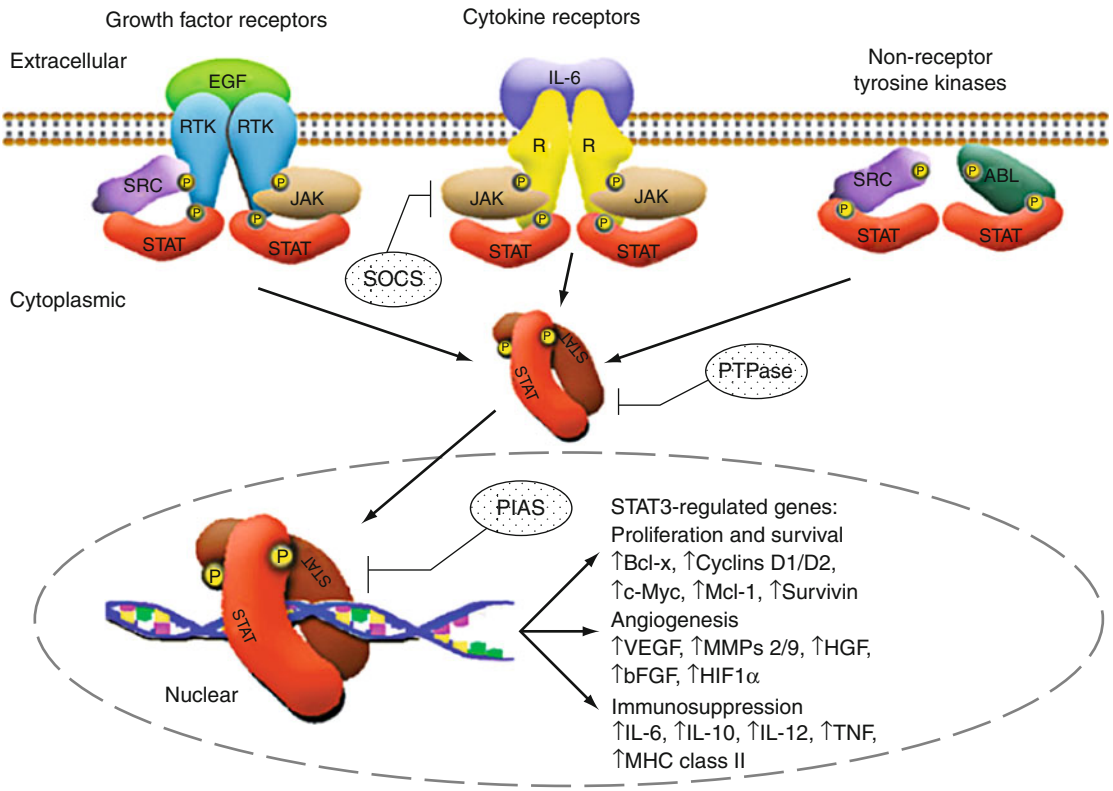
All multicellular organisms possess complex networks of molecular messengers that coordinate vital organ functions. Among the most common mechanisms used by multicellular organisms to ensure appropriate timing and duration of essential biological processes are production and secretion of cytokines and growth factors. Cytokines and growth factors control a wide variety of fundamental biological processes in diverse cell types, including immune responses, cellular differentiation, proliferation, and programmed cell death (▶ Apoptosis; ▶ Apoptosis Signaling). Although differences exist between the biological processes and cell types regulated by cytokines and growth factors, these ligands possess some overlapping functions and share remarkably similar mechanisms of signal transmission. Cytokines and growth factors generally elicit a biological response by binding to receptor proteins located on the outer cell surface. Binding of these ligands to their specific receptors induces a change in the ability of the receptor to recruit and activate cytoplasmic signaling molecules that participate in signal transmission. Modulation of the activity of these cytoplasmic signaling proteins by the receptors initiates a cascade of biochemical signaling events, which ultimately lead to changes in nuclear gene expression that mediate the biological responses (▶ Signal Transduction). Cytokine- and growth factor–induced processes are normally tightly controlled to ensure proper functioning. Aberrant functioning of these pathways results in deregulated signaling and is associated with development of a variety of pathological conditions including ▶ cancer.

STATs in Cytokine- and Growth Factor–Induced Signaling

In response to cytokine- or growth factor–induced activation of signaling, cytoplasmic STATs become phosphorylated on specific tyrosine residues by receptors or receptor-associated proteins possessing tyrosine kinase activity (see Fig. 1) (▶ Receptor Tyrosine Kinases). Tyrosine phosphorylation of STAT proteins allows STAT dimers to rapidly translocate into the nucleus. Once in the nucleus, STAT dimers bind to DNA and control the transcription of specific cellular genes. Under normal circumstances, the biological responses elicited by cytokines and growth factors are transient, and transmission of signaling is terminated through several inhibitory mechanisms, including (1) protein tyrosine phosphatases (▶ PTPase) that dephosphorylate and inactivate STATs, (2) ▶ suppressor of cytokine signaling (▶ SOCS) family of proteins that prevent STAT activation by tyrosine phosphorylation, or (3) protein inhibitors of activated STATs (▶ PIAS), a group of proteins that decrease transcription by binding to and thereby inhibiting STATs. While tyrosine phosphorylation of STAT proteins has been shown to be essential for cytokine-induced signals, activated cytokine receptors generally do not possess kinase activity and are not capable of directly phosphorylating STAT proteins on tyrosine. Cytokines induce activation of STATs indirectly through activation of receptor-associated tyrosine kinases of the Janus kinase (▶ JAK) family. After cytokine binding, STATs are phosphorylated by the receptor-associated JAKs, allowing signal transmission to proceed. Activation of STAT proteins has also been shown to be required for growth factor–induced cellular responses. However, the majority of growth factors that induce STAT activation bind to receptors that possess intrinsic tyrosine kinase activity, and tyrosine phosphorylation of STATs can be directly mediated by the activated receptor protein, receptor-associated JAK proteins, or by additional non-receptor tyrosine kinases such as Src.

Bioactivity

Significantly, constitutive tyrosine phosphorylation and activation of STATs has been detected in cells



Signal Transducers and Activators of Transcription in Oncogenesis. Fig. 1 Constitutive activation of STATs by tyrosine kinases. Growth factors (e.g., EGF) and cytokines (e.g., IL-6) stimulate activation of receptor-intrinsic (RTK) or receptor-associated (JAK, Src) tyrosine kinases. Activated tyrosine kinases phosphorylate receptors to provide docking sites for unphosphorylated STATs. Once recruited, STATs themselves become tyrosine phosphorylated. Oncogenic tyrosine kinases such as SRC and ABL can also phosphorylate STATs independently of receptor engagement. Phosphorylated STAT dimers translocate to the nucleus, bind to specific DNA elements, and regulate gene expression. In normal cells, STAT activation is transient and tightly regulated by inhibitory proteins, including PTPase, SOCS, and PIAS. In cancer cells, constitutive activation of STATs, in particular Stat3 and Stat5, is associated with changes in the expression of genes that control fundamental cellular processes subverted in oncogenesis. *RTK* receptor tyrosine kinase, *R* receptor. See text for details [2]

that have undergone malignant ▶ transformation in response to expression of a variety of viral oncoproteins, which are often themselves constitutively activated tyrosine kinases (▶ Cell Transformation; ▶ Oncogene). Consistent with a role for STATs in oncogenesis, regulation of gene expression by one STAT family member, Stat3, has been shown to be required for induction of malignant transformation by the oncogenic Src tyrosine kinase. Furthermore, expression of a constitutively activated mutant of Stat3 can induce malignant transformation of specific cell types. These results demonstrate that, in addition to signals important to normal cellular functions, activated STATs can also transmit signals critical to oncogenic transformation.

STAT Activation During Progression of Human Cancer

Constitutive tyrosine phosphorylation and activation of STATs, in particular Stat3 and to a lesser extent Stat5, has been shown to occur frequently in a variety of human tumor types including leukemias, lymphomas, multiple myeloma, head and neck cancer, lung cancer, prostate cancer, renal cell carcinoma, colon carcinoma, melanoma, and breast cancer. Frequent activation of specific tyrosine kinase signaling pathways, including activation of growth factor receptor tyrosine kinases, has also been detected in many of these tumor types. These findings suggest that constitutive activation of STAT proteins results from the constitutive activation of tyrosine kinases. Moreover,

because STAT proteins control the transcription of nuclear genes involved in growth control, constitutive activation of STATs may transmit signals essential for oncogenic signaling and contribute to ▶ tumor progression. Among the genes that are regulated by STATs are genes involved in controlling cell cycle progression and programmed cell death (including ▶ Cyclins D1/D2, ▶ c-Myc, ▶ Mcl-1, ▶ BCL-x, and ▶ Survivin) (▶ Cyclin D; Myc Oncogene; Mcl Family; Survivin), genes that are involved in immunosuppression (interleukins-6, -10, -12, TNF, and ▶ MHC class II) (MHC), and genes that also participate in the regulation of angiogenesis (such as ▶ VEGF, ▶ MMPs 2 and 9, ▶ HGF, ▶ bFGF, and ▶ HIF1α) (▶ Vascular Endothelial Growth Factor; Matrix Metalloproteinases; Fibroblast Growth Factors; Hypoxia Inducible Factor-1). Thus, constitutive activation of STATs – in particular Stat3 and Stat5 – in human tumors may contribute to progression of cancer by multiple mechanisms, including proliferation and survival of the tumor cell itself, induction of angiogenesis and suppression of immune cells in the tumor ▶ microenvironment (immunosuppression).

Targeting STATs for Cancer Therapy

Inhibition of STAT signaling has repeatedly been demonstrated to result in growth inhibition and induction of apoptosis in tumor cells harboring constitutive activation of Stat3 or Stat5. The observed dependence of certain tumors on constitutive STAT activation for survival has wide implications for cancer therapy, offering the potential for preferential tumor cell killing.

Targeting the Tumor Cell

Persistent Stat3 and Stat5 signaling within tumor cells directly participates in the development and progression of human cancers by either preventing apoptosis, inducing cell proliferation, or both. Targeting of tyrosine kinase activity upstream of STAT pathways has drawn special attention because of the recent development of tyrosine kinase-selective inhibitors. However, one potential drawback of tyrosine kinase inhibitors is that they block multiple downstream signaling pathways in addition to STAT proteins, increasing the likelihood of undesirable toxicity. Since STAT activation is a point of convergence for many different tyrosine kinases, STATs themselves represent promising targets for the development of cancer drugs. One of the attractive features of STAT proteins for cancer therapy is that there are only two molecular targets, Stat3 and Stat5, as opposed to a multitude of tyrosine kinases. Antisense oligonucleotides and small-interfering RNA (▶ siRNA) that target STATs are just two of the many possibilities to interfere with aberrant STAT signaling (▶ Antisense DNA Therapy). Alternatively, small-molecule inhibitors are being developed to directly interfere with STAT proteins (▶ Drug Design). Recent progress has been made in design of short peptides, ▶ peptidomimetics, and other non-peptide compounds that effectively block Stat3 dimerization and DNA-binding activity both in vitro and in vivo. Importantly, these compounds inhibit cell transformation mediated by activated Stat3 and provide the basis for development of novel drugs for potential cancer therapy.

Targeting the Tumor Microenvironment

Recent evidence suggests that Stat3-positive cancer cells can crosstalk with adjacent "normal" immune cells for the purpose of modifying the tumor microenvironment in favor of the cancer cell. Stat3-positive cancer cells can suppress the production and secretion of pro-inflammatory factors that otherwise would stimulate an antitumor immune response (▶ Inflammation; ▶ Inflammation in Cancer). At the same time, cancer cells can secrete immune-suppressive factors that act on immune cells. Some of the factors secreted by tumor cells, such as IL-6, IL-10, and VEGF, act on both tumor cells and adjacent immune cells by maintaining Stat3 activation in the tumor cell and simultaneously inducing Stat3 activation in immune cells. It has been shown for many immune cells in the tumor microenvironment that Stat3 acts as a negative regulator of immune-stimulating molecules. Stat3 activation in immune cells has been found to inhibit the antitumor function of natural killer cells (NK), ▶ dendritic cells (DC), T-cells, and ▶ macrophages, among other cell types (Natural Killer Cells; Dendritic Cells; ▶ T Regulatory Cells; Macrophages). In the case of macrophages, for example, it has been demonstrated that ablation of both Stat3 alleles leads to increased levels of several pro-inflammatory cytokines similar to those found in cancer cells in which Stat3 signaling is inhibited. Moreover, simultaneous inhibition of Stat3 signaling in immune cells and tumor cells induces a more pronounced antitumor effect, demonstrating the additive nature of blocking Stat3 signaling in both tumor cells and normal cells of the tumor microenvironment. In addition to negatively regulating immune

cell function, some of the factors secreted by Stat3-positive cancer cells, such as VEGF, bFGF, HGF, and MMPs, are pro-angiogenic and facilitate tumor growth by increasing blood supply (tumor angiogenesis).

Summary

Because STATs play essential roles in regulation of cell proliferation and survival, the frequent activation of these proteins in human tumors suggests that they have essential roles in the malignant progression of human cancers. Furthermore, recent studies indicate that STATs also propagate signals between tumor and normal cells in the tumor microenvironment, thereby promoting tumor immune evasion and tumor angiogenesis. Detection of constitutively activated STATs could provide an important marker for activation of oncogenic tyrosine kinase signaling pathways during tumor progression. Currently, characterization of the full complement of STAT-regulated genes that participate in growth regulation and tumorigenesis remains a very active and important area of investigation (► Microarray (cDNA) Technology). These studies have the potential to provide new information critical to understanding how fundamental cellular processes are subverted in tumor cells. Finally, activated STATs in human tumors provide promising targets for the design of novel therapeutics that block STAT functions involved in stimulation of proliferation, prevention of cell death, and induction of immune evasion and tumor angiogenesis. Early results in this area suggest that targeted inactivation of STAT proteins may be an effective approach to halting the growth of various types of human tumor cells. Inhibition of STAT signaling in tumors is predicted to impart therapeutic benefit by inducing growth arrest and apoptosis as well as suppressing tumor angiogenesis and immune evasion. However, targeting STAT proteins for therapeutic intervention in cancer remains to be fully explored.

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Signal Transduction

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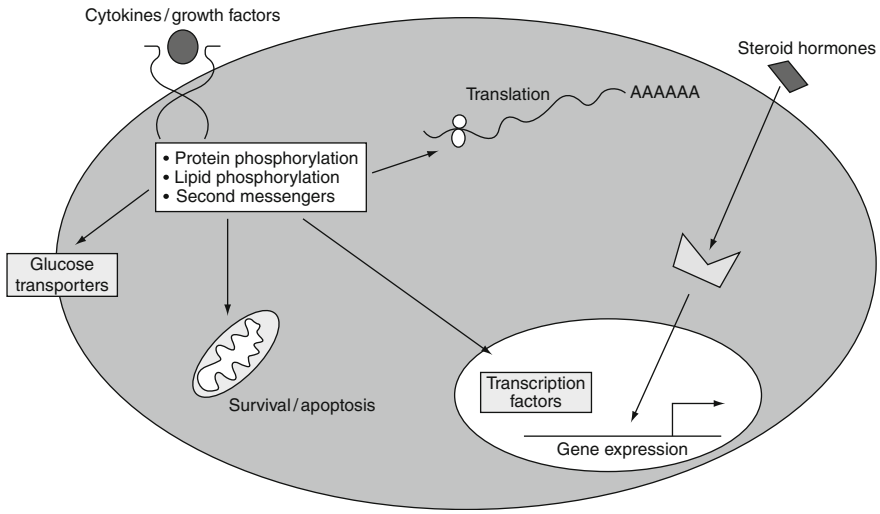
Definition

Signal transduction is the process by which information from extracellular stimuli is propagated within a cell to alter cellular function. These signals frequently modulate gene expression, but they may also directly regulate processes such as cellular survival, metabolism, and macromolecular synthesis.

Characteristics

Starting shortly after fertilization, the phenotype of a cell becomes regulated largely by extracellular cues. Soluble factors such as cytokines and hormones, direct contact between cells, and interactions with the extracellular matrix all provide critical information affecting cellular morphology, proliferation, differentiation, and survival. A central question in cellular physiology is how these extracellular processes are converted into signals that can modulate the phenotype of a cell. A number of mechanisms have evolved by which such stimuli arising outside of a cell can regulate the expression of genes in the nucleus and other critical cellular processes. Several processes are used in a variety of ways by mammalian cells to transmit these signals. Each of these processes is also balanced by feedback mechanisms to modulate the cellular response. In essentially every kind of cancer, an abnormality in one or more signal transduction pathways provides a cell with signals for survival, proliferation, or self-renewal in the absence of the normal physiologic cues. Thus, elucidating signal transduction pathways is critical to understanding tumor pathogenesis



Signal Transduction. Fig. 1 Through signal transduction, extracellular stimuli are converted into biochemical processes that control cellular function. Steroid hormones bind to intracellular receptors which then directly control gene expression. Cytokines, growth factors, and peptide hormones interact with cell surface receptors which then initiate cascades of protein phosphorylation, lipid phosphorylation, and second messenger generation. These mediators then regulate gene expression or directly alter processes such as cellular metabolism, protein translation, and apoptosis

and tumor biology. Furthermore, among the most important advances in cancer therapy in recent years has been the introduction of signal transduction inhibitors that specifically target these molecular defects. These agents hold the potential to have much greater efficacy and much less toxicity than conventional cytotoxic agents.

Receptors

The ability to respond to the extracellular environment is mediated by receptors (Fig. 1). Although dozens of receptor molecules have been described in mammalian cells, from a functional perspective they can be grouped into three categories. First are receptors that are coupled to kinases. Activation of these receptors initiates the transfer of the terminal phosphate of ATP (or GTP) to an amino acid residue in a protein. Most receptor-associated protein kinases have specificity for phosphorylating tyrosine residues. This often leads to the subsequent activation of protein kinases that can phosphorylate serine or threonine residues, or lipid kinases. Thus, the interaction of a soluble ligand with an extracellular receptor can lead to the amplification of the signal through kinase cascades that can modulate cellular function through a number of mechanisms. Receptors may contain intrinsic tyrosine kinase activity, as is the case for polypeptide growth factor receptors such as the receptors for insulin, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and others (▶ Receptor Tyrosine Kinases). Receptors may lack tyrosine kinase activity of their own, but associate with cytoplasmic tyrosine kinases which become activated by the binding of a ligand. Cytokine receptors, such as receptors for interferons, interleukins, oncostatin M, and leukemia inhibitory factor (LIF) associate with tyrosine kinases of the Janus kinase family whose activation propagates the signal.

Activation of receptor-associated tyrosine kinases leads to two major effects. First, it allows the tyrosine phosphorylation of specific substrates whose activity is regulated by this modification. For example, transcription factors of the ▶ signal transducers and activators of transcription (STAT) family are found inactive in the cytoplasm under basal conditions. Once phosphorylated by a tyrosine kinase, STATs form dimers, which allows them to translocate to the nucleus, bind to specific DNA regions, and regulate the transcription of target genes. The second effect of tyrosine kinase activation results from the phosphorylation of the receptor itself and associated proteins. These phosphotyrosine residues form potential docking sites for proteins that have structural domains, such as the ▶ Src homology 2 (SH2) domain, that allow them to specifically bind to phosphorylated tyrosine residues. This can trigger a series of interactions with other

proteins in which tyrosine phosphorylation of proteins performs a "scaffolding" function, allowing other proteins to associate, which then activates their function.

In addition to the phosphorylation of proteins, kinases that can phosphorylate lipids play a key role in signal transduction. For example, ▶ phosphatidyl inositol 3-kinase (PI3 kinase), which is activated downstream of many receptors, leads to the phosphorylation of lipids in the cell membrane which can serve as a docking site for proteins with the lipid-binding ▶ pleckstrin homology domain. The recruitment of proteins such as ▶ Akt to these sites then leads to their activation.

The second family of receptors do not directly modify other proteins, but rather trigger the production of small molecule mediators that diffuse through a cell and modulate cellular function. Such "▶ second messengers" include molecules like cyclic AMP (cAMP), cGMP, calcium ions, and lipids released from cellular membranes (such as diacylglycerol). Thus, for example, the binding of glucagon to the glucagon receptor leads to the generation of cAMP by ▶ adenylyl cyclase associated with the glucagon receptor. cAMP can then activate cAMP-dependent protein kinase, also known as protein kinase A, which phosphorylates a variety of substrates on serine and threonine residues. Among these is the transcription factor ▶ cAMP response element binding protein (CREB), which becomes activated by this phosphorylation, and mediates the expression of a cohort of target genes.

The third functional group of receptors for extracellular signals are not located on the cell surface, but rather are intracellular. The steroid hormone family of receptors makes use of the fact that the ligands with which it interacts are small lipophilic molecules that are cell permeable. For example, hydrocortisone is secreted by the adrenal glands, circulates in the bloodstream, and then directly enters cells where it binds to the glucocorticoid receptor. The receptor and its associated ligand then binds to specific regulatory sequences in the genome and activates or represses the expression of specific target genes.

Targets of Signaling Pathways

While the modulation of gene expression is a key endpoint of signaling pathways and plays a major role in the modulation of cellular behavior triggered by extracellular stimuli, other important targets of signaling pathways are present in a cell. For example, among the serine, threonine kinases activated downstream of cell surface receptors are kinases that can phosphorylate ribosomal proteins. The ribosomal protein S6 can be phosphorylated by members of the p70S6 kinase family or the ribosomal S6 kinase (RSK) family. Phosphorylation of S6 increases the rate of translation of a subset of mRNAs, which may be important for increasing cellular growth and proliferation. Kinases such as the "mammalian target of rapamycin" (mTOR) can directly phosphorylate and affect the function of proteins involved in both translation initiation and elongation.

Signaling pathways can also directly regulate cellular metabolic function. For example, the serine, threonine kinase ▶ Akt, which is activated by a variety of receptors, can phosphorylate the glucose transporter Glut4, which promotes its localization to the cell surface, and enhances glucose uptake in ▶ adipocytes.

For humans and other mammals to develop and function normally, the ability of cells to die through the process of ▶ apoptosis is a critical step. The development of organs such as the brain and the function of the immune system is dependent on the programmed death of large numbers of cells. While there are several mechanisms by which a cell can die, one central process involves families of proteins that regulate mitochondrial permeability. These proteins may serve to promote survival or apoptosis, and their relative levels and activity are a major determinant of whether or not a cell survives. A number of signaling pathways directly regulate these proteins. For example, the serine, threonine kinase Akt can phosphorylate the pro-apoptotic protein BAD (a member of the ▶ BCL-2 family of proteins), which blocks its ability to trigger apoptosis, and thereby promotes the survival of a cell.

Signal Transduction in Cancer Pathogenesis

Given the importance of signal transduction in a range of critical cellular processes, it is not surprising that inappropriate activation of signaling pathways can contribute directly to the development of cancer. Signaling pathways may be activated by a variety of mechanisms. Among the most common mutations in human cancer, and the first identified in a human tumor, are activating point mutations of ▶ Ras family proteins. These mutations lead to the activation of a number of downstream kinases that promote cellular survival and proliferation. Cell surface tyrosine kinases can become activated by overexpression

resulting from gene amplification. This mechanism leads to enhanced production of the ▶ Her2 (ErbB2) protein in ~30% of breast cancers, with the consequent activation of downstream signaling pathways. Receptor tyrosine kinases can also be activated by point mutations, deletions, or internal tandem repeats, all of which can lead to enhanced tyrosine kinase activity in the absence or presence of ligand. Another common mechanism by which tyrosine kinases are activated in cancer is through ▶ chromosomal translocations that lead to the formation of a chimeric gene that encodes a tyrosine kinase with enhanced activity. The prototype for this is ▶ BCR-ABL, a tyrosine kinase resulting from a translocation between chromosomes 9 and 22 (forming the so-called ▶ Philadelphia Chromosome). The tyrosine kinase c-abl is a relatively weak tyrosine kinase that localizes primarily in the nucleus and is involved in responding to DNA damage. By contrast, Bcr-Abl is a highly active kinase which is found in the cytoplasm and phosphorylates and activates a number of key signaling molecules. Bcr-Abl is found in essentially all patients with chronic myeloid leukemia (CML), and a subset of patients with acute lymphoblastic leukemia (ALL).

Most signal transduction pathways have negative feedback systems that limit their effect. However, signaling pathways can become activated during tumorigenesis by the loss of such a negative feedback component. For example, ▶ PTEN is a lipid phosphatase that dephosphorylates inositol phosphates, thereby countering the effect of PI3 kinase. The PTEN gene is frequently deleted in cancers which leads to unrestrained activity of the ▶ PI3 kinase cascade.

Signal Transduction in Cancer Therapy

Current cytotoxic cancer therapies kill cells in a largely indiscriminate manner, and thus have limited efficacy and considerable toxicities. However, increased understanding of how signaling pathways are activated in cancer has presented an opportunity to develop targeted agents which are more effective at inhibiting tumor cells while causing few side effects. Current signal transduction inhibitors generally target activated tyrosine kinases by blocking the ability of ATP to bind to the kinase. Drugs such as ▶ Imatinib mesylate, which inhibits the abl kinase, have been enormously successful in treating ▶ CML, and cause few serious side effects. While much effort is going into the development of kinase inhibitors, there is also an interest in developing inhibitors for signaling proteins without catalytic function. For example, drugs that inhibit the posttranslational lipid modifications of Ras might be able to overcome the effects of an activating point mutation in this protein.

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Signal Transduction Cross-Talk

Definition

Is the mechanism by which activated signaling molecules in a primary ▶ signal transduction pathway can regulate signaling molecules in another primary signal transduction pathway.

▶ Kit/Stem Cell Factor Receptor in Oncogenesis

Signal Transduction Inhibitor-571

▶ STI-571

Signaling Cascades

Definition

Cascades involving enzymes that act one on another to relay molecular signals (hormone, growth factor) or

physical signals (sensory stimuli) from a cell's exterior to its intracellular response mechanisms.

► Signal Transduction

Signalosome

Definition

Functional definition of a multi-protein complex of various signaling elements, whose association and activities are regulated by scaffold proteins. The assembly of the signalosome is modulated in a complex spatio-temporal manner and ensures the specificity and speed of intracellular signal ► transduction.

Signal-Transducer Proteins

Definition

Proteins functioning as units in signaling pathways are described as signal-transducers. They have at least two "working parts," one involved in recognition of an input signal (receptor part) and the other in generation of an output signal (generator) recognized by the downstream components. Some signal transducer molecules can respond to additional input signals that modulate their function (modulator) or could, after a short period, terminate generation of the output signal (timer).

► Inositol Lipids
► Signal Transduction

Silencing Mediator of Retinoid and Thyroid Hormone Receptor

Definition

► SMRT Co-repressor.

Simian Virus 40

Definition

► SV40; a virus of the genus *Polyomavirus* of the family Papovaviridae, originally isolated from kidney cells of the rhesus monkey. SV40 nucleotide sequences have been identified in certain human cancers, such as ► osteosarcomas and ► mesothelioma.

► SV40

Simulation

Definition

Allows precise radiation dose distribution within the prescribed treatment volume by integrating data from the computed tomography scanner or magnetic resonance imager to the three dimensional treatment planning computer system.

► Radiation Oncology

SIN3A

Definition

Transcriptional repressor protein interacting with ► histone deacetylases.

► Chromosomal Translocation t(8;21)

Single Cell Gel Electrophoresis Assay

Synonyms

SCGE.

► Comet Assay

Single Cell Invasion

Definition

Cell–cell adherence is lost and the cells migrate solitarily. This invasion pattern is usually seen in the presence of ► epithelial–mesenchymal-transition or amoeboid transition (change to a leukocyte-like migration pattern) and requires the expression of β1-integrin.

► Podoplanin

Single Cell Microgel Electrophoresis Assay

► Comet Assay

Single Dose Toxicity Studies

Synonyms

Acute toxicity studies

Definition

Are standardized protocols whereby small groups of animals are given a single dose of a chemical usually at several doses to define a dose-response. The top dose is usually expected to be at about the maximum tolerated dose in the particular animal species under study. After euthanasia the animals are subjected to a full autopsy examination and most tissues are examined microscopically for any adverse effects. They are conducted according to Good Laboratory Practice. The so-called LD50 (lethal dose 50) study is no longer performed.

► Preclinical Testing

Single Nucleotide Polymorphism

Definition

Refers to SNP; single nucleotide changes (A, T, C, or G) in the genome sequence. Such changes can sometimes modify the function of a protein and an understanding of these changes can enhance our understanding and treatment of disease.

► MIC-1

Single Stranded Conformation Analysis

Definition

SSCP; method applied for mutation screening that is based on the identification of secondary structure differences between mutated und unmutated single strands of a specific amplified region. Detection is performed after ► PCR within non denaturating gels.

► Leukemia Diagnostics

Single-chain Fv Dimer

► Diabody

Single-Chain Fv Fragment

Definition

Single-chain Fv fragment recombinant antibody molecule composed of the variable light chain domain (VL) and variable heavy chain domain (VH) of an antibody linked by a short and flexible peptide linker.

► Bispecific Antibodies

Siomycin A

Definition

Is a thiazole antibiotic compound that was identified from the culture broth of *Actinomycetes*, that inhibits FoxM1 activity.

► Forkhead Box M1

SIOP

Definition

The Societe Internationale Oncologie Pediatrique (International Society of Pediatric Oncology) is an international organization of clinicians involved in the treatment of childhood cancer.

Sipa-1

Synonyms

Spa-1

Definition

Signal-induced proliferation associated protein 1 is a GTPase-activating protein specific for Rap1 and is encoded by *Sipa-1* gene. There are several proteins with a homologous structure and function, including human E6TP1 (▶ Human Papillomavirus E6-targeted protein 1, also called Spa-L1 in mouse and SPAR in rat), Spa-L2, and Spa-L3.

▶ Rap1/Sipa-1

Sipple Syndrome

▶ Multiple Endocrine Neoplasia Type 2

Sipuleucel-T

Definition

A cell-based vaccine composed of autologous ▶ antigen-presenting peripheral blood mononuclear cells (enriched for a ▶ dendritic cell fraction) that have been exposed to a recombinant protein consisting of granulocyte-macrophage colony-stimulating factor (GM-CSF) fused to prostatic-acid phosphatase (PAP), a protein expressed by ▶ prostate cancer cells. Upon administration, the vaccine may stimulate an antitumor ▶ T-cell response against tumor cells expressing PAP.

▶ Prostate Cancer Experimental Therapeutics

Sir2-like Proteins

▶ Sirtuins

SiRNA

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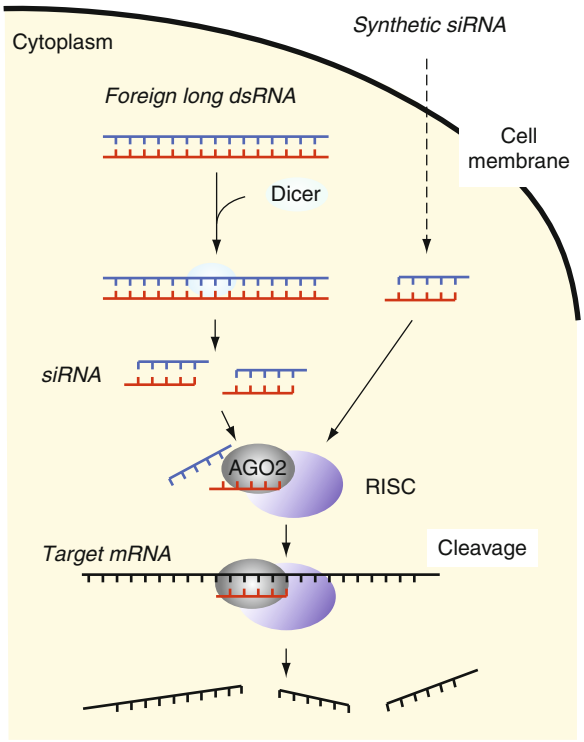
Definition

The small interfering RNA (siRNA) transcripts are 21–25 bp long, double-stranded transcripts as a defense response to nonendogenous double-stranded RNA (dsRNA), leading to sequence-specific mRNA cleavage. This double-stranded induced cleavage was named ▶ RNA interference (RNAi).

Characteristics

siRNA transcripts are 21–25 bp long, double-stranded transcripts that were found in *Caenorhabditis elegans* in 1998 as a defense response to nonendogenous dsRNA, leading to sequence-specific mRNA cleavage. This double-stranded induced cleavage was named RNAi and was soon to be found in other organisms, for example, *Drosophila melanogaster*, and in vertebrates. The main function of the mechanism is believed to a defense system against introduced viral double-stranded sequences, cutting them to pieces and thereby rendering them unable to infect the cell. The gene regulation part was not discovered until recently. RNAi was also quickly adapted for use in the



SiRNA. Fig. 1 Schematic representation of RNA interference (RNAi). siRNA is generated by Dicer cleavage of long double-stranded RNA sequences. After cleavage, the double-stranded siRNA is loaded into the RNA-induced silencing complex (RISC). Synthetic siRNA is directly incorporated in the RISC. During the loading process one strand is peeled off by Argonaute2 (AGO2) leaving a single-stranded siRNA lodged inside RISC. This strand is then used by RISC as a template to recognize the cleavage target in the target mRNA transcript. A full match induces a cleavage

laboratory as a gene-silencing tool for the silencing of specific genes by introducing the specific double-stranded sequence, and has become a common tool today for the study of gene expression and regulation due to its specificity. siRNA is generated by Dicer cleavage of long dsRNA sequences. After cleavage, the double-stranded siRNA is loaded into the RNA-induced silencing complex (RISC). During the loading process one strand is peeled off, leaving a single-stranded siRNA lodged inside RISC. This strand is then used by RISC as a template to recognize the cleavage target in the target mRNA transcript. A full match induces a cleavage in *Drosophila* RISC complexes, Argonaute2 (AGO2) has been identified as carrying the slicing action, but several other proteins are also involved in the RISC complex (Fig. 1).

Clinical Aspects

There are still unknown details regarding the biogenesis and function of siRNA, especially in mammals. The siRNA technique of using siRNA transcripts to knock down gene expression of specific genes has quickly become a popular method that is used for gene function analysis in molecular biology. The method is still being developed to improve the siRNA vectors, both the specificity and the usability of the technique, but already it exhibits a high specificity. The hope of the future is to be able to use siRNA knockdown techniques to treat both genetic disorders and viral infections and, in extension, to be able to use siRNA as a cancer treatment.

The advantage of RNAi technology is that it can be used to target a large number of different genes involving a number of distinct cellular pathways. This is particularly important for a disease as complex as cancer. Most of the RNAi candidate cancer gene targets are involved in pathways that contribute to net tumor growth (either through increased tumor-cell proliferation or reduced tumor-cell death, or both). While mRNAs expressed from mutated cancer ▶ oncogenes can be directly targeted for RNAi intervention, RNAi can also be used to target and silence gene products that negatively regulate the function of endogenous ▶ tumor suppressor genes. Other gene products that can be targeted by RNAi include proteins involved in cellular ▶ senescence, or protein stability and degradation. Although these additional targets are not directly involved in the oncogenesis pathway, they can indirectly contribute to net tumor growth, and therefore represent potential candidates for RNAi intervention.

Delivery

RNAi-mediated gene silencing in mammalian cells can be achieved by transfection, for example, using liposomes or electroporation of synthetic siRNA molecules, or by gene transfer using plasmid- or virus-based expression cassettes with RNA polymerase III promoter encoding dsRNA molecules.

The easiest way to induce RNAi is the use of chemically synthesized siRNAs. However, in addition to siRNAs produced by chemical synthesis, siRNAs may be generated by from long dsRNAs in vitro via recombinant Dicer, by in vitro transcription using T7 RNA polymerase, or siRNAs can be isolated from

Drosophila embryo extracts. Classic transfection of siRNA molecules using different physical methods such as liposome-mediated transfection, electroporation, or single-cell microinjection has been successfully applied. Treatment of mammalian cells with siRNAs typically results in a transient downregulation of the target mRNA after 1–2 days for a duration of 3–5 days. In vivo delivery of chemically synthesized siRNAs to mice was reported by injection into the tail veins. By this approach, a downregulation of 90% of endogenous mRNA transcripts in the majority of liver cells after a single siRNA injection could be achieved. In the liver, the RNAi effect persists for several days.

Although siRNAs are readily taken up into worm and fly cells, most mammalian cells do not efficiently internalize these small molecules. This is even true of cells, such as dendritic cells and ▶ macrophages, which are actively sampling their environment. Therefore, the major obstacle for using small RNAs as drugs is to deliver them into the cytoplasm of cells. An exception may be mucosal tissues. In the lung and vagina, siRNA uptake is extremely efficient and occurs even in the absence of transfection reagents. For clinical indications where siRNAs only need to be delivered to a localized region, such as the eye, pulmonary or vaginal mucosa, or superficial tumors, efficient siRNA delivery and silencing can be achieved by mixing siRNAs with cationic lipid transfection reagents used for in vitro transfection and directly injecting the siRNA–lipid complexes into the relevant tissue or instilling it into the body cavity. A similar approach is certain to apply to the skin. Mixing siRNAs with other molecules known to carry nucleic acids into cells, such as certain cationic peptides, might also be used effectively for local delivery. However, some cell types, such as lymphocytes, dendritic cells, and hematopoietic stem cells, are refractory to transfection using cationic lipids. Therefore, even when these targets might be localized, alternate delivery strategies may be needed.

The first demonstrations of the therapeutic potential of siRNAs for silencing disease-related genes delivered siRNAs systemically by rapid high-pressure intravenous injection. This method leads to transient right-sided heart failure, where elevated venous pressures somehow enable siRNAs to get into cells in highly vascularized organs like the liver, pancreas, and lungs. Nonetheless, this strategy is too risky for human use. It is, however, possible to deliver siRNAs into an organ, such as the kidney, by rapid retrograde injection via catheter into the draining vein. It may be possible to use hydrodynamic injection into a peripheral vein to treat skeletal muscle by blocking venous outflow using a tourniquet. Elevated venous pressures are generated only in the targeted tissue without inducing potentially fatal heart failure. However, a minimally invasive method for delivering siRNAs requires alternate approaches. A variety of strategies that involve complexing siRNAs to cationic polymers or peptides or incorporating siRNAs into nanoparticles or liposomes have been proposed. Alternately, siRNAs can be covalently or noncovalently linked to antibody fragments or ligands to cell surface receptors to limit the delivery of the siRNAs to cells that bear the specific receptor. These strategies probably deliver siRNAs via receptor-mediated endocytosis, although the trafficking of siRNAs into and within cells has not been well studied. The directed delivery of siRNAs into specific cells will decrease the amount of siRNAs needed for the efficient silencing of gene expression in the target organ or tissue and will reduce potential toxicity by preventing targeting of unintended cells and tissues. The optimal delivery strategy may differ between different therapeutic indications and will depend on efficiency and duration of delivery and silencing, lack of systemic toxicity, and lack of immunoreactivity, which would interfere with repetitive treatments. The first examples of effective systemic delivery have only recently been described.

Nonspecific Immune Stimulation

While there is a high degree of specificity associated with RNAi, some effects have been observed that are independent of the specific gene targeted for silencing. In general, 21 bp or longer dsRNAs can lead to a sequence-independent ▶ interferon response. Interferon can also activate the dsRNA-dependent protein kinase (PKR), which phosphorylates and subsequently inactivates the translation factor eIF2, leading to a global inhibition of mRNA translation. The length of the initiating siRNA clearly has some role in triggering the interferon response, with more recent data suggesting that sequences shorter than 19 nucleotides are more likely to escape the interferon antiviral response. It is anticipated that the judicious selection of siRNA sequences together with a greater

understanding of their interactions with any given target gene will resolve this issue.

Similarly, evidence exists that siRNAs can activate dendritic cells and other cells of the immune system through a much more specific and restricted class of receptors, the toll-like receptors, which can recognize foreign nucleic acids including dsRNAs and when activated can send a danger signal to trigger a proinflammatory response. These findings raise the possibility that RNAi reagents may trigger unforeseen immune responses, including autoimmune diseases, in vivo.

Off-Target Interference

Nucleic acid-based gene-silencing molecules may also have effects on genes that are not considered targets, the so-called off-target effects, due to similarities in nucleic acid sequences. The degree of the off-target effect is dependent upon the mode of silencing and the stability of the nucleic acid hybrid. If siRNAs are not carefully selected, siRNAs having partial complementarity to an mRNA target can repress translation or subject unintended mRNAs to degradation. A study that compared the gene-expression profiles created by different siRNAs targeted against the same transcript revealed that in extreme cases, as little as seven nucleotide complementarity between the 5′ end of either siRNA strand to an mRNA can cause a reproducible reduction in transcript levels. Interestingly, it has been found from studies in primitive organisms that off-target effects are not observed when complete dsRNAs are introduced instead of synthetic siRNAs. This may be explained by the fact that the siRNAs derived endogenously from the cleavage of dsRNAs are generated and selected by Dicer and the RISC complex, which may have a proofreading mechanism that protects against the generation of siRNA sequences that might result in the silencing of endogenous genes. Therefore, it is possible that mammalian siRNAs generated from dsRNA precursors through the action of Dicer and the RISC complex may be less prone to induce off-target effects than synthetically designed siRNAs. Several algorithms and software are available to select siRNA target sequences with reduced off-target effects, and it will be important to select siRNA targets with relatively sophisticated sequence comparison tools to minimize potential off-target effects.

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SIRS

Definition

► Systemic Inflammatory Response Syndrome.

SIRTs

► Sirtuins

Sirtuins

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Synonyms

Class III histone deacetylases; Sir2-like proteins; SIRTs

Definition

Sirtuins are a family of protein deacetylases (► Histone Deacetylases) and ADP ribosyltransferases requiring NAD+ for their enzymatic activity.

Characteristics

Biochemical Features of Sirtuins

Sirtuins are protein deacetylases and ADP ribosyltransferases that target a wide range of cellular

Sirtuins. Table 1 Cellular localization and functions of sirtuins

	Localization	Function
SIRT1	Nucleus	Deacetylation of histone and nonhistone proteins
		Regulation of chromatin structure
		Regulation of activity of p53 and several other transcription factors (see text)
		Involved in cellular stress response, survival, senescence, cellular life span, differentiation
SIRT2	Cytoplasm	Deacetylation of α-tubulin
	Shuttles into nucleus	Mitotic checkpoint regulation
SIRT3	Mitochondria	Acetyl-CoA production
SIRT4	Mitochondria	ADP-ribosylation, suppression of insulin signaling
SIRT5	Mitochondria	Not known
SIRT6	Nucleus	Guards genomic instability, target unknown
SIRT7	Nucleolus	Promotes rRNA transcription

proteins in the nucleus, cytoplasm, and mitochondria. Among the substrates are histone proteins, and therefore sirtuins are also referred to as class III ▶ histone deacetylases. Seven human sirtuins (SIRT1–7, see Table 1) have been identified and share a catalytic domain of 275 amino acids. As opposed to ▶ histone deacetylases, sirtuins require ▶ NAD(P)H:quinone oxidoreductase as a cofactor for their enzymatic activity. Therefore, NAD+/NADH ratio and metabolic status of the cell influences the activity of sirtuins. Nicotinamide is a physiological intracellular inhibitor of sirtuins.

Cellular Functions of Sirtuins

Human sirtuins (SIRTs) are structurally related to the silent information regulator 2 (Sir2) family, which are conserved from bacteria to humans and regulate lifespan in lower organisms. Sirtuins are involved in regulation of the cellular stress response, cell cycle progression, chromosomal stability, and aging. SIRT1 knockout mice show multiple developmental defects and die around birth and is thus required for normal embryonal development.

Mammals have seven sirtuins (Table 1). In addition to the intranuclear SIRT1, there is the cytoplasmic SIRT2, the three mitochondrial sirtuins SIRT3, 4, 5, and also within the nucleus the ▶ heterochromatin-associated SIRT6 and the nucleolar SIRT7. Based on sequence homology, the sirtuins can be grouped into four classes. SIRT1, 2, 3 comprise class I, SIRT4 is in class II, SIRT5 is in class III, and SIRT6, 7 are class IV sirtuins. Table 1 summarizes the cellular localization and functions of the seven human sirtuins.

Among the 7 sirtuins, SIRT1 has been most intensively studied, whereas the functions and target proteins of SIRT2–6 are less well understood. SIRT1 regulates the acetylation status of histone proteins and is thereby involved in the control of ▶ chromatin structure and regulation of transcriptional activity. For example, SIRT1 directly deacetylates lysine 16 of histone H4, which is a mark for transcriptional inactive heterochromatin, whereas acetylated lysine 16 is found in transcriptionally active ▶ euchromatin. SIRT1 is a component of the polycomb group repressor complex, a multiprotein complex which controls differentiation and developmental programs in embryonic cells. In addition to its function in regulating chromatin structure, SIRT1 regulates the activity of several cellular proteins via modulating their acetylation status. For example, deacetylation of the ▶ tumor suppressor protein ▶ p53 by SIRT1 results in downregulation of its transcriptional and ▶ apoptosis-inducing as well as ▶ senescence-inducing activity. Other proteins known to be targets of SIRT1 are MyoD (a transcriptional regulator of muscle gene expression and differentiation), FOXO proteins (family of transcription factors that regulate oxidative stress response, cell cycle arrest, DNA repair, and apoptosis), Ku70 (a protein involved in nonhomologous repair of DNA double-strand breaks), PPAR-y (a member of the nuclear receptor superfamily that integrates energy control, lipid metabolism, and glucose homeostasis), and NF-kB (transcription factor that regulates immune response, inflammation, cell proliferation, and cell death). As a result of modulating the function of these proteins, SIRT1 is involved in cell survival, muscle

differentiation, and fat metabolism and is upregulated during caloric restriction. SIRT1 plays a role in regulation replicative senescence and limit of proliferation of normal cells. SIRT1 ensures that damaged DNA is not propagated and that mutations do not accumulate in cells. In recent years, much attention has been paid to the function of SIRT1 in controlling lifespan and longevity.

Sirtuins and Cancer

SIRT1 limits proliferation capacity and induces senescence in normal, non-transformed cells exposed to chronic stress stimuli and genomic insults. Therefore, sirtuins are thought to protect against the development of cancer. Sirtuins are considered as targets for chemoprevention of age-related cancers by promoting their function in guarding genomic stability and senescence.

However, SIRT1 is also found to be overexpressed in a number of cancers such as acute myeloid leukemia (AML), skin, breast, and colon cancer, and is upregulated in chemotherapy-resistant cancer cells. High levels of SIRT1 protect malignant cells from apoptosis via deacetylation and subsequent reduction of the activity of proteins involved in programmed cell death. Among the cancer-relevant substrates of SIRT1 is the tumor suppressor protein p53. SIRT1 deacetylates p53 resulting in repression of its functional activity. Another substrate of SIRT1 is the critical B-cell lymphoma protein 6 (BCL6). Various tumor cell lines cease growth and undergo apoptosis after SIRT1 ▶ knockdown. SIRT1 ▶ overexpression blocks ▶ oncogene-induced senescence. At the ▶ epigenetic level, SIRT1 deacetylates ▶ histone H4 at lysine 16, a modification which is a common hallmark of human cancer. Thus, inhibition of sirtuins appears to be a promising target for certain cancers which are dependent on high levels of sirtuin activity.

Sirtuin Inhibitors and Activators

The biological activity of sirtuins can be modulated by small molecule inhibitors and activators. Nicotinamide, a natural byproduct of the sirtuin deacetylase reaction, is a general inhibitor of all sirtuin family members. Other small molecule inhibitors are sirtinol, M15, and splitomycin. The observation that calorie restriction increases life span through increase of the activity of sirtuins in animal models leads to the screening of compounds with sirtuin stimulating activity. Among the substances identified were the polyphenols quercetin and piceatannol, and resveratrol, a polyphenol found in the skin of red grapes.

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Sister Chromatid Exchange

Definition

SCE; is the mitotic recombination between the two newly replicated sister chromatids, leading to exchange of genetic information.

Sister-Chromatids

Definition

Chromatids of the same chromosome, which are connected by a centromere. Nonsister-chromatids are the chromatids of homologous chromosomes ▶ anaphase-promoting complex.

▶ Securin

Site-Directed Mutagenesis

Definition

Is a mutation created at a defined site of known sequence in a DNA molecule of a wild-type, by a molecular biology technique.

Site-specific Drug Delivery

▶ Targeted Drug Delivery

Sivelestat

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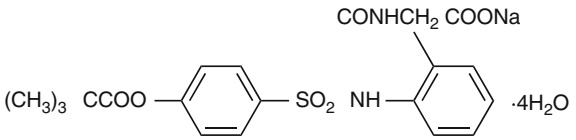
Definition

Sivelestat is a low molecular weight synthetic inhibitor of human ▶ neutrophil elastase (NE) (neutrophil elastase and cancer), which was produced by Ono Pharmaceutical Co., Ltd., in 1991 as ONO-5046, N-[2-{4-(2,2-Dimethylpropionyloxy) phenylsulfonylamino}-benzonyl] aminoacetic acid (MW. 434.47). The structure is illustrated in Fig. 1. This is a potent, specific, and intravenously active NE inhibitor and it is widely used to treat patients with lung injury diagnosed as ▶ SIRS (Systemic Inflammatory Response Syndrome) in Japan at a dose of 0.2 mg/kg/h for 14 days.



Sivelestat. Fig. 1 Structure of sivelestat, ONO-5046, N-[2-{4-(2,2-dimethylpropionyloxy) phenylsulfonylamino}-benzonyl] aminoacetic acid (MW. 434.47)

Characteristics

Mechanisms of Action

Sivelestat inhibits the action of NE which is a member of serine proteases produced by polymorphonuclear neutrophils and monocytes/macrophages. NE degrades a broad spectrum of extracellular matrix (ECM) (▶ Extracellular remodeling) and cell surface proteins, such as elastin, interstitial collagens, proteoglycans, fibronectin, laminin, and type IV collagens. Under normal physiological conditions, the proteolytic activity of elastase released by recruited neutrophils is strictly regulated by antiproteases, such as alpha-1-protease inhibitor and secretary leukoprotease inhibitor. However, under conditions of physiological disturbance, such as the presence of neoplasms, surgical stress, or inflammation, the balance between elastase and antiprotease is disrupted, and the predominant elastolytic activity causes the destruction of peripheral ECM of the lung.

NE also cleaves pro-▶ transforming growth factor-alpha (TGF-α) (▶ Epidermal Growth Factor Receptor Ligands) on the surface of human airway epithelial cells via its proteolytic activity and releases mature, soluble TGF-α on human airway epithelial cells. Release of TGF-α activates the ▶ epidermal growth factor receptor (EGFR) (Epidermal growth factor receptor ligands) signaling cascade (▶ Transduction Of Oncogene), resulting in mucinous secretion in the peripheral airways that might cause severe respiratory complications after major surgical stress or severe inflammatory response.

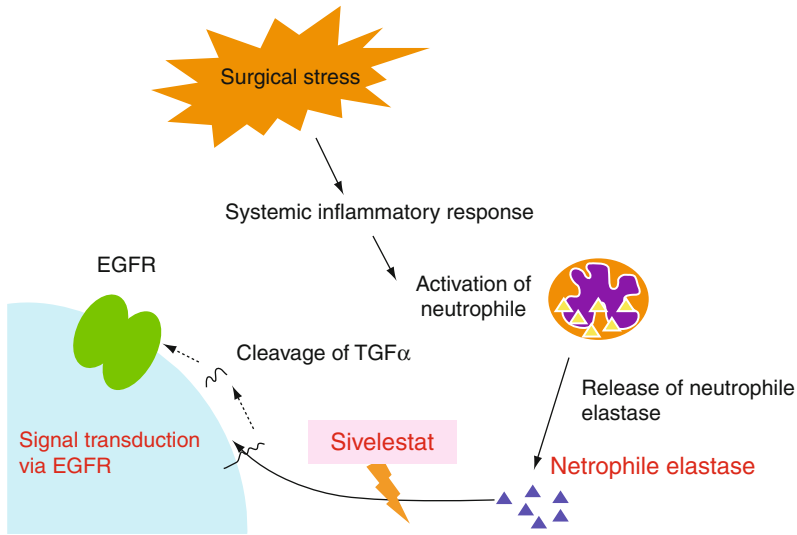
Clinical Aspects

Clinical Application in Practice

Sivelestat is prescribed clinically for the treatment of various inflammatory diseases in Japan. The anti-inflammatory effects of sivelestat are applied for the alleviation of surgical stress, and can reduce morbidity and organ dysfunction, including ▶ acute respiratory distress syndrome (ARDS). Considerable amount of NE is released from activated neutrophils which are induced by systemic cytokines including ▶ TNF-α, ▶ IL-8, and ▶ IL-6 produced by ▶ macrophages in the lung. SIRS is often observed after surgical injury, which influences on the function of leukocytes and endothelial cells leading to the ARDS or ▶ acute lung injury (ALI) with the destruction of the alveolar construction of the lung.

Application to Cancer Treatment as Molecular Targeting Therapy

Rapid recurrence or regrowth of tumors are often observed after major cancer surgery, or perioperative surgical complications with SIRS that involve the release of NE by neutrophils and also by cancer cells.



Sivelestat. Fig. 2 Action mechanism of sivelestat. NE released from activated neutrophils by SIRS cleaves the pro-TGF-α on the cell surface of the cancer cells, which stimulate the growth of the cell via EGFR. Sivelestat blocks the cleavage of the TGF-α

On the other hand, cancer cells have multi-► autocrine growth factor and receptor loops including EGFR- TGF-α system for the development of growth and metastatic potential.

The growth and invasion activity of cancer cells are stimulated by NE and the growth stimulation occurs via the cleaved TGF-α from the cell membrane, which activates the EGFR and then activates the subsequent intracellular signal transduction. Moreover, NE stimulates the release of several growth factors including ► PDGF (platelet derived growth factor) and ► VEGF (vascular endothelial growth factor) that are closely related to the growth and angiogenesis of tumor cells. As mentioned above, sivelestat inhibits the NE-induced release of TGF-α from cell membrane of not only neutrophils but also cancer cells (Fig. 2). Therefore, sivelestat might be a new therapeutic tool for the treatment of anti-inflammatory response and also for molecular targeting therapy (► Molecular Therapy) of postoperative cancer patients by inhibiting the rapid cancer cell growth after resection of tumors accompanied with SIRS.

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Sjögren Syndrome

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Synonyms

Dry eyes syndrome; Dry mouth syndrome; KCS; Keratoconjunctivitis sicca; Sicca complex; Sicca syndrome; Xerophthalmia; Xerostomia

Definition

Sjögren syndrome is a chronic, progressive autoimmune disorder that primarily affects the exocrine glands. The classic signs and symptoms include enlargement of the parotid and lacrymal glands, with mucosal dryness manifested by dry mouth (▶ Xerostomia) and dry eyes (▶ Xerophthalmia) (▶ Keratoconjunctivitis Sicca). Lymphocytic infiltrates replace functional epithelium, which results in decreased exocrine secretions (exocrinopathy).

Numerous features suggest an immunologic etiology. Patients demonstrate hypergammaglobulinemia and have several types of ▶ autoantibodies, such as the rheumatoid factor (RF), antinuclear antibodies (ANA), Ro/SSA and/or La/SSB, and antibodies to salivary duct epithelium.

Characteristics

Sjögren syndrome is named after the Swedish ophthalmologist Henrik Sjögren. However, the disease was addressed during the nineteenth century in a number of case reports, between the years of 1882 and 1924, describing various combinations of dry mouth, dry eyes, and chronic arthritis. Mikulicz reported in 1892 a man with bilateral parotid and lacrimal gland enlargement associated with massive round cell infiltration. Later on, the link between Sjögren syndrome and ▶ malignant lymphoma was described in a classic paper in 1964. More recently, a set of preliminary classification criteria was identified by a European Concerted Action in 1993 and they have been widely accepted. The triad of dry mouth (xerostomia), dry eyes (keratoconjunctivitis sicca), and a connective tissue disease, usually ▶ rheumatoid arthritis (RAN binding protein) or ▶ systemic lupus erythematosus (SLE), is termed secondary Sjögren syndrome. In the absence of a connective tissue disease, the designation primary Sjögren syndrome (or sicca syndrome) is used.

Incidence

Sjögren syndrome is the most common form of autoimmunity disorder and is seen mostly in women (female to male ratio of 9:1) in the fourth and fifth decades of life. Its frequency in RA is 20% and therefore Sjögren syndrome is most commonly seen in this context.

Etiology

The etiology of Sjögren syndrome is unknown but may involve genetic and immunological factors.

1. *Genetic Factors*. A prominent feature of Sjögren syndrome is its genetic predisposition. The polymorphic ▶ major histocompatibility complex (MHC) genes are well-documented genetic risk factors for the development of ▶ autoimmune diseases overall. With regard to Sjögren syndrome, the most relevant MHC complex genes are the class II genes, more specifically the HLA-DR and ▶ DR5 humanized antibody. In whites of northern and western European background, including North American whites, Sjögren syndrome is one of several autoimmune diseases associated with the ▶ haplotypes HLA-B8, DRw52, and DR3. An association with DR2 has been reported in Scandinavians and DR5 in Greeks.
2. *Immunological Factors*. Immunological abnormalities include a marked hyperactivity of B lymphocytes with hyperglobulinemia and serum autoantibodies, some organ-specific such as antibodies to salivary duct epithelium, and others nonspecific such as ANA and rheumatoid factor. Impaired cellular immunity with defective suppressor T cell (▶ Regulatory or suppressor T cells) function has been reported in some patients but not in others. Both ▶ T lymphocytes and B lymphocytes have been identified in the tissue lesions and local synthesis of ▶ immunoglobulins has been demonstrated. Since the lymphocyte infiltration has been demonstrated to be initially periductular in local lymphocyte, cytotoxicity directed against ▶ antigens on salivary gland duct cells has been postulated.
3. *Environment*. Among the possible etiologic and triggering factors involved in Sjögren syndrome, the discussion about a relationship between viral infections causing development of autoimmune reactions began some decades ago. The putative role of different ▶ viruses in Sjögren syndrome can be viewed in the light that salivary glands are a site of latent viral infections. Potential viral triggers include a number of viruses. Among these, ▶ Epstein–Barr virus (EBV) has been widely studied in relation to Sjögren syndrome. A higher prevalence of serum human herpesvirus-6 (HHV-6)-specific antibodies has also been detected

in patients with Sjögren syndrome than in normal individuals (36% vs. 10%). Retroviruses are known to infect cells of the ▶ immune system and cause abnormalities in immune regulation. High serum titers of antihuman T lymphotropic virus type I (HTLV-1) antibodies and a high prevalence of salivary immunoglobulin A (IgA) class anti-HTLV-1 antibodies in patients with Sjögren syndrome were reported endemically in Japan. Hepatitis C virus (HCV) infection in some populations has been frequently (14%) detected in patients with primary Sjögren syndrome. Analysis of the association between chronic lymphocytic sialadenitis and ▶ chronic HCV liver disease showed that histologic features of Sjögren syndrome were significantly more common in HCV-infected patients (57%) compared with controls (5%). Lymphotropic viruses have the potential to trigger the autoimmune process. Some of the immunoreactive regions within the La/SSB protein have been found to have sequence similarities with proteins of EBV, HHV-6, and human immunodeficiency virus (▶ HIV)-1. It seems reasonable that these viruses can promote autoantibody (particularly anti-La/SSB) production through ▶ molecular mimicry or exposure of La/SSB homolog sequences on cellular surfaces after translocation of cryptic self-determinants.

Clinical Manifestations

Presentation

The typical case is of a middle-aged woman who developed sicca symptoms insidiously. Less commonly it presents with parotid swelling or with constitutional symptoms such as malaise and arthralgias. Rarely the presenting feature is cutaneous vasculitis, a cranial neuropathy usually involving the trigeminal nerve, a peripheral neuropathy, or the hyperviscosity syndrome. Sicca symptoms tend to develop more rapidly in primary Sjögren syndrome, when episodic parotitis and involvement of other organs is more common.

Involvement of Exocrine Glands

Ocular symptoms due to reduced lacrimal secretion and desiccation of the cornea and conjunctiva (keratoconjunctivitis sicca) include a gritty or sandy sensation, reduced tearing, photophobia, and the presence of a ropey discharge, particularly at the inner canthus. Recurrent infections are also common. In advanced cases, the cornea may be severely damaged and complications include corneal ulceration and occasionally perforation. Decreased saliva results in dryness of the mouth (xerostomia) that leads to dysphagia, abnormalities of taste, and adherence of food to the buccal surface. Widespread involvement of mucosal glands elsewhere may result in dryness of the nose, posterior pharynx, trachea, bronchial tree, skin, and vagina. Involvement of the respiratory tract leads to an increased frequency of upper and lower respiratory tract infections and otitis media. There is parotid gland enlargement in 50% of cases (80% in primary Sjögren syndrome) sometimes accompanied by pain and fever.

Extraglandular Involvement

Extraglandular lymphoproliferation occurs in 25% of primary Sjögren syndrome, and involves the reticuloendothelial system, kidney, muscle, and lungs. Systemic manifestations are varied and include diffuse interstitial pneumonitis, renal tubular abnormalities, peripheral and cranial neuropathy, nonthrombocytopenic purpura, and the development of pseudomalignant and malignant lymphoid proliferation. Malignant lymphoma occurs more often than can be explained by chance alone, and Sjögren syndrome has often been considered to be a link in the spectrum between autoimmune disorders and lymphoproliferative disorders (Malignant lymphoma, Antonino Carbone and concepts).

Diagnosis

The most commonly used tests for the detection of dry eyes are the Schirmer I and the rose bengal (alternatively Lissamine green dye) and subsequent scoring according to van Bijsterveld. Schirmer I is performed using standardized tear tests strips. In the European classification criteria, ▶ Seckel syndrome 1 is positive when the wetting is less than 5 mm in 5 min. The scoring according to van Bijsterveld detects destroyed conjunctival epithelium induced by dryness. Saliva production tests are simple screening tests for salivary gland involvement in Sjögren syndrome. Saliva, which is produced by the three major and numerous minor submucosal salivary glands, exhibits great flow variations among healthy individuals and in the same individual under diverse conditions. The test should

therefore be standardized; the unstimulated whole saliva collection test is performed for 15 min, and the test is considered positive when 1.5 mL or less whole saliva is collected, being well below the normal mean range. Other tests used to evaluate salivary gland involvement include parotid sialography and salivary gland scintigraphy. The sialography typically shows sialectasis in contrast to the fine arborization seen in normal parotid ductules. In the scintigraphic test, ^{99m}technetium-pertechnate is given intravenously, and in Sjögren syndrome patients, the typical finding is decreased uptake in response stimulation of the parotid and submandibular salivary glands. This test is a sensitive and valid method to measure abnormalities in salivary gland function in the hands of skilled personnel.

SS is Diagnosed on the Basis of Either European Classification

European Classification. (Preliminary criteria for the classification of Sjögren syndrome, Vitali C et al.)

1. Ocular symptoms – Positive response to one of three questions pertaining to dry eyes
2. Ocular signs – Positive Schirmer test (<5 mm in 5 min) or positive rose bengal staining
3. Oral symptoms – Positive response to one of three questions pertaining to dry mouth
4. Salivary gland involvement – Objective evidence of salivary gland involvement; salivary scintigraphy; parotid sialography; unstimulated salivary flow less than 1.5 mm/min
5. Serologic or autoantibody test results – Presence of autoantibodies to Ro (SSA), La (SSB), or both
6. Categories
   ## Primary SS – Presence of any of four of the previous six categories
   ## Secondary SS – Presence of potentially associated connective tissue or autoimmune disease with the first two categories (ocular symptoms and ocular signs) plus any two of the next three categories

Laboratory Abnormalities

Autoantibodies to salivary duct, gastric parietal cells, thyroglobulin, mitochondria, and smooth muscle are frequently present. Rheumatoid factor is found in over 90% and ANA in ~70%. Serum antibodies to two partially characterized cellular antigens Ro (SSA) and La (SSB) are commonly found in primary Sjögren syndrome.

Anti-Ro/SSA and Anti-La/SSB Antibodies

The reported frequencies of anti-Ro and anti-La (► Anti-Ro/SSA and anti-La/SSB antibodies) depend on the methods of detection and referral bias of the center performing the study. Overall, anti-Ro precipitins occur in ~60–75% of primary Sjögren syndrome and are also observed in cases of secondary Sjögren syndrome, irrespective of the association with SLE, progressive systemic sclerosis, rheumatoid arthritis, or primary biliary cirrhosis (PBC). Anti-La antibodies were initially reported to occur in up to 40% of patients with primary Sjögren syndrome. Even higher frequencies were reported when anti-La was analyzed by ► ELISA or ► immunoblotting. Further studies have shown that combined detection of anti-La and anti-Ro antibodies has a higher diagnostic specificity for primary Sjögren syndrome than does anti-Ro alone. Although the pathogenetic role of anti-Ro and anti-La in Sjögren syndrome is not established, positive serology is associated with a high frequency of palpable purpura, leucopenia, lymphopenia, and hypergammaglobulinemia, and with more severe glandular disease. Recent studies also have found salivary enrichment of anti-Ro and anti-La in patients with Sjögren syndrome, suggesting local autoantibody production in salivary glands as well as presence of Ro52, Ro60, and La autoantibody-producing cells in salivary gland biopsy samples from patients with Sjögren syndrome (SSA(Ro) SSB(La) in SS, Tsay GJ and concepts).

Anti-CENP-H Antibodies and SS

Anticentromere antibodies (ACA) have been described in patients with different rheumatic disorders including CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia), PBC, primary Raynaud's disease (PRD), and rheumatoid arthritis (RA). It is generally accepted that there is a strong correlation between ACA and Raynaud's phenomenon. An increased frequency of ACA and anti-CENP-H antibodies was found in patients with SS. SS can be subdivided serologically into two groups: group one with anti-SSA/Ro and/or anti-SSB/La antibodies, and group two with ACA and/or anti-CENP-H antibodies. In summary, ACA and anti-CENP-H antibodies are associated with SS. ACA or anti-CENP-H antibodies can be

used as a serological marker for SS (Anti-CENP-H in patients with Sjögren syndrome, Tsay GJ and concepts).

Pathogenesis

Critical features of pathogenesis include: (1) association with particular class II ▶ histocompatibility (HLA-DR/DQ) antigens; (2) a pattern of particular autoantibodies, including a newly described ▶ antibody against muscarinic M3 receptor for acetylcholine; (3) a high proportion of "▶ high endothelial venules (HEVs)" in the lacrimal and salivary gland biopsies, which express increased levels of ▶ cell adhesive molecules; (4) upregulation of major histocompatibility antigens (▶ H antigens or Histocompatibility Antigens) and adhesive molecules on epithelial cells in salivary and lacrimal glands, probably in response to local production of interferon gamma (▶ IFN-γ); (5) expression of cell surface (▶ Fas, ▶ Fas Ligand) and nuclear molecules (▶ BCL-2, ▶ bcl-x) on glandular epithelial cells and on ▶ lymphocytes infiltrating the glands; (6) secretion of proinflammatory ▶ cytokines (such as ▶ IL-1, ▶ Tumor Necrosis Factor-α (TNF-α), and ▶ IFN-γ) by lymphocytes and/or epithelial cells to perpetuate the inflammatory response; and (7) decreased secretion by the residual glandular acini as a result of their decreased neural innervation and defects in their postsignal transduction.

Immunopathology

Humoral Immunity

A large number of autoantibodies have been reported in both primary and secondary Sjögren syndrome, reflecting both B-cell activation and a loss of immune tolerance in the B-cell compartment (▶ Humoral Immunity). Over the past few years, there has been significant progress in defining the fine specificity of these antibodies and characterizing their target ▶ autoantigens. In some cases, the presence of these antibodies is related to the extent and severity of disease in Sjögren syndrome. The ▶ B cells make up roughly 20% of the infiltrating cell population in affected glands. The B cells produce ▶ immunoglobulins (Ig) with autoantibody activity for IgG (rheumatoid factor), Ro/SSA, and La/SSB. A substantial number of the B cells are of CD5+ phenotype (▶ B-1 cells). Production of IgG predominates in Sjögren syndrome, whereas synthesis of IgA is more abundant in normal salivary glands. Nonorgan-specific autoantibodies anti-Ro/SSA and anti-La/SSB are diagnostically the most important and the best-characterized autoantibodies in primary Sjögren syndrome. The majority of anti-Ro-positive sera also react with the denatured form of a 52-kDa protein termed Ro52, which is structurally distinct from Ro60 and probably does not directly associate with the Ro ribonucleoprotein particle. However, the two Ro proteins colocalize to surface membrane blebs on apoptotic cells, where they may become targets of an autoimmune response. Human ▶ monoclonal antibodies reactive with continuous and conformation-dependent ▶ epitopes on Ro52 have recently been cloned from a patient with primary Sjögren syndrome.

Cellular Immunity

Immunohistologic analysis of lymphoid cell infiltration in exocrine glands in Sjögren syndrome shows a predominance of ▶ T cells or (T lymphocytes) with fewer B cells and macrophages. ▶ Adhesion molecules and activated lymphocyte function-associated ▶ antigen type I (LFA-I) (▶ Leukocyte Functional Antigen (LFA) promote homing and occasionally characteristic cell clustering similar to that of follicular structures of lymph nodes. Expression of the mucosal lymphocyte ▶ integrin $\alpha^E\beta_7$ and its ligand E-cadherin suggest a mucosal origin of a subpopulation of the infiltrating cells. There is an increased expression of HLA-DR/DP/DQ molecules on acinar and ductal epithelial cells, presumably due to local production of IFN-γ by activated T cells. The majority of T cells in the lymphocytic infiltrates are CD4+ T-helper cells (▶ Helper CD4 T cells) with a ▶ CD4/▶ CD8 ratio well over 2. Most of these T cells bear the memory phenotype CD45RO+ and express the α/β T cell receptor and LFA-I, and may contribute significantly to B-cell hyperactivity. There is indication of oligoclonal expansion of certain TCR Vβ family-expressing lymphocytes. The peripheral blood in Sjögren syndrome has yielded findings similar to those in salivary glands, although a difference in magnitude is occasionally evident.

Immune-Mediated Tissue Destruction

Highly upregulated expression of ▶ HLA molecules, and the more recently demonstrated B-7 costimulatory molecules, by salivary gland epithelium in Sjögren syndrome is a potentially effective local antigen-presenting

mechanism whereby HLA antigens could be involved in exocrine glandular destruction mediated directly or indirectly by $CD4^+$ T cells (▶ CD4 T Cells). Such interaction may lead to further production of cytokines and stimulation of B-cell proliferation and differentiation. Indeed, high levels of ▶ interleukin (IL)-1B, ▶ IL-6, and TNF-α, three tissue-destructive cytokines, are produced by epithelial cells. IL-10 and IFN-γ are produced mainly by infiltrating T cells, whereas IL-6 and IL-10 also are produced in increased amounts in peripheral blood. A low capacity to produce ▶ interleukin-2 (IL-2) in Sjögren syndrome might be due to the absence of T cell ▶ costimulatory signals, resulting in the induction of anergy in the responding T cells population, but other explanations also are possible. Even though the mechanism(S) behind the characteristic glandular destruction of Sjögren syndrome salivary glands remains obscure, immunopathologic findings demonstrate that infiltrating ▶ cytotoxic T cells (▶ CTLs) could play a role in this event. On recognition of a proper MHC/antigen complex presented by a ▶ target cell, CTLs induce cell death through one of two main and independent pathways, the perforin-mediated pathway or the ▶ Fas/APO-1/CD95. Interestingly, expression of Fas also has been detected among infiltrating mononuclear cells in salivary glands of MRL/lpr mice, a murine model displaying features similar to those of human SLE and Sjögren syndrome.

Expression of Fas, FasL, Bcl-2, and other ▶ apoptosis-associated genes/proteins has been detected by reverse transcription-polymerase chain reaction (▶ RT-PCR) and immunohistochemical staining of minor salivary glands in patients with Sjögren syndrome. In particular, ductal and acinar epithelial cells, but to some degree also infiltrating mononuclear cells, express abnormal levels of Fas and FasL, especially in cases with heavy mononuclear cell infiltration. Ductal epithelial cells expressing Fas were usually situated inside or close to a dense focus. Most in situ studies have clearly shown a low-grade or even absent apoptosis among infiltrating mononuclear cells. The presence of ▶ granzyme A (▶ Granzymes) in Sjögren glands suggests that rather than apoptosis, the ▶ perforin pathway of CTL-mediated killing may be involved in the destruction of salivary glands. Among the salivary gland-infiltrating T cells, some express activation markers such as CD25, ▶ proto-oncogene products, and HLA-DR, but few T cells proliferate as determined by cell cycle studies. It seems difficult also to stimulate the T lymphocytes in Sjögren syndrome with the autoantigens Ro/SSA and La/SSB. These findings suggest that many cells are of memory T cell phenotype; either few of them are autoantigen-specific, or alternatively, many of them are in a state of anergy. In both cases, lack of stimulation of T cells also will hamper the apoptotic signals.

Immunopathogenesis (Summary)

The etiology and pathogenesis of Sjögren syndrome is still a matter of speculation, although several hypotheses prevail. Nevertheless, there is considerable evidence that some as-yet-unknown initiating factor(s) set against the appropriate genetic background may evoke immunologically mediated inflammatory mechanisms, which result in the chronic exocrine gland lesions. T cell-mediated autoimmune responses (▶ Cell-Mediated Immunity or cell-mediated immune response) in the glandular tissue and dysregulated apoptosis are currently considered to be of central importance in the pathogenesis. A plethora of autoantibodies has been linked to this autoimmune exocrinopathy, although their role is not always well defined. Accordingly, B-cell activation is a very consistent immunoregulatory abnormality in Sjögren syndrome.

Treatment and Prognosis

At present, treatment for most patients is essentially symptomatic. The patient should be seen regularly by a rheumatologist as well as by an ophthalmologist and a dentist to prevent and treat the consequences of mucosal dryness, in addition to extraglandular manifestations and other associated complications.

Artificial tears often alleviate the patient's complaints, and are of importance in preventing corneal damage and conjunctivitis. The use of topical steroids is not recommended because of a high risk of secondary bacterial and viral infections in the eye. Another treatment option for dry eye is "punctal occlusion" by using a variety of "plugs" to occlude the punctual openings at the inner aspects of the eyelids. With this procedure, the instilled artificial tears will remain in the eye for a longer time. The management of dry mouth aims to prevent and treat infections, gum disease, and dental caries. To reduce the risk of caries, it is necessary to keep good oral hygiene and use sugarless sweets and chewing gums to stimulate residual salivary flow. Artificial saliva products and special

toothpaste is advocated. Eradication of oral candidiasis usually provides significant improvement of oral symptoms.

Oral pilocarpine has recently been shown to be a safe treatment and to provide significant subjective and objective benefits for patients with Sjögren syndrome with symptoms associated with xerostomia. Another potential therapy includes systemic use of interferon-α (IFN-α), which may be of benefit for the symptoms associated with xerostomia.

Hydroxychloroquine may be useful as an immunomodulating agent reducing immune activation and lymphoproliferation and is sometimes used in patients with Sjögren syndrome. Administration of systemic steroids also has been suggested to improve the signs and symptoms of Sjögren syndrome, but they are mainly used for treatment of severe extraglandular complications such as pulmonary and renal involvement. Serology can be useful in predicting the subsequent outcome and complications in patients with primary Sjögren syndrome. The presence of anti-Ro/SSA antibodies may identify patients with systemic disease, and in anti-Ro/SSA/anti-La/SSB-positive patients, the relative risk of developing non-Hodgkin lymphoma has been reported as high as 49.7 after 10 years' follow-up. The development of extraglandular manifestations seems to be influenced by a number of factors including the MHC HLA B8 and DR3 expression. Spontaneous symptomatic improvement has been described in 12% of patients with primary Sjögren syndrome, especially in elderly patients with some clinical overlap with SLE.

SS and HCV

Patients with SS-HCV and B-cell lymphoma are clinically characterized by a high frequency of parotid enlargement and vasculitis, an immunologic pattern overwhelmingly dominated by the presence of RF and mixed type II cryoglobulins, a predominance of mucosal-associated lymphoid tissue (MALT) ▶ lymphomas, and an elevated frequency of primary extranodal involvement in organs in which HCV replicates (exocrine glands, liver, and stomach). Recently, there has been growing interest in the association of chronic HCV infection with both lymphoproliferative and autoimmune diseases. Several studies have found a higher prevalence of lymphoproliferative disorders in patients with HCV, although others have found no significant association. In addition, the specific tropism of HCV for many extrahepatic cell types has recently suggested a link between HCV and the development of autoimmune diseases, although this extrahepatic replication is not supported by all studies. The sialotropism of HCV may explain the close association with SS and sicca syndrome, whereas its lymphotropism links the virus with the synthesis of cryoglobulins and with lymphoma development. This extrahepatic tropism suggests the probability of the development of both autoimmune and lymphoproliferative processes in some patients with chronic HCV infection. In primary SS, lymphoma seems to be triggered by RF-secreting B cells closely associated with the 17109 and G-6 idiotypes, whereas in patients with HCV, a possible association with an antibody response to the envelope protein E2 of the virus has been postulated. The coexistence of both RF-positive processes (SS and HCV) in the same patient might enhance the possibility of developing diseases related to B-cell proliferation (cryoglobulinemia and low- and high-grade lymphoma). The most frequent type of B-cell lymphoma found in patients with SS-HCV was MALT lymphoma. The predominance of MALT lymphoma in SS-HCV and primary SS but not in HCV suggests an important role for SS in the lymphomagenesis of patients with SS-HCV (Treatment of SS-HCV, Robert G and concepts). Two therapeutic options should be highlighted as future options. The first is the use of monoclonal agents against B cells (▶ Rituximab or epratuzumab), which have been successfully used to treat not only ▶ B-cell lymphomas but also cryoglobulinemic vasculitis, with the aim of controlling the marked B-cell hyperreactivity observed in patients with SS-HCV. The second is the use of antiviral agents (▶ Interferon and ▶ ribavirin), which aims to eradicate the virus as the main causative agent of this B-cell hyperactivity, and which was successfully used in some of our patients. A combination of anti-B-cell and anti-HCV agents may be a promising option for the successful treatment of B-cell lymphomas in patients with SS-HCV.

Does SS Cause Lymphoma?

A small percentage of people with Sjögren syndrome develop lymphoma, which involves salivary glands, lymph nodes, the gastrointestinal tract, or the lungs. Persistent enlargement of a major salivary gland

should be carefully and regularly observed by your doctor and investigated further if it changes in size in a short period of time. Other symptoms may include the following. (Note that many of these can be symptoms of other problems, including Sjögren syndrome itself. Nevertheless, it is important to see your doctor if you have any of these symptoms so that any problem can be diagnosed and treated as early as possible.)

1. Unexplained fever
2. Night sweats
3. Constant fatigue
4. Unexplained weight loss
5. Itchy skin
6. Reddened patches on the skin

If you are worried that you might develop lymphoma, talk to your doctor to learn more about the disease, the symptoms to watch for, any special medical care you might need, and what you can do to relieve your worry.

SS and Lymphoproliferative Disorders

Sjögren syndrome is a chronic organ-specific autoimmune disease characterized by lymphocytic infiltration into the salivary and lacrimal glands. About half of primary SS patients develop systemic disorders. Primary SS can be divided into three stages according to the extent of organ damage and the course of the disease. In stage I (~45% of cases), patients have only sicca syndrome and do not experience any systemic involvement, even after 10 years. In stage II (~50% of cases), patients experience lymphocytic organ damage, which may involve the pulmonary, renal, hepatic, hematologic, and/or dermatologic systems, among the others. Finally, in stage III (~5% of cases), patients develop malignant ▶ lymphomas. Lymphomas in salivary glands are thought to arise from lymphoepithelial lesions in which there are close interactions among epithelial cells, T cells, and B cells. The B cells in the lesions become activated through the interaction between CD40L and CD40. The progression from polyclonal lymphoproliferation to monoclonal lymphoproliferation, to MALT lymphoma, and finally to high-grade malignant lymphoma is regarded as a multistep process. Antigenic activation of B cells, together with oncogenic events, including ▶ p53 inactivation and bcl-2 activation, may play important roles in B-cell monoclonal proliferation and malignant transformation. The rheumatoid factor clone is regarded as a candidate B-cell clone that undergoes transformation (Lymphoproliferative disorders in Sjögren syndrome, Yasufumi Masaki and concepts).

Future Directions

The search for susceptibility genes in families with Sjögren syndrome is ongoing with the same approach as in the other chronic autoimmune diseases and with utilization of two major strategies: the position-independent candidate gene approach with mutation screening of suspected disease-related genes and full genome scanning (microsatellite analysis) in humans as well as in animal models to determine susceptibility chromosomal regions, which later will be used in a positional candidate gene strategy. Another challenge in Sjögren syndrome will be to stratify the disease process including genetic and environment triggers. Identification of new genetic markers and better characterization of novel autoantibodies (e.g., those directed against muscarinic receptors in exocrine glands) may lead to the development of better diagnostic and prognostic tests in Sjögren syndrome including its systemic complications. Sjögren syndrome is considered to represent an ideal disease to study the mechanisms underlying autoimmunity because its manifestations are both organ-specific and systemic. The significance of such studies is underlined by the high prevalence of Sjögren syndrome as a common but often neglected systemic autoimmune disease often found in the female and aging population.

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Skeletal Complications

- ▶ Bone Loss Cancer Mediated
- ▶ Skeletal-Related Events

Skeletal Secondary Tumors

- ▶ Bone Tropism

Skeletal-Related Events

Definition

Refers to the major complications of tumor bone disease (i.e., pathologic fracture, spinal cord compression, hypercalcemia).

- ▶ Skeletal Complications
- ▶ Zoledronic Acid

Skeletrophin

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Synonyms

Mib-2; Mind bomb homolog 2; Putative NF-kappa-B-activating protein 002N; ZZANK1

Definition

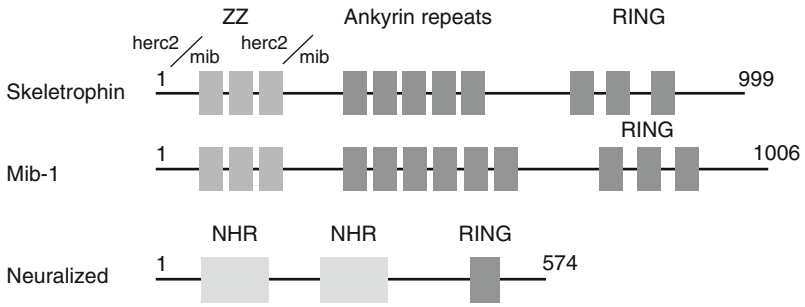
Skeletrophin is one of three ubiquitin ligases, the substrate of which is a cytoplasmic region of Notch ligands and positively regulates ligand-dependent Notch activation. The gene maps to human chromosome position 1p36.32-33, where tumor-suppressor factors for neuroendocrine tumors are encoded. Although at least six alternative splicing forms have been found in the public database, the major human product, which is expressed in many tissues, is composed of 999 amino acids and is approximately 110 kDa. Skeletrophin is localized in the cytoplasm and is widely expressed in various adult tissues, with particularly strong expression in skeletal muscle, heart, and brain tissue. There are also alternative splicing forms expressed specifically in the brain, and forms expressed specifically in the developing brain.

Characteristics

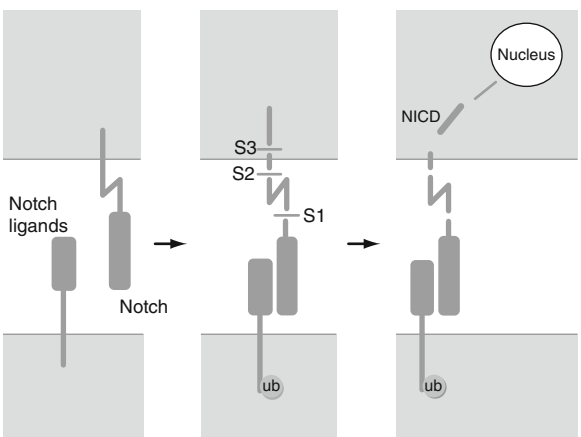
Skeletrophin has two mib/herc2 domains, a ZZ domain, ankyrin repeat domains, and two RING-HC motifs (Fig. 1). A self-ubiquitination assay revealed that a typical RING-HC motif at the C-terminal mediates ubiquitin ligase activity in vitro. However, both of the RING-HC motifs are believed to be essential in vivo. Both skeletrophin and its paralogue, mib-1 (also known as DIP-1, Fig. 1), also cause mono-ubiquitination of the intracellular domain of Notch ligands. In some tissues, including the brain, both skeletrophin and mib-1 are expressed; however, in other tissues, especially at developing tissues, skeletrophin is expressed only at low levels, with predominant expression of mib-1. Another ubiquitin ligase, termed Neuralized, also causes mono-ubiquitination of Notch ligands.

The other ubiquitin ligases, Neuralized, also target intracellular region of Notch ligands. They have two neuralized homology repeat domains (NHRs) and C-terminal RING domain. NHR is believed to be important for ligand binding.

Notch ligands are internalized to the cytoplasm by mono-ubiquitination. In general, mono-ubiquitin-mediated endocytosis removes ligands or receptors from the cell surface membrane, therefore abolishing signal transmission. In contrast, endocytosis of Notch ligands positively mediates ligand-dependent Notch activation, when Notch ligands bind to Notch (Fig. 2). It is thought that endocytosis of Notch ligands may alter the molecular structure of the bound Notch molecules and expose the proteinase cleavage sites, indicated by S1 in Fig. 2, of the extracellular domain of Notch. This process is linked to the generation of the



Skeletrophin. Fig. 1 Schematic representation of molecular structures of skeletrophin and its paralogue mib-1 (mind bomb homologue-1). Skeletrophin and mib-1 have two and three RING-HC motifs, respectively. A typical RING-HC motif at the C-terminus of both skeletrophin and mib-1 mediates ubiquitination in vitro. Two unique herc2/mib domains are found at the N-terminus. Distinct molecular functions of herc2/mib are unclear; however, this domain is often found to be coexistent with the RING motif. A novel zinc-finger domain (ZZ domain) is found in dystrophin and in the first half of skeletrophin and mib-1. The ZZ domain is a putative calmodulin binding site; therefore, it is thought that skeletrophin and mib-1 may be regulated by Ca^{2+} concentration. Ankyrin repeats are a conspicuous feature of ankyrin and act as a binding site for various molecules



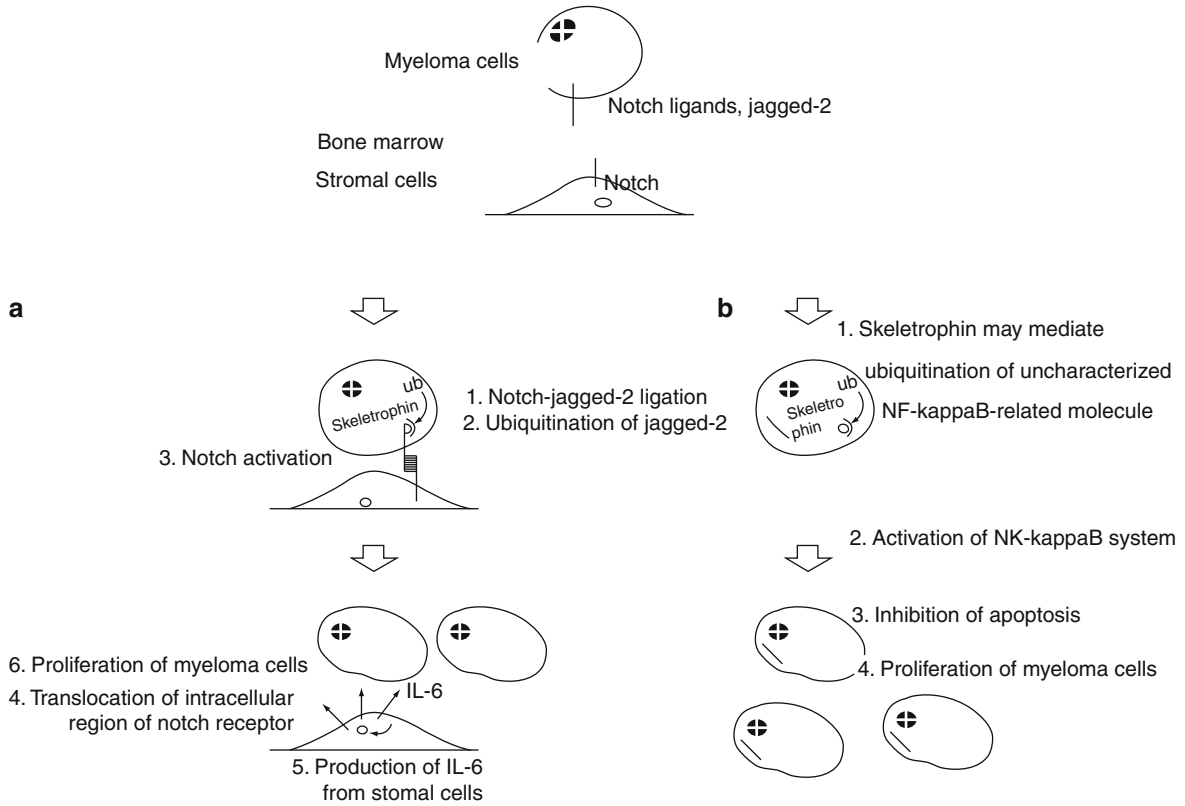
Skeletrophin. Fig. 2 Mono-ubiquitination of the intracellular region triggers endocytosis of Notch ligands. Ligand binding to the Notch receptor results in a conformational change, leading to the unmasking of the proteinase cleavage site, indicated by S2 in the figure. Subsequently, the extracellular domain of Notch undergoes proteolytic cleavage, and is then further processed at the juxtamembrane proteolytic cleavage site to generate the Notch intracellular domain (NICD). Finally, NICD moves into the nucleus and mediates transcriptional signaling

intracellular Notch domain, which transmits the ligand-dependent Notch activation signal. Mono-ubiquitination-mediated endocytosis is generally followed by lysosomal protein degradation or recycling of surface membrane proteins. Skeletrophin may also be involved in the degradation or recycling of Notch ligands that are not bound to Notch. However, the precise mechanism that determines whether skeletrophin activates or abolishes ligand-dependent Notch activation remains largely unknown.

Clinical Aspects

Ligand-dependent Notch activation has a dual antagonistic role in promoting and suppressing tumorigenesis. Skeletrophin also plays a role in both tumor progression and tumor suppression.

Skeletrophin is overexpressed in multiple myeloma, especially from patients whose disease is at an advanced stage and where there are osteolytic bone lesions present. In general, ubiquitin ligase and its substrate, the target molecule, are not constitutively co-expressed in a cell; however, both skeletrophin and its substrate, Jagged-2, a Notch ligand, are constantly and abundantly expressed in many myeloma cells. This aberrant co-expression of skeletrophin and Jagged-2 is caused by epigenetic molecular events in myeloma cells. Both *skeletrophin* and *jagged-2* contain a CpG-rich promoter, harboring CpG islands, which, when hypomethylated, are activated in numerous myeloma cells (Fig. 3). As a result, skeletrophin constitutively acts to cause mono-ubiquitination of the intercellular region of Jagged-2, causing ligand-dependent Notch activation in bone marrow stromal cells, which directly contact with myeloma cells. Finally, activated bone marrow stromal cells secrete cytokines, including IL-6, and create an adequate microenvironment for myeloma progression (Fig. 3a). Jagged-2 is a cell surface membrane protein with a transmembrane domain, whereas in many



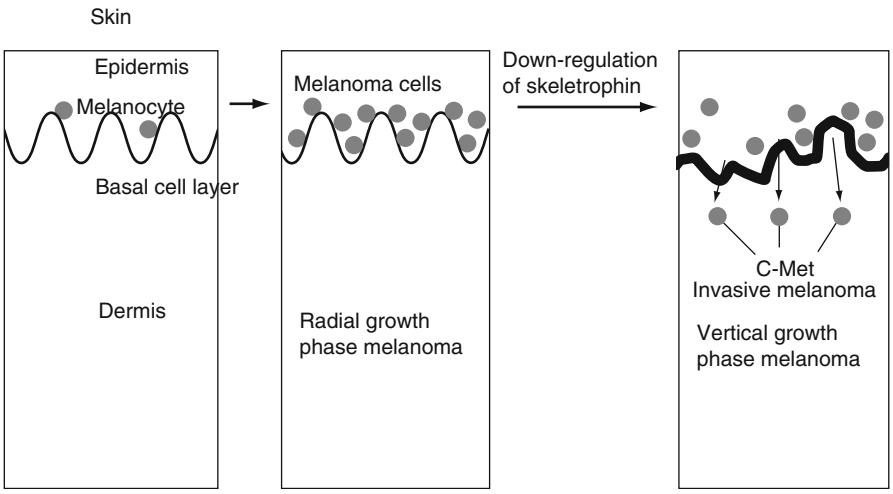
Skeletrophin. Fig. 3 The pathological role of skeletrophin in multiple myeloma. (**a**) Indirect effect through bone marrow microenvironment. Myeloma cells adhere to stromal cells. Overexpression of skeletrophin and its substrate, Jagged-2, in myeloma cells facilitates ligand-dependent Notch activation in stromal cells. Activated stromal cells secrete various cytokines including IL-6 to promote myeloma. (**b**) Direct mechanism of skeletrophin in multiple myeloma. Overexpression of skeletrophin activates NF-kappaB signaling and protects myeloma cells against apoptosis

myeloma cells, Jagged-2 is localized to the cytoplasm, as a result of endocytosis following skeletrophin-mediated-mono-ubiquitination.

Skeletrophin is also characterized as a putative NF-kappa-B-activating protein, designated 002N. Several independent transcriptional screenings have detected skeletrophin as a powerful NF-kappa-B activator among over 15,000 clones. The anti-apoptotic effect of skeletrophin also contributes to progression of myeloma, especially in patients receiving chemotherapy (Fig. 3b). Notably, the molecular structure of the RING-HC motif in skeletrophin is very similar to that of apoptosis-mediating ubiquitin ligases, such as IAP-1, 2, and XIAP. These findings suggest that skeletrophin has an anti-apoptotic effect and promotes tumor progression by targeting an unknown molecule, other than Notch ligands.

In contrast, skeletrophin suppresses malignant melanoma progression, especially tumor invasion (Fig. 4). Skeletrophin is expressed in normal melanocytes, almost all benign nevi, and in many noninvasive melanoma cells. Skeletrophin is silenced by various epigenetic events, including hypermethylation, in many invasive malignant melanomas at the invasion stage or the vertical growth phase. Skeletrophin downregulates transcription of the Met oncogene, which encodes the hepatocyte growth factor receptor, and plays a role in the determination of the invasive phenotype of many malignant tumors. Restoration of skeletrophin in invasive melanoma has been shown to suppress tumor invasion both in vitro and in vivo.

To date, three molecular events have been determined to repress skeletrophin expression in melanoma. First, aberrant hypermethylation of the promoter region of *skeletrophin* on a CpG island is found in many invasive melanomas. Second, downregulation of an activator protein-2 (AP-2), which is known to have a tumor-suppressor role in melanoma, also



Skeletrophin. Fig. 4 Silencing of skeletrophin in melanoma contributes to tumor invasion. Skeletrophin is expressed in melanocytes, a normal component of melanoma, and in noninvasive melanoma cells at the radial growth phase. In contrast, various epigenetic events downregulate skeletrophin in invasive melanoma at the vertical growth phase. The silencing of skeletrophin allows melanoma cells to express c-Met. C-met encodes

downregulates the transcription of skeletrophin. Third, overexpression of Snail, a zinc-finger transcriptional factor, is responsible for silencing of skeletrophin by binding to an E-box in the *skeletrophin* promoter. Notably, Snail is known to be overexpressed in invasive melanomas. In addition, as well as being one of the most common karyotypic lesion seen in melanoma, loss of alleles of 1p36 has been detected as a late event, at the invasive and metastatic phase, in melanoma progression. Therefore, loss of heterozygosity may be important for downregulation of skeletrophin in melanoma.

It has long been speculated that a gene encoding tumor-suppressor factor in invasive melanomas is located at human chromosome position 1p36.3. Recent studies indicate that this tumor-suppressor factor may be skeletrophin itself. Moreover, it is believed that uncharacterized genes, which encode the suppressor molecule for various neuroendocrine tumors including neuroblastoma, are located at 1p36.3. Since melanoma is a malignant tumor of neural-crest-derived melanocytes, skeletrophin might be a tumor-suppressor factor for various neuroendocrine tumors.

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Ski

Definition

Was first identified as a viral oncogene from the avian Sloan-Kettering retrovirus, which transforms chicken embryo fibroblasts. Elevated levels of c-ski have been detected in several human tumor cell lines derived from neuroblastoma, melanoma, and prostate cancer. Ski appears to bind to DNA and be part of the ▶ histone deacetylase complex.

▶ Smad Proteins in TGFβ Signaling

Skin Cancer

Definition

Refers to cancers involving the skin. About 80% of these skin cancer cases are ▶ basal cell carcinoma (BCC), 16% ▶ squamous cell carcinoma (SCC), and 4% ▶ melanoma.

Skin Carcinogenesis

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Definition

A multistep process by which a number of distinct phases occur to form malignant skin tumors. The first step in *skin carcinogenesis* is ▶ initiation, which is a reversible process during which genetic mutations, gene activation or inactivation occur. Examples of initiation are mutations in the v-Ha-*ras* oncogene or inactivation of the p53 tumor suppressor gene. The next phase of carcinogenesis is ▶ tumor promotion, characterized by a reversible phase of clonal expansion of initiated cells containing mutations/inactivated genes, with a dysregulation of apoptosis of the initiated cells, as well as by accumulation of epigenetic changes such as DNA methylation, inflammation characterized by infiltration of activated leukocytes, production of growth factors, cytokines, reactive intermediates, including oxygen-free radicals and nitrogen radicals which stimulate formation of DNA damage, with inhibition of DNA repair enzymes. These alterations become irreversible and first lead to development preneoplastic papillomas, which are benign skin lesions. Simultaneous with tumor ▶ promotion, vascular permeability occurs, which is the first stage of skin ▶ tumor-associated angiogenesis, which is in itself a multistep process, during which vasculature develops to provide oxygen and nutrients to expanding preneoplastic lesions. With continual accumulation of genetic mutations, the next stage of carcinogenesis, ▶ tumor progression occurs, which is characterized by the accumulation of genetic mutations leading to conversion of preneoplastic skin lesions to malignancy, which are primarily squamous cell carcinomas (Fig. 1).

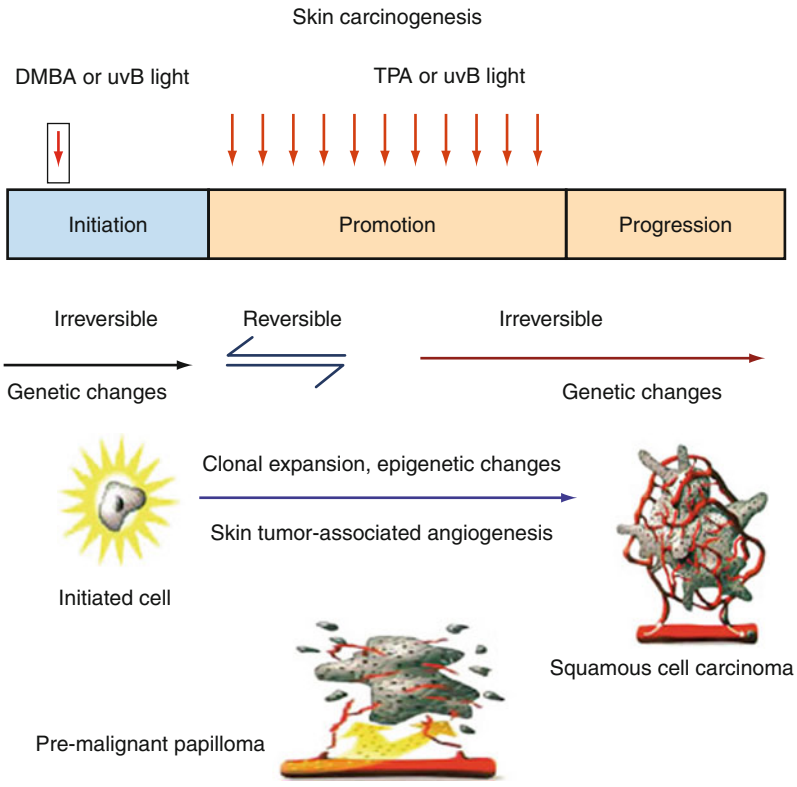
Characteristics

In 1775, Dr. Percival Pott, a British surgeon, made the observation that there was a high incidence of scrotal cancer in chimney sweeps compared to that of the general population. This observation is recognized to be the first report of chemical carcinogenesis, suggesting that exposure to environmental agents, such as soot and coal tar, may be directly linked to development of cancer in humans. In addition, this observation also led scientists to evaluate the carcinogenic process using a variety of different models and to identify the specific compounds and/or physical agents that are involved in the growth of human tumors.

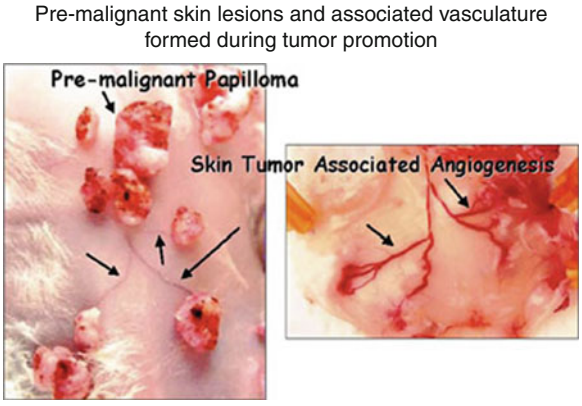
Thus began the development of rodent models to study what is now understood to be the multistep process of carcinogenesis. The most widely used models of multistage carcinogenesis has been in mouse skin, which have been used over the past 70 years to identify and define distinct and sequential stages of mouse skin carcinogenesis, which is a process that includes multiple steps including tumor initiation, tumor promotion, and tumor progression (Fig. 2).

The development and use of mouse skin carcinogenesis models have led to the appreciation that the majority of tumors of epithelial origin in humans arise due to a multistep process, with the majority of experimental models developed using the skin model as an example. There are now multiple models available that have been used to identify the genetic, molecular, and cellular basis of rodent lung tumors, gastrointestinal tumors, oral and head and neck tumors, as well as hepatocellular tumors and breast tumors that are based on the commonly held view that human tumors develop as a result of a multistep process.

There are two primary mouse skin models that have been used to define the genetic, molecular, cellular, and genetic basis of skin carcinogenesis. The first of these models is based on the topical application of a single dose of a chemical carcinogens, primarily polycyclic aromatic hydrocarbons, such as ▶ 7, 12 dimethylbenz[a]anthracene (DMBA), followed by multiple topical exposures to an agent such as the



Skin Carcinogenesis. Fig. 1 Schematic of the multistep process by which squamous cell carcinomas develop following exposure to either chemicals such as DMBA and TPA or to multiple exposures to sunlight that contains ultraviolet light in the 290–320 nm range, defined as uvB light. Note that this process includes not only proliferation but also includes genetic alterations, epigenetic changes, increased vascular permeability, and tumor-associated angiogenesis



Skin Carcinogenesis. Fig. 2 Photomicrographs of premalignant skin lesions and the dermis underlying the skin lesions which contain vasculature to each premalignant skin lesions which provides oxygen and nutrients. These are the classical hallmarks of the process of multistage skin carcinogenesis leading to formation of squamous cell carcinomas

► tumor promoter, 12-0-tetradecanoylphorbol-13-acetate (TPA), which is the active ingredient of croton oil, first used in multistage skin carcinogenesis studies.

The other model of skin carcinogenesis commonly used is based on induction of skin tumors following multiple exposures to wavelengths of light that are contained in solar radiation, primarily ultraviolet light in the 290–320 nm wavelength range of light, known as Ultraviolet Light B (uvB) light. Although humans do develop skin tumors from exposure to chemicals, the vast majority of human skin tumors occur due to multiple exposures to solar irradiation, within an increase in skin tumors, due to the depletion of the ozone layer.

Exposure of the skin to a single dose of a carcinogen such as DMBA, a polycyclic aromatic amines derived from fossil fuel by-products followed by multiple exposures to the tumor promoter TPA or to multiple exposures of the skin to uvB light induces both genetic changes and epigenetic alterations, which significantly alters the normal organized pattern of keratinocyte proliferation and differentiation, ultimately resulting in development of skin malignancies.

Genetic Basis of Skin Carcinogenesis

These two mouse models of multistage skin carcinogenesis have been used to identify and define distinct and sequential stages of mouse skin carcinogenesis, which include tumor initiation, promotion, and

progression. Although exposure of mice to either chemicals or to uvB light results in formation of skin tumors, these different agents induce different genetic mutations.

In the chemical skin carcinogenesis model which uses DMBA as the ▶ initiator and TPA as the tumor promoter to induce skin tumor formation, the first step is tumor initiation, which is accomplished by topical application of a single subcarcinogenic dose (25–200 nmol) of DMBA to the dorsal skin of genetically susceptible mice. Exposure of the skin to carcinogen results in formation of mutations in the Harvey-ras (Ha-*ras*) oncogene at codons 12, 13, 59, and 61 of epidermal keratinocytes, which are then considered to be "initiated" cells.

Initiation

The formation of genetic mutations by either a chemical, such as a polycyclic aromatic hydrocarbon, 7,12 dimethylbenz[a]anthracene (DMBA) or benzo[a]pyrene (BP), derived from incomplete combustion of fossil fuels or as a byproduct of smoking tobacco, respectively, which induce mutations in such areas as codons 12, 13, 59, and 61 of the v-Ha-ras oncogene. Physical entities, such as solar irradiation, particularly ultraviolet light (uv) in the 290–320 nm wavelength range (uvB), induce inactivation of the p53 tumor suppressor gene by formation of mutations in specific codons including codons 151/152, 245, 248, 278, 286 within exons 5–9 of this gene.

Promotion

Promotion is a process by which chemical or physical agents, such as 12-0-tetradecanoylphorbol-13-acetate (TPA) or uvB light, stimulate clonal expansion of initiated cells and alter the differentiation pattern of specialized epithelial cells of the skin, epidermal keratinocytes. In addition to these cells undergoing tumor promotion, the stem cells of the skin located in the interfollicular "bulge region" of hair follicles in the dermis also undergo proliferation. Initiated cells undergoing clonal expansion do not undergo the normal process of terminal differentiation, which is a specialized form of programmed cell death, i.e., apoptosis, that normally occurs in epidermal keratinocytes that contain damaged DNA. In contrast to chemicals such as DMBA and BP that act primarily as initiators, uvB light acts both as an initiator as well as a tumor promoter, and is therefore considered to be a "complete carcinogen."

As an initiator of multistage skin carcinogenesis, uvB light induces inactivation of the p53 tumor suppressor gene due to formation of mutations primarily by formation of pyrimidine 6–4 pyrimidone photoproducts at codons 151/152, 245, 248, 278, and 286 of the p53 gene, with the frequency of photoproducts very high at codon 286. The importance of the mouse model of ultraviolet light–induced multistage skin carcinogenesis to human tumorigenesis is based on the fact that ~50% of all human tumors contain defects in the p53 tumor suppressor gene, although the codons that are hot spots for p53 mutations may differ from those induced by uvB light, indicating that there is genetic heterogeneity in human tumors and in mouse models used to study human carcinogenesis. This clearly defines the uvB light–induced model of skin carcinogenesis as having high relevance to the genetic basis of human skin cancer, as well as to other types of human cancers of epithelial origin.

With the development of transgenic mouse models, the genetic basis of both uvB-induced skin carcinogenesis, which is p53 inactivation, and chemical carcinogenesis induced by DMBA/TPA, which is based on v-Ha-*ras* mutations/activation, has been verified. Other multistage models of carcinogenesis have been developed based on these two mouse models of skin carcinogenesis, such as models for understanding the genetics of cervical cancer induced by human papillomas virus and the genetic basis for colorectal carcinoma using the adenosis polyposis coli gene.

Cellular and Molecular Basis

Ultraviolet light in the 290–320 wavelength range, (uvB) light is a complete carcinogen. This physical agent therefore acts as an initiator and induces mutations in specific codons of the p53 tumor suppressor gene, with clusters of p53 mutated cells formed at very early times after uvB exposure. In addition, uvB light also acts as a tumor promoter to induce clonal expansion of initiated cells, with a dysregulation of apoptosis stimulating this process. A number of different types of DNA damage have been shown to be present in skin exposed to uvB light, including cyclobutane pyrimidine dimers, pyrimidine (6–4) pyrimidone photoproducts, and formation of oxidative DNA adducts such as 8-oxo-deoxyguanine. Epidermal keratinocytes which contain these mutations in the p53 tumor suppressor

gene that leads to its inactivation are resistant to apoptosis through the dysregulation of Fas–Fas ligand interactions, thereby allowing keratinocytes with p53 mutations to have a selective advantage in undergoing clonal expansion, first leading to actinic keratosis and subsequently to development of squamous cell carcinomas.

In skin carcinogenesis induced by DMBA/TPA, DMBA serves as the initiator of mutations in the v-Ha-ras oncogene and tumor promotion is accomplished by repeated topical application of a tumor promoter, such as 12–0 tetradecanoylphorbol-13-acetate (TPA), to initiated skin for 20–30 weeks. TPA stimulates clonal expansion of initiated *ras*-mutated keratinocytes, ultimately leading to outgrowth of preneoplastic skin papillomas. The changes in the skin induced during tumor promotion include epidermal and follicular hyperplasia, altered differentiation patterns of epidermal keratinocytes and interfollicular bulge region "stem cells," with an associated resistance to apoptosis and simultaneous increase in genes associated with cell survival, dermal inflammation characterized by infiltration of activated leukocytes that produce reactive oxygen and nitrogen intermediates, leading to DNA damage and alterations in DNA repair. In addition, other critical factors, such as production of growth factors and their receptors, cytokines and chemokines, phosphorylation and subsequent activation of signal transduction molecules, as well as induction of immediate early genes, have been examined for their essential role in multistage skin carcinogenesis.

The last phase of mouse skin carcinogenesis is tumor progression, which occurs in a small subset of preneoplastic papillomas that acquire additional genetic mutations and subsequently undergo malignant conversion from preneoplastic lesions to form malignant squamous cell carcinomas.

Skin tumor-associated angiogenesis is a process that is now recognized to be essential for skin carcinogenesis. The primary growth factors involved in stimulating the vascular permeability and development of new blood vessels surrounding the developing skin lesions are in the family of vascular endothelial growth factor proteins. Further studies which better define other factors involved in skin tumor-associated angiogenesis may hold promise for development of novel strategies to inhibit survival of preneoplastic skin papillomas and to block their progression to squamous cell carcinomas.

Relevance to Human Disease

Skin tumors are one of the most prevalent types of tumors diagnosed in humans. Experimental rodent models of multistage skin carcinogenesis have been developed to define the genetic, molecular, and cellular basis of this multistep process by which human skin tumors form. These mouse models have also provided the basis for development of rodent models for other tumors of epithelial origin. The two models of multistage skin carcinogenesis described above have allowed identification of the molecular pathways involved in this process and provide models for identification and development of chemotherapeutic agents for treatment of skin cancer patients. In addition, these models of skin carcinogenesis have also been used extensively to evaluate the activity of natural products and their active ingredients for prevention of skin cancer as well as are currently being used to define the role of stem cells in skin tumor carcinogenesis.

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Skin Prick Testing

Definition

A method of testing to determine atopy and the allergens responsible.

► Allergy

Skinny Needle

► Fine Needle Aspiration Biopsy

Skin-Sparing Mastectomy

Definition

Surgical removal of the breast tissue with preservation of the overlying skin.

► Oncoplastic Surgery

Skipper-Schabel Model

► Log-Kill Hypothesis

Skipper-Schabel-Wilcox Model

► Log-Kill Hypothesis

Skp2

Definition

S-phase kinase-associated protein 2; is part of the ► SCF complex.

► Ubiquitin Ligase SCF-Skp2

Skp-2

Synonyms

Skp2

SL-1

► Stromelysin-1

SLD

Definition

Sphingolipid, the class of lipids based on sphingosine and similar amines.

► Sphingolipid Metabolism

SLE

Definition

► Systemic Lupus Erythematosus.

SLe^x

Definition

Sialyl Lewis x, a naturally occurring glycan of white blood cells and vascular endothelium, necessary for selectin-mediated ► adhesion, and typically overexpressed on tumor cells.

► Glycobiology

Slit

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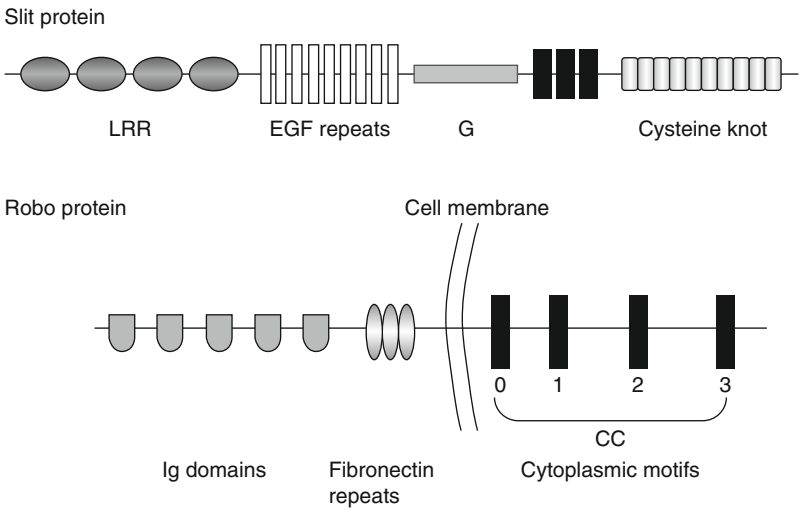
Definition

The *Slit* family of secreted proteins has been shown to function in ► axon guidance and neuronal ► migration. There are three Slit proteins in mammals,



Slit. Fig. 1 Schematic depicting the structure of prototypical Slit and Robo proteins. The mammalian Slit protein contains an N-terminal signal peptide, four leucine-rich repeats (LRRs), nine (in vertebrates) EGF repeats, and a C-terminal cysteine knot. A typical Robo1 receptor consists of five extracellular immunoglobulin (Ig) domains, three fibronectin repeats, and a conserved intracellular region of four cytoplasmic motifs. Robo3 (also known as Rig1) is missing one of the cytoplasmic motifs and Robo4 encodes only two Ig domains, two fibronectin repeats, and two cytoplasmic motifs

and while Slit1 expression is mostly restricted to the nervous system, Slit2 and 3 are also expressed in other organs.

Characteristics

A typical Slit protein contains an N-terminal signal peptide, four leucine-rich repeats (LRRs), seven (in *Drosophila*) or nine (in vertebrates) EGF repeats, a laminin G domain, and a C-terminal cysteine knot (Fig. 1). The receptor for Slit is the transmembrane protein ▶ Robo (Roundabout), and four *ROBO* genes have been identified. Typically, Robo proteins, including Robo1, consist of five extracellular immunoglobulin (Ig) domains, three fibronectin repeats, and a conserved intracellular region of four cytoplasmic motifs (Fig. 1).

Slit function in the Nervous System

Expression of Robo proteins has traditionally been associated with migrating axons in the developing nervous system. Examples include expression in commissural axon growth cones after ventral midline crossing and expression in olfactory bulb axons on route from the olfactory epithelium to the primary olfactory cortex. In the *Drosophila* spinal cord, Slit functions as a short-range cue to prevent ipsilateral projecting commissural fibers from crossing the midline, and contralateral projecting commissural fibers from recrossing the midline. A combinatorial code of Robo receptors on medial, intermediate, and lateral axons helps control lateral positioning in response to a Slit gradient in the CNS. In addition, Slit proteins act as chemorepellents in axon guidance in the mouse visual system as well as in neuronal migration and axon guidance in the mammalian forebrain both in vitro and using knockout mice deficient in Slit1 and/or Slit2 in vivo.

Slit-Robo Signaling

Studies examining Slit-Robo signaling can be divided into three basic categories. The first paradigm involves the ▶ risk factors and the regulation of the actin cytoskeleton to generate a turning response.

Evidence has accumulated suggesting an important role for regulation of the actin cytoskeleton machinery in Slit-mediated repulsion. The *Slit-Robo-GAPS* (▶ srGAPs) facilitate hydrolysis of Cdc42 leading to actin depolymerization. Studies have demonstrated opposing roles for Rac in the regulation of axon repulsion in Drosophila. For example, Slit stimulation leads

to recruitment of the SH3-SH2 adaptor protein Dreadlocks (Dock) and the p21-activated serine-threonine kinase (Pak) to the Robo receptor CC2 and CC3 cytoplasmic motifs. Recruitment of this complex increases Rac activity to regulate axon repulsion at the CNS midline. In contrast, Vilse, a conserved family of RhoGAPs has been shown to promote hydrolysis of RacGTP, and less efficiently, Cdc42GTP to mediate Robo repulsion in Drosophila tracheal cells and axons. The contradictory models can be explained in terms of a temporal model of Slit effectors where sequential interaction with the Robo receptors leads to a sustained turning response.

In addition to the involvement of the RhoGTPases, a role for the Abelson kinase (Abl) and its substrate Enabled (Ena) in Slit-mediated repulsion has been elucidated. Abl binds to CC3 and phosphorylates a tyrosine in CC1; whereas, Ena associates with CC2 to regulate the repulsive effect of Slit. Genetic and biochemical evidence suggests that Abl and Ena play opposing roles in Robo-mediated repulsion where Abl antagonizes Slit-Robo signaling and Ena promotes the repulsive effect. In addition, Abl has also been linked with a supramolecular complex consisting of Robo and N-cadherin that facilitates inactivation of N-cadherin-mediated adhesion in response to Slit. This mechanism uncouples the association of N-cadherin with the actin cytoskeleton and is accompanied by a loss of growth cone traction and axon extension.

Another mechanism associated with Slit-Robo signaling is the "silencing" of the Netrin receptor DCC. Activation of the Robo receptor leads to the silencing of Netrin1's attractive effect through direct binding of Robo's cytoplasmic domain to that of the DCC receptor without a concomitant affect on the stimulation of growth cone extension rate in embryonic Xenopus spinal axons. This hierarchical organization contributes to the finely tuned controlled mechanisms guiding growth cones to their final targets.

Slit Function Outside the Nervous System

Although the function of Slit in axon guidance and neuronal migration is well characterized, other developmental roles have been demonstrated. For example, homozygous knockout mice for the first Ig domain of the Robo1 gene frequently die at birth due to respiratory failure and inadequate lung maturation. Survivors demonstrated severe lung hyperplasia and bronchial abnormalities suggestive of early lung cancer. Human patients with horizontal gaze palsy with progressive scoliosis (HGPPS) were reported to have mutations in Rig1/Robo3, and functional studies have shown defects in commissural hindbrain projections and pontine nuclei. Furthermore, Slit2 and 3 and Robo1 and 2 expression have been detected in nonneuronal cells including pulmonary mesenchyme and airway epithelium, kidney, heart, spleen, thymus, and lymph nodes. The temporal and spatial distribution of Slit and Robo mRNAs in fetal and adult tissues suggest that these genes may be associated with functional organization and cell motility during development.

Slit Proteins and Cancer

Secreted proteins that guide neuronal and glial cell precursors in the developing central nervous system have also been linked with tumorigenesis. Long-range chemotropic factors including netrins, semaphorins, ephrins, and the Slit family of proteins are known for their roles in neuronal and glial cell migration. These molecules play an important role in neurodevelopment and it is reasonable to assume that they also influence tumor progression in the nervous system.

Slit-Robo has been shown to be involved in tumor angiogenesis and PI3K signaling has been implicated in this process. Other studies have identified Slit2 as a potential tumor suppressor gene in gliomas, lung, breast, and colorectal cancer, as well as in neuroblastoma.

Slit and Medulloblastoma

Slit has also been shown to play an important role in nonneuronal cells, as an inhibitor of leukocyte chemotaxis and promoter of tumor-induced angiogenesis and endothelial cell attraction. ▶ Invasion of brain tumor cells has made primary malignant brain neoplasms among the most recalcitrant to therapeutic strategies. Slit2 inhibits the invasion of ▶ medulloblastoma, but not ▶ glioblastoma (multiforme) cell invasion, in a variety of in vitro models. For example, time-lapse videomicroscopy indicated that Slit2 reduced medulloblastoma invasion rate without affecting cell direction or proliferation. Both medulloblastoma and glioma tumors express Robo1 and Slit2, but only medulloblastoma invasion is inhibited by recombinant Slit2 protein. Downregulation of activated Cdc42 may contribute to this differential response.

A role for Rig1/Robo3 in controlling midline crossing of hindbrain precerebellar neurons and axons has been elucidated. Human patients with horizontal gaze palsy with progressive scoliosis (HGPPS) were reported to have mutations in Rig1/Robo3, and functional studies have shown defects in commissural hindbrain projections and pontine nuclei. Furthermore, early and late stage chick cerebellar rhombic lip fragments are repelled by Slit2. Medulloblastoma cells are thought to arise from external granular layer cerebellar precursors derived from the rhombic lip, thus providing an obvious developmental parallel with Slit2 inhibition of medulloblastoma cell invasion. Slit also inhibits CXCR4-induced motility in breast cancer cells and CXCR4 antagonists have been shown to inhibit medulloblastoma tumor growth in vivo.

Slit2 expression has been shown to be downregulated in some gliomas with methylated SLIT2 promoter compared to gliomas and normal brain samples showing no methylation of this promoter. Slit2 and Robo1 are expressed by a variety of glioma and medulloblastoma cell lines and primary tumors. Most CNS neurons in the rat brain express at least one Robo and one Slit during their development, and that levels are maintained from the embryonic to the adult stage. Neurons expressing Robo mRNA could be unresponsive to Slit if molecules or mechanisms regulating Robo expression and function were present.

In light of the evidence demonstrating a role for Slit in leukocyte chemotaxis, angiogenesis, and now, medulloblastoma invasion, it will be necessary to further functionally characterize the intracellular mechanisms mediating nonneuronal Slit effects. The variability in the cell types and models employed will inevitably lead to differences in the intracellular mechanisms responsible for Slit-mediated effects. Selective neurodevelopmental cues such as Slit may provide significant insights into tumor invasion and outline the need for detailed assessments of how to implement this strategy for other tumor types.

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Sloughing of Cells

▶ Exfoliation of Cells

Slu

▶ Snail Transcription Factors

Slug

Definition

Snail2; is a zinc-finger factor that directly represses the transcription of the ▶ E-cadherin gene by binding to E-boxes (consisting of the sequence 5´-CANNTG) in the proximal E-cadherin promoter.

▶ Calreticulin

SM

Definition

Sphingomyelin; a phosphosphingolipid, the choline ester of ▶ ceramide-1-phosphate.

▶ Sphingolipid Metabolism

SM-5887

▶ Amrubicin

SMA

Definition

Smooth muscle actin.

SMA- and MAD-related Protein 4

► Deleted in Pancreatic Carcinoma Locus 4

SMAC/Diablo

Definition

The second mitochondria-derived activator of caspase/direct IAP (inhibitor of apoptosis protein) binding protein with low pI. It inhibits the ► inhibitor of apoptosis (IAP) family and promotes caspase activation.

► PUMA

SMAD

Definition

Transcription factor family involved in cell proliferation and differentiation control; regulated by tumor growth factor β (TGFβ).

► Smad Proteins in TGF-Beta Signaling

Smad Binding Element

Definition

SBE; was initially defined by a consensus sequence of two inverted repeats of GTCT. More recent data suggest that a single GNCN repeat may also be sufficient for Smad-DNA binding. Due to the low complexity of this consensus sequence, it is highly likely for this consensus to occur within any promoter sequence suggesting that direct binding of the DPC4-R-Smad complex to DNA is primarily important to stabilize the interaction of the complex with other DNA-binding partners.

Smad Proteins in TGF-Beta Signaling

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Definition

The Smad proteins are a family of structurally related molecules which perform a pivotal function in the transforming growth factor-β (► TGF-β) superfamily intracellular cascade. This cytokine superfamily includes TGFβ, activins, and ► bone morphogenetic proteins (BMP) and regulates a broad scale of biological responses, including cell fate and ► extracellular matrix production. TGFβ superfamily members signal through heteromeric complexes of transmembrane type I and type II serine/threonine kinase receptors. Upon ligand binding, type II receptor phosphorylates type I receptor, thus activating its kinase. The activated type I receptor then propagates signals to downstream targets such as the Smad proteins. Smads (for Sma and ► Mad proteins from Caenorhabditis elegans and Drosophila, respectively) are currently divided into three classes:

- The receptor-activated Smads (R-Smads) transiently interact and become phosphorylated by specific activated type I receptor. In mammals, Smad1, Smad5, and Smad8 are specifically involved in BMP signaling, and Smad2 and Smad3 are restricted to TGFβ/activin pathway.
- The common-mediator Smad4 proteins (Co-Smad) form heteromeric complexes with either BMP or TGFβ/activin pathway–restricted Smad. These complexes then translocate to the nucleus where they control expression of target genes.
- The inhibitory Smads (I-Smads), namely, Smad6 and Smad7, prevent the activation of the R- and

Smad Proteins in TGF-Beta Signaling. Table 1 Smad synonyms

Smad protein	Other names				*Xenopus*	*C. elegans*	*Drosophila*
Smad1	MADR1	Bsp-1	DWF-A	hMAD1	Xmad1		Mad
Smad2	MADR2	JV18-1		hMAD2	Xmad2		Sma 2
Smad3				hMAD3			Sma 3
Smad4		DPC4		hMAD4	Xmad4	Sma 4/DAF-3	Medea
Smad5			DWF-C				
Smad6							Dad
Smad7							
Smad8	MADH6						
Smad10	Smad4β						

Co-Smads through competition with R-Smads for binding to the activated type I receptor. Another mechanism has also been proposed for Smad6; this protein can compete with Smad4 for interacting with receptor-activated Smad1, yielding an apparent inactive Smad1-Smad6 complex (Table 1).

Characteristics

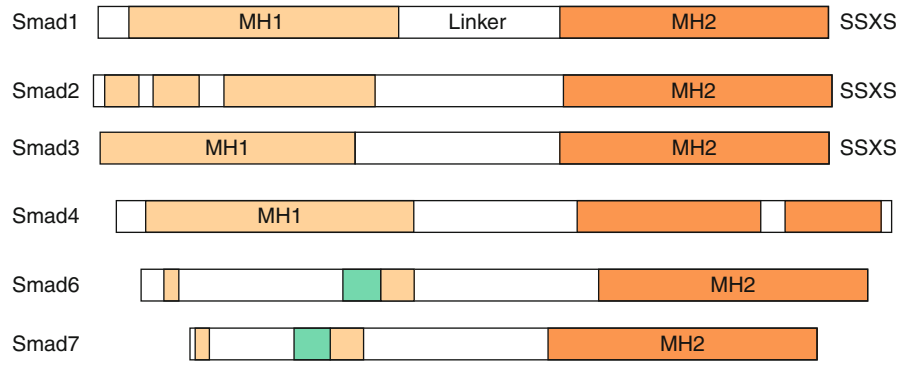
Smad proteins share two highly conserved domains named Mad-Homology domain 1 and 2 (MH1 and MH2) on the N- and C-terminal part of the proteins, respectively. The crystal structure of both domains has been determined. The MH1 and MH2 domains are adjoined by a divergent proline-rich linker region.

Smad proteins do not appear to contain any intrinsic enzymatic activity but rather exert their function through protein–protein or protein–DNA interactions. The MH2 domain mediates the association with other Smads, interaction with activated type I receptors (for R- and I-Smads) and various transcription factors, for example, forkhead activin signal transducer (FAST). Furthermore, the MH2 domain enables the interaction of Smad proteins with various transcription co-activators or co-repressors. The MH1 domain also mediates protein–protein interactions with transcription factors, for example, ▶ JUN. Importantly, the MH1 domains of Smad3 and Smad4, but not of Smad2, are able to bind directly to a 5′ GTCT DNA sequence through a β hairpin motif. As Smads bind to DNA with rather low affinity and specificity, these proteins appear to need the interaction with other DNA binding partners to regulate TGFβ target gene expression. In the basal state, MH1 and MH2 domains mutually inhibit each other functions, probably because of a physical interaction. In R-Smads, cytokine-triggered C-terminal serine phosphorylation relieves this auto-inhibition. The nonconserved linker region contains several peptide motifs that participate in Smad activity regulation (Fig. 1).

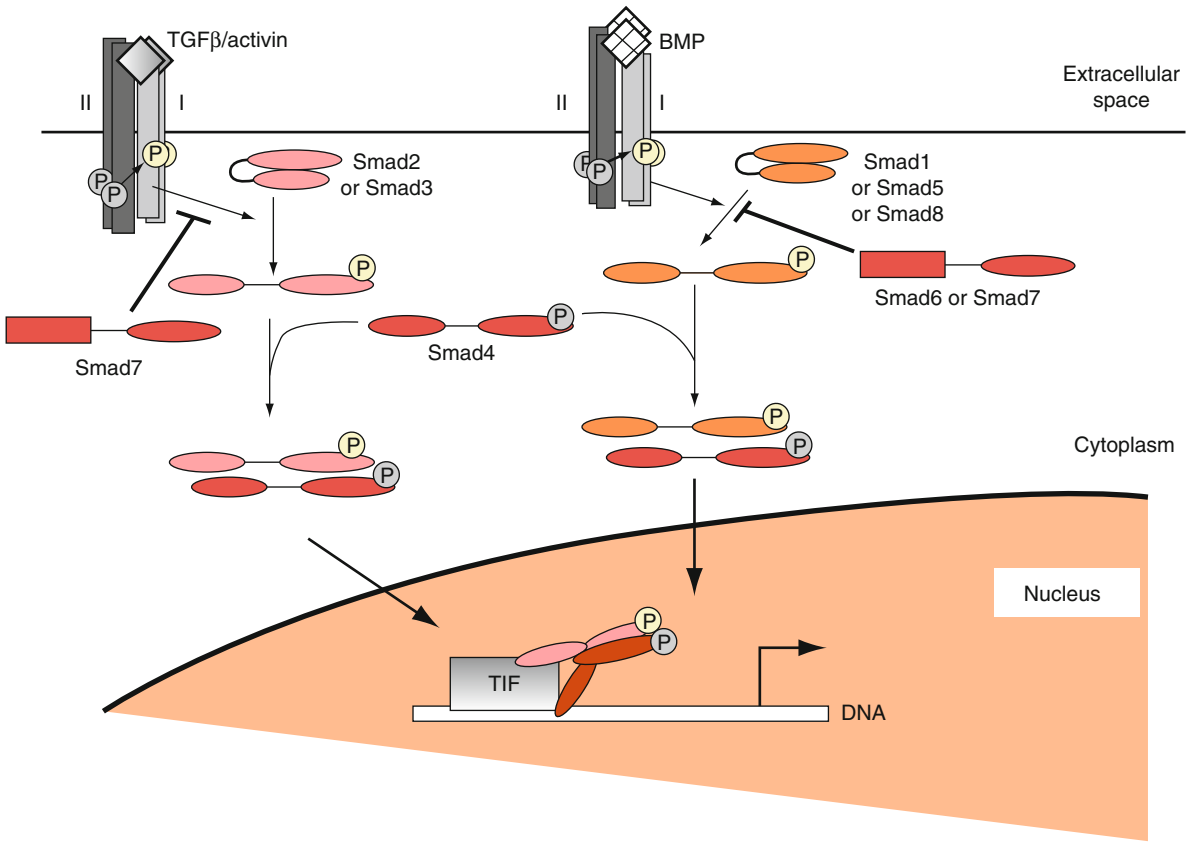
Cellular and Molecular Regulation

In the absence of cytokine stimulation, R- and Co-Smad monomers are mainly localized in the cytoplasm, whereas I-Smads are predominantly nuclear. Smad anchor for receptor activation (SARA), a protein anchored to membranes, presents the unphosphorylated R-Smads to the TGFβ-activated receptor complexes. This SARA/R-Smad interaction targets the Smad proteins to the plasma membrane and promotes the cytokine intracellular cascade. The type I receptor-mediated R-Smad phosphorylation triggers homo- and heteromerization with Smad4 and induces a nuclear accumulation of these proteins. Thus, the R-Smad localization prior to or after activation of the pathway is an important feature of TGFβ superfamily signaling. For example, activated ▶ RAS induces the phosphorylation of R-Smads in their linker region through ▶ MAP kinase activation, thus preventing Smad translocation to the nucleus. As a result, oncogenic RAS inhibits TGFβ signaling. Interferon γ (IFNγ) also inhibits TGFβ signaling by abrogating R-Smads nuclear translocation. In this case IFNγ promotes the expression of Smad7, an inhibitory Smad that prevents TGFβ-restricted Smad activation.

Smad transcriptional activity is also regulated in the nucleus where Smad interacts with several proteins that promote or repress their activity. For example, the oncogenic ▶ Evi-1 protein interacts with Smad3 through its MH2 domain and abrogates Smad3 binding



Smad Proteins in TGF-Beta Signaling. Fig. 1 Structure of Smad proteins. Pathway-restricted Smads (R-Smads) are phosphorylated by the activated type I receptor on the two most C-terminal serine residues in the SSXS motif. The common-mediator Smad (Co-Smads) contains various small insertions in the MH1 and MH2 domains. The antagonistic Smads (I-Smads) lack most of the conserved MH1 domain. *Brown boxes* indicate regions that are highly conserved between Smad proteins. *Green boxes* are regions of similarity in I-Smads only



Smad Proteins in TGF-Beta Signaling. Fig. 2 Schematic representation of the TGFβ/Smad pathway. Cytokine binding leads to the formation of a heteromeric receptor complex in which type II receptor phosphorylates and activates type I receptor. Pathway-restricted Smads are then phosphorylated by the type I receptor and form complexes with the common-mediator Smad4. These heteromeric complexes enter the nucleus where they participate, in combination with other transcription factors (TF), in the regulation of target genes. Inhibitory Smads bind to the activated type I receptor thus preventing R-Smads activation

Smad Proteins in TGF-Beta Signaling. Table 2 Smad gene characteristics

Smad protein	Human chromosome	Number of exons	Mutation in human cancer
Smad1	4q28-31		
Smad5	5q31	8	
Smad8	13q12-14		
Smad2	18q21.1	11	Colorectal, lung
Smad3	15q21-22	9	
Smad4	18q21.1	11	Pancreas, colorectal, lung, ovary
Smad6	15q21-22		
Smad7	18q21.1		

to DNA, thus reducing its transcriptional activity. Smads also recruit co-activators like ▶ CBP/p300 that promote Smad transcriptional activity or corepressors as the oncoprotein ▶ Ski. These repressors recruit histone deacetylases to Smad complexes.

R-Smad are activated by phosphorylation; however, no phosphatase has yet been implicated in turning off TGFβ signaling. When R-Smad proteins enter the nucleus, they appear to activate their own degradation through ▶ ubiquitin-mediated proteolysis (Fig. 2).

Clinical Relevance

The gene encoding Smad4 was originally cloned as a tumor suppressor gene and called deleted in pancreatic cancer 4 (▶ DPC4). Smad4 appears to have a role in the late stages of a subgroup of colorectal cancers and many pancreatic cancers. In heterozygous mice carrying mutations of Smad4 and ▶ adenomatous polyposis coli (▶ APC) genes on the same chromosome, loss of heterozygosity and reduplication of the gene carrying the mutations results in intestinal polyposis with more malignant phenotypes than the simple APC heterozygotes (Table 2).

The Smad2 gene is located on the same chromosome region as Smad4 and is also frequently mutated or deleted in colon cancer. The inactivating mutations found in Smad2 and Smad4 mostly affect the MH2 domain in regions important for protein–protein interactions. However, the real role of these proteins in the cancer process has not yet been clearly defined. Mutations in other Smad proteins have so far not been found in human tumors. Nevertheless, one of the mouse Smad3-deficient strains develop metastatic colorectal cancer.

TGFβ is involved in several pathologies; however, the implication of Smad proteins in these disorders remains to be elucidated.

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SMAD4

▶ Deleted in Pancreatic Carcinoma Locus 4

SMAD-4/DPC4

▶ Trefoil Factors

Small Animal Positron Emission Tomography

▶ Positron Emission Tomography

Small Bowel Mesentery

Definition

Leaf of connective and fatty tissue connecting the small bowel to the posterior abdominal wall. As well as physically supporting the intestine, it carries the blood, nerve, and lymphatic supply.

▶ Desmoid Tumor

Small Cell Carcinomas

Definition

Are at the highly aggressive pole of the spectrum of ▶ neuroendocrine tumors.

Small Cell Lung Cancer

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Synonyms

Oat cell carcinoma

Definition

Lung cancer consists of two major types:

- ▶ Non-small cell lung cancer (NSCLC)
- Small cell lung cancer (SCLC)

About 80–85% of all ▶ lung cancer patients have NSCLC and the rest of the 15–20% have SCLC. In 2010, the American Cancer Society has estimated that 222,000 new cases of lung cancer will be diagnosed in the USA; of which 35,000 will have SCLC. Even though both are lung cancers, they are considered as separate cancers and the management of each of these two cancers is different. It is important to recognize that the treatments applicable for non-small cell lung cancer may not be applicable for small cell lung cancer patients.

Characteristics

SCLC is characterized by large central mass and/or bulky mediastinal ▶ adenopathy. These tumors have a high propensity to metastasize. The median age of lung cancer patients is 70 years. Among all types of lung cancer, SCLC has the highest association with smoking and almost never arising in absence of smoking history (▶ Lung Cancer and Smoking Behavior). SCLC is also characterized by its ability to produce substances with neuro-hormonal activity, and/or be associated with antibodies directed against neural and muscle antigens that produce a large spectrum of ▶ paraneoplastic syndromes.

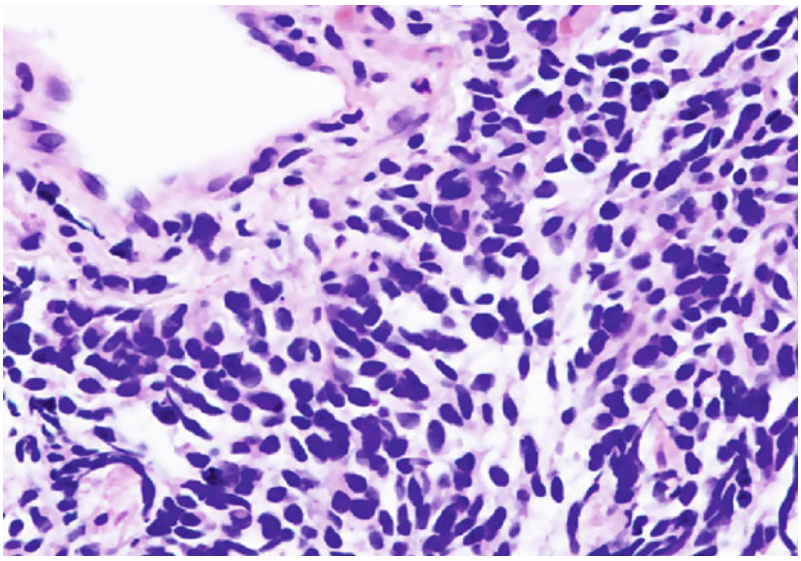
Pathology of SCLC

SCLC is a tumor formed by cells with very high nucleus-to-cytoplasm (N/C) ratio, a scant rim of cytoplasm, and finely granular nuclear ▶ chromatin with absent or very rare ▶ nucleoli (Fig. 1). Cells are round to spindly, often exhibit molding, crush artifact, and extensive ▶ necrosis, and display an exceedingly high ▶ proliferation rate. Cells grow in nests, usually separated by ▶ desmoplastic ▶ stroma. The diagnosis can be challenging particularly when extensive crush artifact is present. SCLC has to be distinguished from neuroendocrine lesions of lesser histological grade, including various forms of ▶ carcinoid, which have similar cytological and architectural features, but do not display both necrosis and high ▶ mitosis activity of SCLC. Although diagnosis rests on morphological, light microscopic features alone, as per World Health Organization (WHO) recommendation, ▶ immunohistochemistry stains are utilized to clarify the diagnosis in difficult and challenging situations. SCLC may be present in association with NSCLC component, and ▶ Large Cell Neuroendocrine Carcinoma (LCNE).

Clinical Presentation

Nearly all the patients with SCLC have symptoms at the time of diagnosis. The common symptoms that many patients have at the time of diagnosis are chest pain, cough, and shortness of breath. Some patients have fatigue and weight loss at the time of diagnosis. Patients may also have symptoms of ▶ Paraneoplastic syndromes related to the sites of ▶ metastasis. Thus patients may develop headache or blurring of vision due to brain metastases or may have abdominal pain from spread to the liver or bone pain from spread to the bones. However, just because the cancer has metastasized does not mean the patient will have symptoms related to that body part at the time of diagnosis. Patients generally have symptoms for only a short period of time before diagnosis. This is related to the relatively rapid growth of this cancer (Table 1).

Paraneoplastic syndromes are changes in different body parts that can occur in cancer patients not related to direct spread of the cancer. Almost 50% of small cell lung cancer patients could have one or more



Small Cell Lung Cancer.
Fig. 1 Cytology of SCLC. Cells have very high nucleus-to-cytoplasm (N/C) ratio, a scant rim of cytoplasm, and finely granular nuclear ▶ chromatin with absent or very rare ▶ nucleoli. Cells are round to spindly, often exhibit molding, crush artifact, and extensive ▶ necrosis, and display an exceedingly high ▶ proliferation rate. Cells grow in nests, usually separated by ▶ desmoplastic ▶ stroma

paraneoplastic syndromes. Some of the common paraneoplastic syndromes, including ssss, cerebellar ataxia, and ▶ Cushing syndrome, are listed in Table 2. Symptoms of paraneoplastic syndrome may precede the diagnosis of small cell lung cancer. Many symptoms from the paraneoplastic syndromes improve with treatment but they may not. Worsening of the symptoms from paraneoplastic syndromes indicates progression of cancer

Small Cell Lung Cancer. Table 1 Symptoms of SCLC

Symptoms	Frequency (%)
Cough	30–60
Chest pain	30–45
Shortness of breath	20–40
Weight loss/loss of appetite	20–40
No symptoms	< 5

Small Cell Lung Cancer. Table 2 Paraneoplastic syndromes associated with SCLC

Paraneoplastic syndrome	Symptoms
SIADH	Low sodium, dizziness from the low sodium
Cerebellar ataxia	Lack of balance
Cushing syndrome	Excess amount of steroids in the body leading to "moon" face, increase in weight

Staging of SCLC Patients

Staging an SCLC patient is to find out the extent of the cancer and the general condition of the patient, since treatment of a patient is based on the stage of the cancer in that patient. Following tests are done in patients who are diagnosed with small cell lung cancer or suspected to have small cell lung cancer.

- Blood work – Complete blood count, blood chemistry such as sodium level, liver and kidney tests
- ▶ CT scan of the chest (should include the liver and adrenal glands)
- ▶ MRI or ▶ CT scan of the brain
- Bone scan

PET scan is not an approved test for small cell lung cancer in the USA even though it is for non-small cell lung cancer. If a PET scan is done then a bone scan is not needed but a brain scan is still required since the brain is not scanned on a routine PET scan.

The reason these scans are done is because the areas in the body that small cell lung cancer can spread to are lymph nodes in the chest (hilar lymph nodes and mediastinal lymph nodes), the brain, other portions of the same lung or the opposite lung, pleura (lining of the lungs), adrenal glands, liver, and bones. But it is important to recognize that small cell lung cancer can spread to any other part of the body such as the kidneys. It is also important to recognize that not in every patient will the cancer spread to all these areas.

Like ▶ NSCLC, SCLC also has a ▶ TNM staging system that classifies tumors into four stages. However, many physicians still utilize an older staging

system that classifies all SCLCs into limited stage or extensive stage.

- Limited Stage – Small cell lung cancer is considered to be limited if the cancer is detected only in the lung or the lung and in the hilar or mediastinal lymph nodes. Limited stages consist of stage I, II, and III. About 25% of all small lung cancer patients have limited-stage small cell lung cancer at diagnosis.
- Extensive Stage – Small cell lung cancer is considered extensive stage if the spread of the cancer can be detected in other parts of the body. Extensive stage consists of stage IV.

About 75% of all small cell lung cancer patients have extensive stage at diagnosis.

Therapy

Limited Stage of SCLC

The standard therapy for patients with limited-stage small cell lung cancer is concurrent chemotherapy and radiation therapy. Chemotherapy is utilized in these patients due to the high metastatic potential of SCLC and due to the high response rate with chemotherapy. Radiation therapy is included in the treatment of limited-stage SCLC if the entire disease in the chest can be included in a single radiation port. This generally includes the primary tumor, ipsilateral and contralteral hilar, and mediastinal lymph nodes and ipsilateral supraclavicular lymph nodes.

Chemotherapy consists of ▶ cisplatin (60–75 mg/m2) or ▶ carboplatin (AUC5-6) given on the first day and ▶ etoposide (100 mg/m2) given on days 1–3. The chemotherapy is repeated every 3 weeks for a total of four cycles. Radiation therapy can be administered once daily for 6–7 weeks (60–70 Gy) or can be given twice daily for 3 weeks (45 Gy). The primary adverse effects of concurrent therapy are esophagitis, fatigue and ▶ cytopenia (neutropenia and thrombocytopenia), and possibly neutropenic fever. A 5-year survival of limited-stage SCLC patients with concurrent therapy is about 20%.

Prophylactic Cranial Radiation

Almost 50% of small cell lung cancer patients can develop brain metastasis. Chemotherapy can go to all different parts of the body but one area it does not reach in sufficient amount is the brain due to the blood–brain barrier. Thus, any brain micrometastases would not be adequately treated. Therefore, patients with limited-stage small cell lung cancer who have concurrent therapy are treated with prophylactic radiation to the brain. This radiation is given from Monday–Friday for about 3 weeks. ▶ Meta-analysis of trials evaluating PCI shows that overall survival is improved by about 5%. There is a small chance that such a radiation may affect the cognitive functions of the brain in the future but this risk is felt to be low and the benefits of properly treating the cancer that may have spread to the brain outweigh the risk of cognitive impairment.

Patients that have completed treatment for limited stage SCLC are evaluated with physical examination every 3 months. Many physicians also order CT scans at intervals of 3–6 months for the first 1–3 years. The utility of obtaining such scans is unclear.

Extensive-Stage SCLC

Although SCLC can metastasize to any part of the body, the most common areas of SCLC metastases are lung, adrenal glands, liver, bones and bone marrow, pleura, and the brain. The standard treatment of metastatic SCLC is ▶ combination chemotherapy with a platinum agent (▶ cisplatin or ▶ carboplatin) and ▶ etoposide. Patients are treated with four cycles administered every 3 weeks. Disease assessment is conducted every two cycles. The response rate with this combination is about 60%. Patients tend to derive clinical benefit soon after starting chemotherapy. Therapy is not continued beyond four cycles because studies have shown that there is no added advantage in continued therapy. The median progression-free survival with current therapy is about 5 months and the median survival is about 10 months. The common adverse effects of this combination are fatigue, nausea, and ▶ cytopenia with possibly neutropenic fever.

In a Japanese study, the combination of ▶ irinotecan and cisplatin was found to be better than cisplatin and etoposide in SCLC patients. However, two studies conducted in the USA could not confirm this advantage. Therefore, cisplatin (or carboplatin) and etoposide remain the current standard of care.

Recurrent SCLC

Almost 75–80% of the patients with limited-stage SCLC and all the patients with extensive-stage SCLC

eventually suffer recurrence. The approved therapy for these patients is treatment with topotecan based on randomized studies that have shown improvement over supportive care alone and similar benefit as a combination treatment of ▶ cyclophosphamide, ▶ adriamycin, and ▶ vincristine.

Topotecan is usually administered over 5 days every 3 weeks. Topotecan can be administered intravenously or orally. Some physicians administer topotecan on a weekly basis, though the every 3 week schedule is the only approved schedule. The main adverse effects of topotecan are cytopenias with possibly neutropenic fever, fatigue, nausea, and possibly diarrhea. The disease is assessed usually after every two cycles. The treatment is usually continued till progression or development of unacceptable adverse events. The median survival in patients treated with topotecan is about 8 months.

In patients who have disease stability for 6 months or longer do derive clinical benefit from re-treatment with frontline therapy.

Patients, who do not derive clinical benefit from topotecan or do so but then the cancer starts progressing, can be treated with other chemotherapy drugs but there is no strong, good-quality data to support the use of other chemotherapy drugs following the use of ▶ Cisplatin (or ▶ Carboplatin) and etoposide in first-line therapy and topotecan in second-line therapy. Some of the agents utilized are ▶ gemcitabine, ▶ taxanes (▶ Docetaxel and ▶ Paclitaxel), and Doxil.

Management of Brain Metastases

SCLC patients with evidence of brain metastases require whole brain radiation to treat the brain metastases. The blood–brain barrier does limit the amount of chemotherapy that reaches the brain. Therefore, chemotherapy cannot be relied upon for the treatment of brain metastases. Due to the high sensitivity of small cell lung cancer to chemotherapy, even the limited amount of chemotherapy that does reach the brain can shrink the brain metastases. However, the effect from the chemotherapy alone is not sufficient and these patients do need brain radiation therapy.

The timing of the brain radiation could vary. If the patient has evidence of brain metastases on the scans but has no symptoms from the brain metastases, chemotherapy can be initiated first with close monitoring of the brain metastases and the brain radiation can be done following the completion of the four rounds of chemotherapy. However, if the patient has any symptoms that are related to the brain metastases, these patients should be first treated with whole brain radiation and then treated with chemotherapy. Depending upon the patient's condition, the doctors may decide to start with chemotherapy and the whole brain radiation together.

Prophylactic Cranial Radiation in Extensive-Stage SCLC – The potential for spread to the brain in small cell lung cancer patients is very high. Almost 50% of the patients have evidence of brain metastases at diagnosis or during the course of their disease. As stated earlier, although chemotherapy can reach all the body parts, the amount of chemotherapy that reaches the brain is limited.

A recent European study demonstrated that patients with extensive-stage SCLC who have no evidence of brain metastases benefit from receiving brain radiation following the completion of frontline chemotherapy. The benefit is in the form of improving survival and reducing the potential for development of brain metatstases.

Based on these results, small cell lung cancer patients are considered for brain radiation following the completion of chemotherapy. However, it is important to mention that due to certain reservations regarding this trial, the strategy of brain radiation following chemotherapy is not applied to all patients.

Conclusion

SCLC is one of the most rapidly growing non-hematological malignancies and is very commonly associated with ▶ paraneoplastic syndromes. Almost all the patients are current or former smokers and almost all the patients have symptoms at presentation. Majority of the patients have metastatic disease at presentation. Due to the high propensity to metastasize and high chemosensitivity, systemic chemotherapy is a component of therapy in all patients. Patients with limited-stage SCLC are treated with chemotherapy and radiation. The 5-year survival in limited-stage SCLC patients is about 20%. Patients with *limited-stage* SCLC following completion of therapy are treated with prophylactic cranial radiation due to the high risk of brain metastases. The median survival in *extensive-stage* SCLC patients is about 10 months. The frontline therapy of extensive-stage SCLC is cisplatin (or carboplatin) and etoposide, while patients with recurrent SCLC are treated with topotecan.

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Small Cell Lung Carcinoma

Synonyms

Oat cell lung cancer

Definition

A type of ▶ lung cancer in which the cells appear small and round under the microscope. Small cell lung cancer often grows quickly and spreads to other parts of the body sooner than other types of lung cancer.

▶ Temsirolimus

Small Cell Neuroendocrine Carcinoma

▶ Extrapulmonary Small Cell Cancer

Small GTPases

Definition

A group of proteins related to Ras which function in signal transduction. They are active when binding guanosine 5´-triphosphate (GTP), and inactive when binding guanosine-diphosphate (GDP). Small GTPases have intrinsic ▶ GTPase activity i.e., the enzymatic ability to hydrolyze GTP to GDP, but this activity is regulated by GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs).

▶ Semaphorin

Small GTP-binding Proteins

▶ Rho Family Proteins

Small Interfering RNA

Definition

siRNA; is a molecule consisting of 20–25 double stranded RNA molecules that are used to interfere with the expression of a specific gene.

▶ RNA Interference

Small Molecule

Definition

Organic compounds of low (typically under ~1,000 g/mol) molecular weight.

▶ Small Molecule Screens

Small Molecule Drugs

Karl-Heinz Thierauch

Definition

A *small molecule drug* in cancer therapy is a molecularly defined chemical entity of low molecular weight, which is applied to a patient with the intention to heal or *palliate* primary ▶ *proliferative disease*. Or it is directed to prevent secondary consequences of the disease, which are brought about by specific *secretory* activity of cancer cells or their general deleterious effect on organ and system functions. A very important aspect is the alleviation of pain.

Characteristics

Differences to Large Molecule Drugs

The molecular weight of a small molecule drug is largely found to be below 1,000 Da, as often it has to pass the cell membrane to reach the target of activity.

Large molecule drugs were often less defined as they were of biological origin e.g., due to ▶ *posttranslational modifications* and impurities. With the increasing power of analytical and preparative techniques and with the advent of molecularly exactly defined ▶ *oligonucleotides*, the distinction of exactly defined small molecule drugs from less defined large molecular weight drugs is fading, though antibodies or viruses will remain an independent class of therapeutics. Another distinction between small molecular drugs and those of large molecular weight which once was absolute now becomes relative: the possible permeation of the drug into the cell acting on intracellular targets. Whereas appropriate small molecules might diffuse or be transported by dedicated membrane proteins into the cells, for large molecules biomimetic uptake mechanisms need to be devised like the binding of a drug conjugate to receptors or toxins, followed by an internalization of the receptors or toxins, respectively. In other cases viral infections might be feasible. So the natural mechanism is used as a Trojan horse or piggyback.

High-Throughput Screening

Many small molecule cancer drugs were derived from natural compounds and used either directly or after some chemical modifications. A second important source came from deeper knowledge of cellular ▶ metabolism: ▶ Ligands and coenzymes were modified to obtain antagonists of the corresponding processes. These techniques were expanded with the advent of ▶ high-throughput screening. Mechanistic targets were selected by target identification groups. Binding and functional assays interfering with the relevant mechanism were then adapted to small-scale screening encompassing between 96 and 1,536 samples in a plate. Even larger scales are applied experimentally, e.g., with various fluorescence read out techniques (e.g., time resolved fluorescence, fluorescence polarization). With appropriate assays up to several million compounds, contained in structurally diverse compound libraries, are tested for their interference with specific ▶ drugable targets (receptors, enzymes) in a few weeks. Also, screens using cultured cells are ongoing in a high-throughput mode.

Structure Determination and Modeling

The optimization of the compounds is either at random using the intuition of the medicinal chemist or now increasingly based on the intimate structural knowledge of the respective drug target, which can be obtained from x-ray crystal structure or NMR-derived structure determinations. Model calculations are performed fitting chemicals into the drug-binding pocket of the target structure and preparing co-crystals of ligands with the target structure to obtain potent compounds. It is a major goal to obtain a high selectivity of a drug for the target chosen, without affecting similar cellular structures necessary for general cell functions. At this point chemical optimization efforts are key.

Physicochemical Properties and Drug Uptake

Small molecule drugs are applied according to their properties by all routes used for general drugs, i.e., oral, rectal, s.c., i.m., i.v., nasal, or by inhalation to mention the most important ones, and appropriate metabolic properties guide its availability for action at the required locus. The small molecule drugs used in cancer therapy need to have the physicochemical properties of general small molecule drugs, like appropriate solubility, logP values, and pKB, all the metabolic characteristics like stability toward liver and gut metabolism. Physicochemical properties are predicted in model calculations, which in turn are used to predict pharmacokinetic behavior. Optimized molecules will be taken up in the gut and by cells and a useful ▶ distribution in the body is found. So an appropriate duration of exposure can be secured in adequate concentrations at the locus of desired activity. Depending on the target, a drug may need to pass into the cell by diffusion or an active transport process. Of special importance is their behavior toward elimination from cells by drug transporters, which may become upregulated in the gut preventing its uptake or as a means of cancer cells to acquire ▶ resistance toward therapy. It may also lead to an accelerated excretion in the gut or kidney. The molecular properties also govern their potential to interact with other drugs influencing their kinetics by inhibition of cytochrome P450 isoenzymes or by induction of metabolizing enzymes (Drug drug interaction).

Drug Formulations

The drugs need to be formulated according to their specific use, e.g., poorly soluble agents like taxanes are formulated for i.v. application as solutions in high amounts of cremophor carriers, which themselves are not neutral to the body, but provoke reactions requiring a premedication for a better patient tolerability. Efforts are made to improve formulations avoiding toxic responses due to recipients. This is done with, for example, taxanes such that the drug substance is dissolved in less awkward carriers or in vitamin E or albumin for i.v. applications permitting higher concentrations with better tolerability and thus efficacy. For others, slow release formulations are prepared to improve their efficacy through a prolongation of drug availability at relevant concentrations. This also might prevent toxicities originating from peak concentrations shortly after application, which are cut off by a slow release formulation.

Side Effects

Selectivity translates into fewer side effects. However, the aim of selectivity will sometimes be compromised by the need to affect several targets by one drug in order to obtain sufficient therapeutic efficacy. Effects which are due to the interaction with one targeted structure in different locations are class effects. All compounds interfering with this target will have the same side effects, as this cannot be avoided structurally. Only differential availability needs or periods of blockade may be a way to decrease such effects, if no local application is possible. The tendency to increase hypertension is, for example, a typical class effect of VEGF signal inhibitors, which is independent from the antiangiogenic effect and accompanies it, irrespective of the specific drug used. Avoidable side effects are contained in the specific structure of a molecule and are called compound-specific side effects.

Some Examples for the Mechanism of Cancer Drug Action

Small molecule cancer drugs traditionally interfere with ▶ cell proliferation, one cardinal distinction to normal cells, through damage of DNA or the synthetic process of DNA synthesis. Examples of these drugs are bleomycin and *cis*-platinum, which result in ▶ DNA double strand breaks, or fludara, which interferes at various points with nucleoside metabolism. Vinca alcaloids or taxanes block tubulin modifications, which are key for nuclear division and cellular transport. With deeper knowledge of cell biology, the cell fate is blocked at very specific sites. So, certain kinases are required for ▶ signal transductions orchestrating the process of the ▶ cell cycle culminating in the separation of the chromosomes to form two new nuclei. If this process is disturbed by specific kinase inhibitors, many cancer cells will respond with a standard program resulting in self-abortion, also called ▶ apoptosis, thus slowing down cancer growth or even reducing its size.

The kinase inhibitors may also interfere with the activity of enzymes which are constitutively activated in cancer cells by mutational events, e.g., bcr-abl in chronic myelogenous leukemia. Some drugs only hit specific mutants but not others, which may result in resistance; therefore, drugs are investigated for their activity against major mutants found in human disease. In future it may be possible to discover compounds, which are specific for major mutants of the enzymes found in cancer, resulting in even higher specificity with less adverse effects as normal cells containing the same enzymes are not affected.

Some cancers depend on paracrine or autocrine growth factor signal transduction and small molecules might block protein functions in the signal chain, inhibiting signal flow downstream of that point. This is true, e.g., for EGF receptor signal inhibitors at the level of the receptor kinase, e.g., gefitinib, which inhibits the growth stimulus for epithelial cells.

Antagonists of nuclear hormones, which block signaling via or downregulate nuclear receptor proteins, are effective in the therapy of breast and prostate tumor disease.

Another promising branch of small molecule therapeutics attempts to induce a more ▶ differentiated and less invasive phenotype of cancer cells. This was originally applied to leukemia cells with the intention of inducing terminal differentiation and is continued with retinoids for AML and other solid tumors. Investigation is now directed toward the inhibition of the epithelial to mesenchymal transition in solid tumors. In recent years, small molecule drugs have been developed against stromal components of tumors with little or no effect on tumor cells themselves, like VEGF signal interrupting and antiangiogenic tumor therapeutics.

Interference with chromatin modification like methylation or acetylation recently became an

important field of drug research as appropriate test systems are now available.

Systemic Effects of Tumor Growth

Other small molecule drugs block deleterious effects of tumors secreting active products. Hormones or cytokines are discharged from neuroendocrine tumors in excessive manner, which are therapeutically antagonized by specific signal blockers to prevent a flooding of the body with the specific hormone signal. In the case of prolactinomas, treatment with dopamine might be beneficial or in the case of anorexia, a frequent companion of progressive proliferative diseases, megestrol might be helpful. Anticoagulants are advisable in some cases as the incidence of thrombi and emboli is increased in many tumor patients. Standard analgesics are administered if indicated. If they are not effective, as in the case of bone metastases in prostate cancer, antiproliferative therapy is also applied for the purpose of pain relief.

Worldwide, very many new small molecule drugs are intensively investigated with the latest available techniques in newly discovered pathways linked to cancer. They promise a breakthrough for the benefit of cancer patients, prolonging their life for a certain period of time. It is essential that such prolongation of life occurs at a decent quality of life to become a real benefit for the patient and not only one for the health industry.

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Small Molecule Screens

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Synonyms

Chemical biology screen; Chemical genetic screen; Compound screen; High-throughput screen

Definition

A small molecule screen is a procedure in which small molecules (typically organic compounds with a molecular weight under ~1,000 g/mol) are systematically tested for their ability to activate, perturb, or modify a target or a biological process of interest.

Characteristics

The goal of small molecule screening is to identify compounds that modulate a particular biological process, and thus, can be used as, or developed into, tools for further medical research and/or ▶ small molecule drugs (▶ molecular therapy). Typically, an assay is developed into a "▶ high-throughput" screen, meaning that it is optimized for rapidly assaying thousands to hundreds of thousands of compounds in parallel and in an automated fashion. Large amounts of data are collected with the help of robotics, liquid handlers, and processing software. "Hits," compounds that produce the desired assay result, are hence identified for further study.

Small molecule screening is often the starting point for identifying chemical tools. Developing a chemical probe to inhibit and study a protein, for example, offers several advantages over mutating a gene. A chemical probe can be used for rapid inhibition in different cell lines, organisms, and species, and at varying time points or at different times during the cell cycle or

development. Furthermore, protein inhibition may be reversed by removing the compound, and compounds can be developed for versatile specificity (either inhibiting a specific protein or a family of proteins). In classical genetics, however, genes are not easily turned on or off at will, and mutating/deleting a gene that is essential for survival may lead to early embryonic lethality. The application of chemical tools and ideas to biological problems is known as ▶ chemical biology.

Small molecule screening has now become a very common method for identifying compounds that produce therapeutically desirable biological phenotypes. These compounds can be further modified to optimize their potential as drug candidates. Previously, drugs were discovered either by opportune testing or by identifying the active compounds in traditional medicines (▶ Chemotherapy of Cancer; Progress and Perspectives).

Types of Screens

Screens are classified into two types: phenotype-orientated or target-based. In ▶ phenotype-orientated screens, compounds are screened for the ability to induce a particular phenotype. Further work is usually required before the target(s) of the compound is/are known. Little a priori knowledge is required about the mechanism by which the desired phenotype is produced. Examples of phenotype-orientated screens include screens for compounds that alter cell division, metabolism, ▶ adhesion, viability, or protein localization. Phenotype-orientated screens are a component of ▶ forward chemical genetics; one may discover novel biological mechanisms involved in producing the desired phenotype by further evaluating the compounds' mechanisms of action.

▶ Target-based screens are performed to identify compounds that more directly alter a particular target. For example, one may purify a protein and then screen for compounds that perturb the function of this protein. The compound can subsequently be used to perturb the protein's function in vivo, and the consequent phenotype can be observed. This methodology is known as ▶ reverse chemical genetics.

In either phenotype-orientated or target-based screens, counter screens or cross screens may be performed to determine if the hits from the first screen, or primary screen, will affect related targets or phenotypes. Multitargeted compounds are often called "promiscuous" compounds.

"Small" Molecules

Screening may be performed with siRNAs, peptidomimetics, natural compounds, synthetic compounds, or other molecules. The term "▶ small molecule" typically refers to organic compounds with "small" molecular weights. There is no clear definition for "small," but estimates range from under 2,000 g/mol to under 300 g/mol; most commonly, "small" refers to under 500 g/mol or 1,000 g/mol. Small molecules are commonly used for screening because organic compounds are often able to cross the plasma membrane of cells, and because most marketed medicines have been derived from this class of agents.

Collections of compounds are known as libraries. Pharmaceutical companies have proprietary libraries containing millions of compounds. Only recently have collections of organic molecules numbering in the thousands to hundreds of thousands become commercially available for academic use. Example libraries include the Prestwick Chemical Library (Prestwick Chemicals; 1,120 off patent compounds, 85% marketed drugs), LOPAC^{1280} (Sigma-Aldrich; 1,280 pharmacologically active compounds), Spectrum Collection (MicroSource Discovery Systems; 2,000 biologically active compounds), Maybridge Screening Collection (Maybridge; 56,000 drug-like organic compounds), and EXPRESS-Pick (ChemBridge Corporation; over 435,000 drug-like small molecules).

In the past century, compound libraries were assembled one compound at a time during the synthesis of drug candidate variants or by purifying compounds from natural products (e.g., plants, fungi, bacteria, and other organisms). Recently, progress in ▶ combinatorial chemistry (the synthesis of a large number of new chemical compounds by combining various sets of compound "building blocks"), automation, and chemical diversity has allowed for rapid diversity-orientated synthesis. Novel small molecule libraries can more readily be created, increasing the rate of production from a few hundred compounds per year per chemist, to millions of compounds per year. At the current time, combinatorial libraries contain simple compounds, meaning that they have one or fewer ▶ stereocenters (a carbon atom with four distinct functional groups). Natural compounds are typically more

complex, and it is thought that compounds with more stereocenters might provide superior target-binding specificity. Proteins, for example, are very stereochemically complex.

Screening Technology and Tools

Small molecules are typically more stable in their dry form, but must be dissolved for screening. Although there is neither a universal solvent nor any solvent proven for long-time compound storage, dimethyl sulfoxide (DMSO), a dipolar aprotic solvent, is commonly used. Dilutions of the DMSO-dissolved compounds may be performed in another solvent, such as in water or phosphate-buffered saline.

Compounds are placed in small plastic rectangular containers called "plates." Each plate contains a grid of individual open divots, called "wells," in a 2:3 rectangular matrix. Screening facilities typically have each compound created by the lab or obtained from a commercial source placed in a different well. The contents of each well and plate are carefully cataloged. Stock plates, or "mother" plates, are not used directly in experiments. Rather, these plates contain concentrated amounts of each compound for storage. "Daughter" plates are copies of the stock plates created by pipetting a small amount of liquid (often microliters) from each well of the stock plate into the corresponding well of an empty plate. The compounds in the daughter plates can be diluted and/or further aliquoted into other plates. The plates used for screening are named assay plates, and their compound contents may be either directly derived from the stock plates, or from other aliquots. Some assay plates may contain mixtures of different compounds in each well to decrease the assay time and cost. In any case, the experimental materials, such as cells or proteins, must be added to the assay plate wells.

In order to decrease the assay cost, length of time, and amount of required materials, a significant amount of effort over recent years has been placed on minimizing the assay volume. In the late 1970s, 96 well plates were used, and a few hundred microliters of assay materials were required in each well. Currently, 384 well plates are in common use, with each well requiring tens of microliters. Some screens are now being performed in 1,536 well, 3,456 well, or even 9,600 well plates, minimizing the assay volume from a few microliters to a few hundred nanoliters.

Accurate, reproducible, and rapid screening of large numbers of compounds necessitates the use of automated robotic systems. These systems are typically built around a core of one or more automated liquid handlers. In addition to a pipetting tool, robotic liquid handlers usually have a gripper tool to manipulate sample containers, assay plates, or pipette tip boxes on a work deck. The handlers are able to transfer liquids, wash tips, and move microplates without human intervention. An example of a liquid handler is the Biomek FX Laboratory Automation Workstation (Beckman Coulter). Other components of automated robotic systems may include incubators, storage containers, liquid dispensers, mixers, plate readers, or other assay equipment (such as automated digital microscopes for high content screens). One or more tracked robotic arms move the microplates from one station to another, and sophisticated software algorithms control almost every aspect of this system. Many screening centers currently have the capacity to screen over 100,000 compounds per day (depending on the assay).

Screening Assays

Numerous assays have been used in small molecule screening. Many have been designed so that hits can be detected by standard plate readers that detect changes in absorbance, fluorescence, or electrochemiluminesence (ECL). Some example screens, among the countless types and variations possible, are described below.

In vitro assays include enzymatic, fluorescent polarization, fluorescence resonance energy transfer, AlphaScreen, scintillation proximity, and biophysical assays. In an enzymatic assay, compounds that inhibit the activity of an enzyme (e.g., from cleaving a fluorogenic substrate) can be screened. In fluorescent polarization assays (FPA), a fluorescent dye is used to label a small peptide (or other molecule), and its speed of rotation is measured. When this peptide is bound to a protein (or other molecule) of equal or greater size, the speed of rotation will decrease significantly. Thus, FPA can be used to identify compounds that inhibit a fluorescently-labeled peptide, for example, from binding a particular protein. In fluorescence resonance energy transfer (FRET), a protein (or other molecule) is first labeled with a particular dye molecule, named a "donor." Then, a protein that interacts with the first protein is labeled with a dye molecule that is excited by

the donor, and accordingly named an "acceptor." When the donor molecule is excited, it will in turn excite the acceptor molecule only when the interacting proteins are in close proximity. FRET can thus be used to screen for compounds that inhibit the binding of two proteins (or other molecules). In a FRET variation known as time-resolved FRET (TR-FRET), fluorescent dye signals are read in a time-resolved manner, reducing assay interference and increasing data quality. AlphaScreen (Perkin Elmer) is similar to FRET in that donors and acceptors are used; however, the interacting proteins (or other molecules) are conjugated to beads. A laser is used to excite a donor bead which produces singlet state oxygen. The singlet state oxygen activates the acceptor bead, which has a chemiluminescer and fluorophores. Scintillation proximity assays also use beads; the donor being radiolabeled, and the acceptor containing a scintillant. Finally, biophysical techniques, such as NMR and X-ray diffraction, have been powerful in vitro tools for detecting compounds that bind to a particular protein.

Cell-based assays include bioluminescence resonance energy transfer (BRET), in which one protein is fused to a bioluminescent donor, and another protein, which interacts with the first protein, is fused to an acceptor fluorophore. When the two proteins are in close proximity, energy transfer can be detected; this assay is similar to FRET, but occurs in a cellular context. Cell-based assays are more commonly used for phenotype-orientated screens, such as in identifying compounds that affect cell division, metabolism, adhesion, transporters, or viability.

▶ High-content screens are cell-based assays that utilize a combination of automated digital microscopy and flow cytometry to collect information about spatial or temporal changes in cellular processes. For example, a nuclear protein can be fused to a fluorescent tag, and an automated high content screening apparatus can be used to detect hit compounds that induce a change in protein localization (e.g., to the cytoplasm).

▶ Virtual high-throughput screen or in silico screen, are being performed using advances in molecular modeling, combinatorial chemistry, and molecular biology. For example, computers are used to screen virtual libraries for their ability to bind to a model of a protein. Virtual high-throughput screening is also used to identify compounds that may or may not potentially possess desirable absorption, distribution, metabolism, excretion, and/or general pharmacokinetic properties.

Screens in whole organisms, such as in bacteria, *Danio rerio* embryos, *Xenopus laevis* embryos, and *Caenorhabditis elegans* have been performed, although screens in larger animals (e.g., mice) are difficult due to the inability to maintain, treat, and observe thousands of these animals in an efficient manner. For screening a single compound on multiple cell lines, the National Cancer Institute has used the hollow fiber assay, in which different human tumor cells are placed within biocompatible hollow fibers. These hollow fibers are implanted subcutaneously or intraperitoneally in mice, and the mice are then treated with the test compound. After treatment, the fibers are removed and the compound's ability to affect different tumor cell lines and to penetrate different physiological compartments can be assessed. This assay, however, is relatively low throughput.

The Screening Epilog

Whether screening for chemical probes or for drug discovery, much research remains to be performed after the initial compound hits are identified. First, the assay is repeated under the same conditions, on the hit compounds to confirm the results. Then, ▶ dose–response curves may be generated using the same assay, thereby providing IC or EC values. Orthogonal testing may be performed, in which the confirmed hit is tested using a different assay. This assay is often closer to the target physiological condition. Functional assays, or secondary assays, may be performed; for example, compounds identified via an in vitro screen may need to be tested in a cellular environment, in the presence of membranes or other physical barriers. For phenotype-based screens, the target(s) of the compound(s) must be identified; an often laborious procedure. Various techniques have been developed for this purpose, including covalent labeling of the compound followed by compound-bound protein purification from cellular extracts, and peptide sequencing.

Even after compounds are tested and shown to possess the desired level of target or phenotype specificity, it is very unlikely that the process has revealed a single perfect chemical probe or drug candidate. Likely, several compounds will show some degree of activity. If these "lead" compounds share similar chemical features, one can identify a

► pharmacophore, the set of structural (electronic and steric) features in a molecule that are responsible for the molecule's biological activity. Compound analogs and structure–activity relationships (SAR) will be used to identify or synthesize new compounds that have improved activity, specificity, and ADME (absorption, distribution, metabolism, and excretion) properties.

Conclusions

Small molecule screening is a commonly used method for identifying chemicals probes and drug leads. This type of screening is a key component in the emerging field of chemical biology, with the objectives to identify a small-molecule modulator for each individual function of every macromolecule, and to translate basic research into improved clinical outcome.

► Anti-inflammatory Drugs
► Apoptosis-Induction for Cancer Therapy
► Aromatase Inhibitors
► Imatinib
► Personalized Cancer Medicine
► Vascular Disrupting Agent and Cancer

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Small Round-cell Tumor

► Desmoplastic Small Round Cell Tumor

Small-Molecule Inhibitors

Definition

Refers to a class of drugs specifically designed to inhibit certain proteins (such as kinases) in the cell that have been implicated in disease.

SMARCB1

Definition

► Tumor suppressor hSNF5/INI1.

► hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

SMAX1

► Androgen Receptor

Smelting

Definition

Chemical reduction to produce a metal from its ore.

► Lead Exposure

SMN

Synonyms

Second malignant neoplasm

Definition

Survival of motor neuron; is a protein associated with spinal muscular atrophy.

Smoking

Definition

Refers to the habit of consuming smoke generated from tobacco in cigarettes, cigars, and pipes. Major cause for cancer, emphysema, and heart disease.

► Tobacco Carcinogenesis
► Tobacco-Related Cancers

Smoothened

Definition

Abbreviated Smo in mouse; SMO or SMOH in human; The *Smoothened* gene encodes a protein with homology to a G-protein coupled receptor. The smoothened protein forms part of the receptor complex for hedgehog proteins.

► Hedgehog Signaling

SMR

Synonyms

Soluble mesothelin-related proteins

► Mesothelin

SMRT Co-repressor

Definition

The POZ domain of Bcl-6 is associated with the silencing mediator of retinoid and thyroid hormone receptor (SMRT), which was originally isolated as a corepressor of some nuclear receptors without a ligand. SMRT is a component of a larger multiprotein complex including mSin3A and ► histone deacetylase (HDAC). The recruitment of an HDAC-containing complex is a common transcriptional repression mechanism used by transcription factors belonging to various functional classes.

► BCL6 Translocations in B-Cell Tumors

SN-38

Definition

The anticancer ► prodrug ► irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is hydrolyzed into its active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin) by carboxylesterases. Stabilization of the covalent Topo I-DNA complex by SN-38 is a critical step in its anticancer action where by Topoisomerase I-mediated DNA breaks are induced via prevention of DNA religation.

► Irinotecan
► Pharmacogenomics in Multidrug Resistance
► Topoisomerases

Sna

► Snail Transcription Factors

SNAG

Definition

Transactivation domain of Snail proteins, 7–9 amino acids located at the N-terminal region that is conserved between ► snail transcription factor and ► Gli proteins.

► Snail Transcription Factors

Snail Transcription Factors

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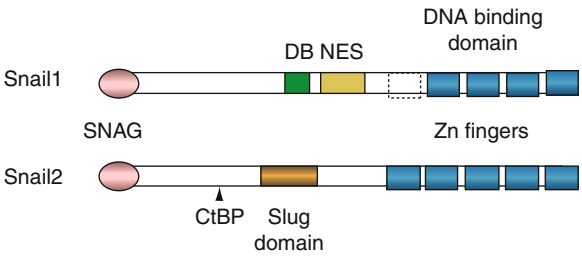
Synonyms

Snail1, Snail2, and Snail3 were previously called Snail, Slug, and Smuc, respectively; Additional synonyms for Snail1: SnR (chick); SNAILH (human); Sna; Additional synonyms for Snail2: hSlug (human),



Snail Transcription Factors. Fig. 1 Schematic representation of the main structural domains of Snail1 and Snail2 proteins. *Blue boxes*, zinc fingers at the C-terminal region; dotted box indicates the presence of a fifth zing finger in Snail1 protein in some invertebrates; *orange box*, the specific domain present in Snail2 proteins; DB (*green box*), destruction box; NES (*yellow box*), nuclear export signal; SNAG (*pink box*), N-terminal transactivator domain; CtBP, interacting domain of C-terminal binding protein

Xslug (Xenopus), Slugh (mouse); Slu; Human symbols for Snail1 and Snail2: SNAI1, SNAI2; SNAIL-like and SNAILP both refer to a human-specific Snail1 retrogene inserted in chromosome 2

Definition

Snail genes are zinc finger transcriptional repressor of the Snail gene superfamily that participate in developmental and pathological ▶ epithelial-mesenchymal-transition (EMT) processes. Snail1 was initially characterized as a potent repressor of ▶ E-cadherin, a major anti-invasive molecule in carcinomas. Snail1 is thus proposed as a potent inductor of tumor invasion, a process that frequently occurs associated to EMT. Besides E-cadherin repression, Snail1-mediated EMT requires the set in motion of a complex genetic program leading to the downregulation of additional epithelial gene markers and to the upregulation of mesenchymal and migratory genes.

Characteristics

Structural Organization

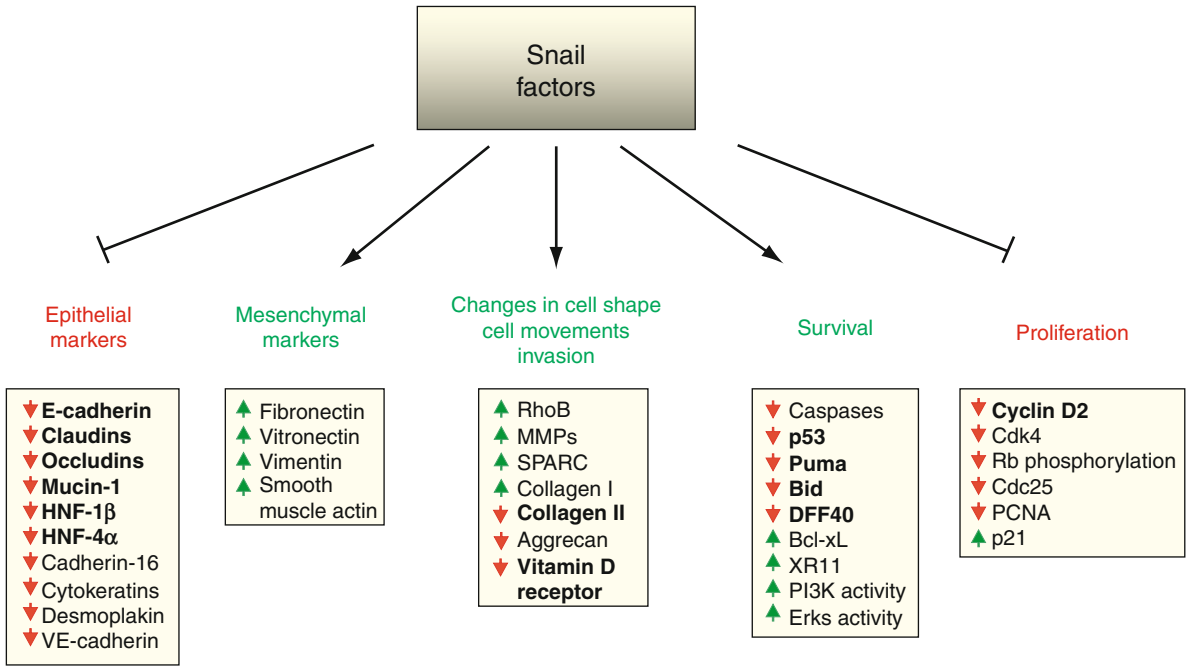
Snail factors share a common organization: a highly conserved carboxy-terminal region, containing from four to six zinc fingers (C_2H_2 type), and a much more divergent amino-terminal region (see Fig. 1). Although three *Snail* genes (Snail1 to Snail3) and a human-specific *Snail1* retrogene (Snail-like) have been described, the vast majority of information available on their function as transcription factors and their involvement in embryonic development and tumor progression is restricted to Snail1 and Snail2. Their zinc fingers function as the sequence-specific DNA-binding domains that recognize consensus ▶ E2-box type elements C/A(CAGGTG). The repressor domain is located at the N-terminal part in the so-called ▶ SNAG domain, conserved between Snail/Gfi proteins. The central region of the Snail proteins is characterized by a serine-proline rich region highly divergent between Snail members. While Snail2 contains the so-called Slug domain in the Ser-Pro rich region, whose function remains elusive (Fig. 1b), two different functional domains have been identified in that central region of vertebrate Snail1, a ▶ nuclear export signal (NES) and ▶ a destruction box (DB) domain (Fig. 1).

Transcriptional Repression

The transcriptional repression mechanism of Snail1/Snail2 has been established for E-cadherin and many other targets (Fig. 2). In all cases, Snail-mediated repression requires interaction with E2-boxes, but with different binding affinities (Snail1 > Snail2). The precise mechanism for Snail1-mediated E-cadherin repression requires the recruitment of specific corepressor complexes containing the mSin3 corepressor and histone deacetylase (HDAC1) and HDAC2. In addition, histone and DNA methylases can be recruited by Snail1 to the repressor complexes to mediate epigenetic silencing of the E-cadherin gene, and potentially of other target genes. The possibility of Snail factors acting as transcriptional activators should be also considered, since Snail2 seems to positively regulate its own promoter during embryonic development in *Xenopus* and at least human Snail2 and Drosophila snail contain a transcriptional activation domain as assayed by transfection.

Regulation of Expression and Functional Activity

Expression of Snail1/Snail2 factors is regulated by a plethora of signals, most of them actively participating in induction of developmental EMT. Among them, growth factors activating ▶ receptor tyrosine kinases (RTK) and ▶ MAPK pathways (like fibroblast growth factor (FGF), epidermal growth factor (EGF), ▶ hepatocyte growth factor/scatter factor (HGF/SF), or oncogenic ▶ Ras); ▶ TGF-β/BMP signals and ▶ Wnt/β-catenin



Snail Transcription Factors. Fig. 2 Snail targets. Snail induces a full EMT through the downregulation of epithelial markers, the upregulation of mesenchymal markers and the acquisition of invasive properties. In addition, Snail regulates cell division and survival. Decreased cell division favors invasion versus tumor growth. Altogether, the ability to move, invade, and survive confers Snail-expressing cells a selective advantage to delaminate from the primary tumor and to form distant metastasis. Direct targets are shown in bold

pathways are major inducers of vertebrate Snail1/Snail2 expression. Significantly, crosstalking between distinct signaling pathways are important for Snail1 induction in different systems, particularly those involving TGFβ, Ras/RTKs, ▶ Notch, and/or Wnt/β-catenin pathways. Additional transcriptional regulatory mechanisms can be exerted through steroid receptors in some particular cellular systems, like in breast mammary carcinoma cells in which ligated ▶ estrogen receptors downregulate expression of Snail1 gene through activation of the specific MTC complex. Significantly, Snail factors can exert an autoregulatory control, underlining the necessity of tight regulation of Snail levels.

Besides transcriptional regulation, functional activity of Snail factors can be modulated at posttanscriptional levels. Phosphorylation of Snail1 by GSK3β (▶ Wnt signaling) in Ser residues located at the central DB and NES domain induces Snail1 nuclear export and cytoplasmic degradation, while potential modification of neighboring Lys residues, phosphorylation by Pak1, and/or interaction with specific ▶ zinc transporters (like LIV1) positively control Snail1 nuclear localization, protein stability, and repressor activity. Much less is known on posttranscriptional regulatory mechanisms of Snail2, except for the induction of proteasome degradation mediated by Ppa2.

Additional Snail Functions

Apart from their implication in EMT, Snail factors can regulate other cellular processes, related to cell proliferation and cell survival with important implications during embryonic development, tumor progression, and other pathologies. In some particular cell/tissue contexts, Snail1 expressing cells have a low proliferative potential, exhibiting a partial G1/S cell cycle arrest, a property that may ease migration of cancer cells during tumor progression. For Snail targets related to cell proliferation, see Fig. 2.

Snail factors also participate in protection against cell death induced by different external cues, thus acting as survival mediators. Significantly, the pro-survival action of Snail1/Snail2 factors has been demonstrated during embryonic development and in human carcinoma cells under genotoxic stress induced by chemotherapeutic agents and in radioresistance of hematological precursor cells.

Snail1/Snail2 negatively regulate p53 (► p53 Protein)-dependent (like ► Puma) and p53-independent genes (for additional targets see Fig. 2). Thus, Snail factor expression can contribute to the acquired resistance to ► apoptosis of tumor cells, a characteristic that might be crucial for the metastatic (► metastasis) process. It is very likely that the Snail-mediated pro-survival/resistance function is associated with EMT, as observed in hepatocytes in culture. Nevertheless, the different functions of Snail factors such as induction of EMT, reduced proliferation, and survival can be dissociated in some cellular contexts, at least during embryonic development.

Clinical Significance

SNAIL1 expression was originally detected in several carcinoma cells associated to E-cadherin downregulation and invasiveness. Importantly, SNAIL1 expression was also detected at the E-cadherin negative invasive regions of mouse skin carcinomas (► skin carcinogenesis), human breast carcinomas and hepatocarcinomas (► hepatocellular carcinoma), supporting its in vivo implication in induction of tumor invasion. Further studies in different tumor series indicate SNAIL1 expression associated to lymph-node status and/or distant metastasis in breast and ovarian carcinomas (► ovarian cancer), colorectal tumors (► colon cancer) and squamous cell carcinomas, while SNAIL2 has been implicated in ► melanoma metastasis. SNAIL1/2 expression in different tumor series correlates with E-cadherin downregulation and/or induction of several ► matrix metalloproteinases (MMPs). The expression of specific MMPs (i.e., MMP2, MMP9) has been found to be transcriptionally upregulated by Snail1/2 in different cell model systems, further supporting the implication of Snail factors as active invasion inducers through the coordinated regulation of the molecular players of the process. The recent identification of SNAIL1 at the tumor–stroma interface of restricted tumor areas strongly support the implication of SNAIL1 at focally restricted invasion areas where the EMT processes are most likely to occur. Interestingly, SNAIL1 expression has been associated to tumor recurrence in breast carcinoma and to poor prognosis in hepatocarcinomas, while SNAIL2 has been recently associated to poor prognosis or overall patient survival in several tumor types (i.e., colon carcinomas and squamous cell carcinomas). It should be important to analyze in the near future whether the implication of SNAIL1/SNAIL2 in tumor recurrence might be related to the prosurvival function of Snail factors, or perhaps to a potential contribution of Snail factors to cell stemness.

Further studies in large tumor series using highly specific anti-SNAIL1 antibodies, recently developed, (and hopefully anti-SNAIL2 in the near future) are certainly required. This, together with additional functional studies in cellular models and, most importantly, in defined mouse model systems of tumor progression, will contribute to get a complete understanding of the clinical relevance of Snail factors in human tumors.

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SNAILH (Human)

► Snail Transcription Factors

SNCA

Synonyms

NACP; PARK1; PARK4

Definition

A gene on human chromosome 4 that encodes the α-► synuclein protein.

SNCB

Definition

A gene on human chromosome 5 that encodes the ► synuclein β protein.

SNCγ

Definition

A gene on human chromosome 10 that encodes the ▶ synuclein γ protein. Also known as ▶ BCSG1.

SnoRNA

Definition

Small Nucleolar RNAs, are small RNAs (between 60 and 300 nucleotides long) found in the nucleolus. They function as guides for site specific modifications of ribosomal RNA, 2´-O-methylation and pseudouridine formation. More than 300 different ones have been described in humans.

▶ Cajal Bodies

SNP

Definition

Single nucleotide ▶ polymorphism; single base variation in the DNA, with a population frequency of > 1%.

▶ Metabolic Polymorphisms and Cancer Susceptibility

SNP Array

Definition

High-density arrays containing thousands of single nucleotide ▶ polymorphisms (SNPs).

▶ Modifier Loci

snRNA

Definition

Small nuclear RNA, the RNA components of the snRNPs involved as part of snRNPs in mRNA splicing.

▶ Cajal Bodies
▶ Pre-mRNA Splicing

SnRNP

Definition

Small nuclear ribonucleoprotein; a complex composed of small nuclear RNAs (snRNA) and proteins. snRNPs are important for splice site recognition and catalysis of the splicing reaction.

▶ Pre-mRNA Splicing

SOCS

Definition

The ▶ suppressors of cytokine signaling (SOCS) family of proteins are key physiological regulators of cytokine responses. Several SOCS proteins have been implicated in the negative regulation of cytokine signaling pathways including STATs.

▶ Signal Transducers and Activators of Transcription in Oncogenesis
▶ Suppressors of Cytokine Signaling

SOD

Definition

▶ Superoxide dismutase.

▶ Photodynamic Therapy

Sodium Chloride

▶ Salt Intake

Soft Tissue Sarcoma

▶ Non-Rhabdomyosarcoma Soft Tissue Sarcomas

Solar Light Induced Cancer

▶ Photocarcinogenesis

Solar Ultraviolet Light

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Definition

Solar ultraviolet light is ultraviolet light emitted by the sun.

Characteristics

The sun emits ultraviolet radiation as part of the electromagnetic spectrum. It is usually subdivided, rather arbitrarily, into UVA (400–315 nm), UVB (315–290 nm), and UVC (200–290 nm). UVA has been further subdivided into UVA1 (400–340 nm) and UVA2 (340–315 nm). More than 95% of the sun's UV radiation reaching the earth's surface is UVA. Practically all of the UVC, and much of the UVB, is absorbed by the oxygen and ozone in the earth's atmosphere, so that ultraviolet radiation below 290 nm is virtually undetectable at ground level. Nevertheless, the remaining UV radiation can still be absorbed by biological molecules (DNA, proteins, lipids) and elicit photochemical and photobiological responses. A light-absorbing molecule is called a chromophore. Upon absorption of the radiation's energy, this chromophore is elevated to an excited state. Ensuing photochemical reactions may either change the chromophore directly, or, through energy transfer in a so-called photosensitized reaction, indirectly change a molecule other than the chromophore.

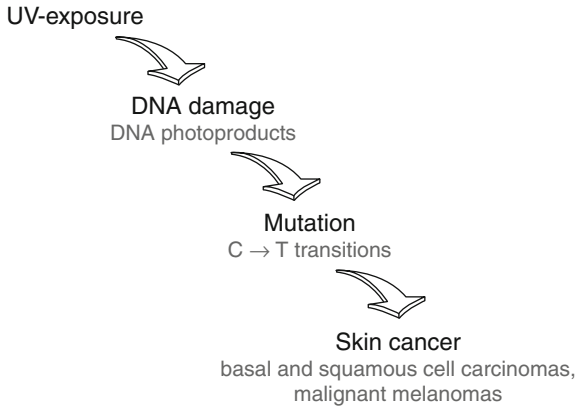
Effects of Exposure to Solar Ultraviolet Light on Skin

Exposure of skin to solar ultraviolet light has both short-term and long-term effects. Visible short-term effects are sunburning and/or tanning. Long-term effects are photoaging and induction of skin cancers (▶ skin carcinogenesis). Skin cancers clearly linked to sunlight exposure are ▶ basal cell carcinomas and ▶ squamous cell carcinomas, and ▶ malignant melanomas. An individual's tendency to develop sunburn and tanning after sun exposure correlates with the individual's susceptibility to long-term effects as well. Therefore, those individuals with higher acute sun sensitivity are generally also more at risk for developing skin cancers after chronic UV exposure.

Within the skin, the depth of penetration of UV light is wavelength-dependent – i.e., the longer the wavelength, the deeper the penetration. While UVA readily reaches the dermis, including its deeper portions, most of the UVB is absorbed in the epidermis, and only a small proportion reaches the upper dermis. UVC, if it reached the earth's surface, would be absorbed or reflected predominantly in the stratum corneum and in upper layers of the epidermis. Both short-term and long-term effects of exposure to UV light are wavelength-dependent. Previously, it was thought that only UVB is carcinogenic, and only UVA causes photoaging. Today, however, we know that both UVA and UVB cause skin cancer and photoaging. The relative contribution of each, however, continues to be a matter of controversy.

Photocarcinogenesis involves a stepwise accumulation of specific genetic changes in a single cell, with subsequent clonal expansion. Usually, it takes decades until a tumor arises. It is commonly accepted that UV-induced skin cancers develop along a photocarcinogenesis chain of events that involves (Fig. 1):

- DNA damage formation after exposure to solar ultraviolet light



Solar Ultraviolet Light. Fig. 1 Photocarcinogenesis chain of events

- Mutation formation following DNA damage formation
- Malignant transformation following mutation formation

DNA Damage Induced by Solar Ultraviolet Light

Different wavelengths of UV light induce different types of DNA damage. UVC and UVB are capable of exciting the DNA molecule directly and subsequently generating ▶ DNA photoproducts. DNA photoproducts are dimers, formed by covalently binding two adjacent pyrimidines in the same polynucleotide chain. The two major types of pyrimidine dimers are ▶ cyclobutane pyrimidine dimers and ▶ 6,4-photoproducts.

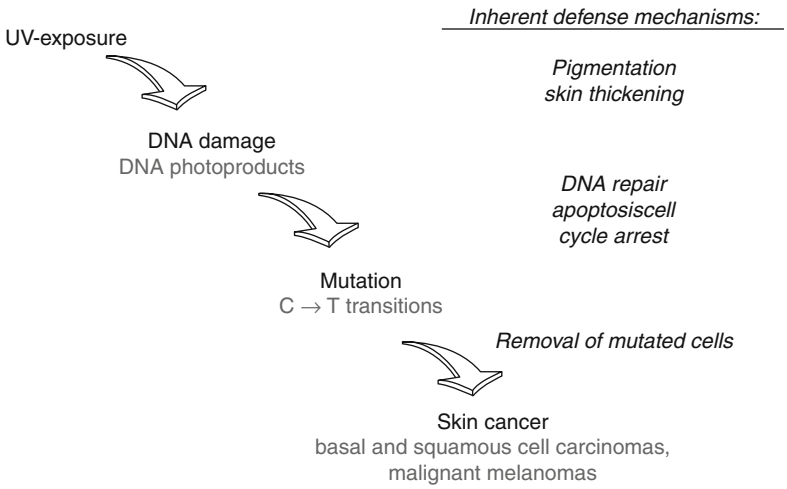
▶ Cyclobutane-pyrimidine dimers (CPDs) are the most common DNA photoproducts formed with solar ultraviolet irradiation of the skin. They are generated upon saturation of the 5,6 double bonds and formation of a four-membered cyclobutyl ring. Cyclobutane-pyrimidine dimers are observed at all possible di-pyrimidine sites, with the thymine–thymine dimer (T–T) being the most common, followed by C–T and T–C dimers. C–C dimers are the least common. The formation of CPDs is not a random phenomenon: it is influenced by the sequence and conformational context of the affected DNA sequence.

The 6,4-photoproduct is a non-cyclobutane di-pyrimidine photoproduct, which is formed upon covalent linkage between the C-6 position of one pyrimidine and the C-4 position of the 3′ adjacent pyrimidine. The T–C (6–4) dimer is the most common dimer of this type, but C–C and T–T dimers are also observed after UV irradiation. Upon further irradiation with UV wavelengths between 280 and 360 nm, the normal isomers of 6,4-photoproducts can be converted to their Dewar valence isomers, which are less mutagenic than the normal isomers but may still contribute to solar mutagenesis. A few other rare DNA photoproducts have been described, such as complex purine lesions and pyrimidine hydrates, but their physiological significance in the photobiology of human skin is unknown.

The absorption maximum of DNA is at 260 nm. This makes UVC the most effective wavelength for the induction of DNA photoproducts in naked DNA. However, *in vivo*, due to the absorption of shorter wavelengths in upper layers of the epidermis, 300 nm (UVB) is the most effective wavelength for inducing DNA photoproducts in the basal layer of the epidermis, the anatomical location from which most skin cancers arise. While UVA is much less effective in generating DNA photoproducts, UVA-induced DNA photoproducts may still have a significant contribution to sunlight-induced mutation formation, because of the much higher abundance of UVA in sunlight, as compared to UVB.

UV radiation can also damage DNA indirectly. After absorption of photons by chromophores other than DNA, energy can be transferred either to DNA (type I photosensitized reaction), or to molecular oxygen, with ▶ reactive oxygen species in turn being able to damage DNA (type II photosensitized reaction).

UV-induced reactive oxygen species include singlet oxygen, and probably other nonradical and radical reactive oxygen species, such as hydrogen peroxide and the superoxide radical. Even the highly reactive hydroxyl radical may be formed by a reaction of hydrogen peroxide with nuclear metals through a Fenton reaction. This oxidative stress not only affects DNA, but also membranes and proteins. The relative contribution of each (oxidative membrane damage, oxidative protein damage, ▶ oxidative DNA damage) to the different biologic effects of UV irradiation has not been well established. Singlet oxygen and the other reactive oxygen species react predominantly with guanine and generate several DNA changes including the mutagenic and well-studied 7,8-dihydro-8-oxyguanine (8-oxoG).



Solar Ultraviolet Light.
Fig. 2 Inherent cellular defense mechanisms against the photocarcinogenesis chain of events

It remains a matter of debate, whether the mutagenic properties of UVA (in particular UVA1: 340–400 nm) are mediated by ▶ oxidative DNA damage or by the weak ability of UVA to form a few pyrimidine dimers.

Mutations Induced by Solar Ultraviolet Light

Cancer development requires the accumulation of numerous genetic changes in a single cell. Solar ultraviolet light induces mutations in several key genes involved in skin cancer development, e.g., ▶ *ras* oncogenes, *p53* (▶ p53 protein, histological and clinical aspects), and *PTCH* tumor suppressor genes. The *p53* tumor suppressor gene encodes a 53 kDa transcription factor that plays a critical role in cellular DNA damage responses. Mutations in this gene can be found in most cutaneous squamous cell carcinomas and their precursors (actinic keratoses). In addition, chronically sun-exposed skin harbors many keratinocyte clones with *p53* mutations, which are undetectable by light microscopy. This indicates that *p53* mutations are an early event in the pathogenesis of UV-induced cutaneous squamous cell carcinomas.

It is well established that different types of UV-induced DNA damage can lead to the formation of a variety of different mutations, either during attempts by the cell to repair (▶ repair of DNA) or to replicate these lesions. Most of the *p53* mutations in cutaneous squamous cell carcinomas and its precursors are C → T single and CC → TT tandem transition mutations at dipyrimidine sites. This spectrum of *p53* mutations is very different from *p53* mutations found in malignancies of internal organs; the latter do not have the preponderance of C → T or CC → TT mutations. This, together with the fact that pyrimidine dimers most commonly cause C → T and some CC → TT mutations, provides convincing evidence for a crucial role of pyrimidine dimers in cutaneous photocarcinogenesis. Therefore, C → T, and especially CC → TT mutations have been termed "UV-signature mutations." They are, in fact, "signature mutations" for DNA photoproducts.

Protection Against Photocarcinogenesis

To ensure that most of the damage inflicted by sun exposure will not lead to the formation of skin cancer, UV-exposed cells have several lines of defense against the photocarcinogenesis cascade Fig. 2.

First line of defense – to avoid formation of DNA damage: In order to prevent DNA damage as a consequence of UV exposure, the human epidermis is protected by melanin, the expression of which can be increased in response to ultraviolet light (tanning response) to better protect against subsequent UV exposures. Melanin is a mixture of different polymerized pigments that absorb UV radiation. It is produced by melanocytes and transferred to keratinocytes, where it covers the upper pole of the nucleus in a microparasol to protect the nuclear DNA from the damaging effects of ultraviolet light. Skin can also increase its thickness, which reduces UV exposure of the basal layer, and it contains antioxidative enzymes, which quench reactive oxygen species and reduce the formation of oxidative DNA damage.

Second line of defense – to avoid mutation formation at sites of DNA damage: To counteract potentially mutagenic effects, UV-induced DNA damage requires excision and replacement of damaged nucleotides by DNA repair pathways. No correction procedure is absolutely exact and error free. If it were, UV-induced skin cancers would not occur in DNA repair–proficient individuals.

DNA photoproducts (pyrimidine dimers) are mutagenic, but they can be repaired by the nucleotide excision repair (NER) pathway. As exemplified by the hereditary disorder ▶ xeroderma pigmentosum, a defect in this repair pathway increases UV sensitivity and UV mutagenesis in cells, and nonmelanoma and melanoma skin cancers *in viv*o. The NER pathway has become well understood, in part through the identification and characterization of the different xeroderma pigmentosum genes.

Individual cancer and skin cancer risks are determined not just by a pronounced NER deficiency, as in xeroderma pigmentosum, but also by more subtle variations in DNA repair efficiency, e.g., as a consequence of polymorphism in DNA repair genes. Likewise, a decline in DNA repair efficiency with age has been linked to the increasing risk of skin cancer with advancing age.

Cells from patients with xeroderma pigmentosum variant have an intact NER, yet a phenotype that is indistinguishable from the other xeroderma pigmentosum complementation groups. Cells from these patients do not have a deficit in repairing DNA photoproducts, but a deficiency in what they do with unrepaired DNA photoproducts during replication in the S-phase of the cell cycle. Replicative DNA polymerases usually stall at unrepaired DNA lesions and detach from the DNA strand. For this situation, cells have a number of specialized DNA polymerases that are able to bypass different kinds of DNA damage and extend replication forks through damaged sites. Different polymerases perform this "translesional DNA synthesis" (▶ translesion DNA polymerases and cancer) with variable fidelity.

Due to a mutation in the pol-η gene, cells from patients with XP variant lack the particular ability of DNA polymerase-η to bypass thymine–thymine dimers with correct insertion of two A residues. This indicates that NER does not always repair all DNA lesions and that the function of a high-fidelity translesional DNA polymerase is crucial for maintaining genomic stability if cells enter S-phase with unrepaired DNA damage. In cells from patients with xeroderma pigmentosum variant, NER can remove most of the thymine–thymine dimers, but, because polymerase-η is missing, any remaining dimers are more likely to be bypassed by polymerases that insert incorrect residues. This causes a UV-▶ mutator phenotype. If DNA polymerase-η or any other specialized translesional DNA polymerase fails to bypass DNA damage during S-phase, the cell is faced with a stalled replication fork. In these cases, DNA recombination repair, which utilized strand invasion from the sister chromatid, can resolve the stalled replication forks.

Following formation of DNA damage by solar ultraviolet light, cells undergo significant changes. The transcription factor p53 (▶ p53 protein; biological and clinical aspects), which is both upregulated and activated following exposure to solar ultraviolet light, plays a pivotal role in these DNA damage responses. Genome-protecting effects of p53 in response to UV exposure are manifold:

- It mediates a cell cycle arrest that allows for more time to repair DNA damage and prevents replication of damaged DNA.
- With overwhelming DNA damage it may induce apoptosis (an affect often seen as sunburn cells, which are apoptotic keratinocytes formed after high-dose sun exposure) and thereby prevent survival of damaged cells.
- It augments DNA repair capacity.

Loss of these protective responses with acquired inactivating p53 mutations results in a UV-mutator phenotype. This explains why p53 plays such a prominent role early in photocarcinogenesis.

Third line of defense – to prevent that cells with mutations expand clonally and form skin cancers: Even after mutations have fixed the inflicted damage for the lifetime of the affected cells, the organism still removes most of these cells, for example, through immune surveillance. However, solar ultraviolet light has several immunosuppressive effects, which contribute to the carcinogenic properties of solar ultraviolet light. Ultraviolet light is therefore a double-edge sword with regard to photocarcinogenesis: Not only does it generate DNA damage that entails mutation formation and malignant transformation, its immunosuppressive properties and the induction of specific tolerance to UV-induced skin tumors also reduces the

ability of the host immune defense to recognize and remove malignant cells. The latter also impairs the immune surveillance of cells infected with oncogenic viruses (such as certain HPV types commonly found in squamous cell carcinomas of transplant patients) and may thereby further promote skin cancer formation.

In addition to the intrinsic protective mechanisms against the chain of events leading to the formation of skin cancer, there are extrinsic protective agents and behaviors that can help individuals to reduce their individual skin cancer risk. In order to prevent or reduce UV irradiation of the skin, one can avoid the sun, especially around noontime, stay in the shade, wear protective clothing, and/or wear sunscreens. Sunscreens are topical preparations which attenuate UV radiation before it enters the skin by reflection, absorption, or both. Sunscreens not only protect against the acute skin injury of sunburn, but also against UV-induced immune suppression, photoaging, and skin cancer. The sun protection factors (SPF) of sunscreens, however, which indicate by what factor sunburn is prevented by sunscreen use, do not correlate well with protection factors for other non-erythema endpoints. Therefore, the SPF cannot be regarded as a reliable guide to non-erythema and chronic endpoints.

Topical application of DNA repair enzymes has been shown to increase DNA repair in skin cells and to accelerate removal of DNA photoproducts. In patients with xeroderma pigmentosum, such applications have been reported to reduce the occurrence of actinic keratoses. This indicates that it may be possible to prevent the formation of mutations after the introduction of DNA photoproducts with the use of such "enzymatic sunscreens."

Hereditary Disorders with Increased Photocarcinogenesis

Many disorders with an increased skin cancer risk after exposure to solar ultraviolet light are characterized by a deficiency in the intrinsic protective mechanisms discussed above. For example, men with androgenetic alopecia have a much higher risk of developing skin cancers on the scalp than men who have retained their UV-protective hair cover. Lack of protective melanin, as in oculo-cutaneous albinism, increases the amount of DNA damage in the basal layer of the epidermis after UV irradiation and, subsequently, the risk of skin cancer.

The different DNA repair defects of xeroderma pigmentosum and the DNA damage-processing defect of xeroderma pigmentosum variant (see above) generate a UV-mutator phenotype, with an increased chance that DNA damage will result in the formation of a mutation.

Some cases of familial melanoma are caused by a germ-line mutation in the CDKN2A locus, which encodes two gene products, $p16^{INK4a}$ and $p14^{ARF}$. Intact p16 induces a G1 cell cycle arrest by inhibiting ▶ cyclin-dependent kinases 4 and 6, which in turn inhibit the phosphorylation of the ▶ retinoblastoma protein. Loss of $p16^{INK4a}$ function, therefore, entails a loss of the G1 checkpoint, leading to abnormal proliferation, unrestricted progression into S-phase, and, importantly, no cell cycle arrest after UV irradiation. $P14^{ARF}$ is an upstream regulator of p53, another important factor mediating UV-induced growth arrest. The disruption of cell cycle control with loss of CDKN2A does not, however, explain why loss of p16/p14 function predisposes mostly to malignant melanoma, and only to a much lesser degree to internal neoplasias, namely, pancreatic cancer. A recent finding that loss of $p16^{INK4A}$ or $p19^{ARF}$ function also impairs the ability of affected cells to repair DNA photoproducts, leading to a UV-mutator phenotype, might be the missing link, explaining why alteration in CDKN2a predisposes mainly to UV-induced tumors.

Similarly, p53 protects against UV mutagenesis by inducing a cell cycle arrest after UV-induced damage, by inducing apoptosis in cells with overwhelming UV-induced DNA damage, and by stimulating DNA repair by directly binding to DNA repair enzymes. Consequently, loss of p53 function has been shown to result in a UV-mutator phenotype and reduced repair of UV-induced DNA damage. Li–Fraumeni syndrome is characterized by a germ-line mutation of the *p53* gene, and predisposes to malignancies of various internal organs and cutaneous melanoma.

Patients with the nevoid basal cell carcinoma syndrome, who develop multiple basal cell carcinomas, especially in UV-exposed areas, harbor germ-line mutations in the *PTCH* gene. PTCH belongs to the ▶ hedgehog signal transduction pathway that transmits extracellular growth and differentiation signals to the nucleus. While many of the *PTCH* mutations in sporadic basal cell carcinomas are UV signature mutations (C → T and CC → TT), their frequency is

lower than in *p53*, and it is unclear whether sporadic *PTCH* mutagenesis is purely UV induced.

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Solitary Bone Plasmacytoma

► Plasmacytoma

Solitary Extramedullary/Extraosseous Plasmacytoma

► Plasmacytoma

Solitary Plasma Cell Myeloma

► Plasmacytoma

Soluble Mesothelin-related Proteins

► SMR

Solute Carrier Transporters

Definition

SLC transporters; Are a large group of membrane transporters that function as secondary-active or passive transporters in the translocation of ions and small molecules, including drugs, across biological membranes. The group comprises more than 370 members in 51 families.

► Membrane Transporters

Somatic Cells

Definition

Cells other than those of the gamete-forming germ line.

► Adult Stem Cells
► Somatic Tissue

Somatic Cross-Over Point Mapping

Definition

Is a method exploiting the fact that certain genetically unstable cells, e.g., in ► Bloom syndrome, show excessive DNA cross-overs within the BLM gene itself. This leads to restoration of BLM function in cells inheriting two different mutations in BLM.

Somatic Hypermutation

Definition

Somatic hypermutation is a process by which somatic mutations are introduced at a high rate into the variable region parts of immunoglobulin genes. This process is specifically activated in germinal center B cells. As a result of somatic hypermutation, antibody variants are generated that differ by a few aminoacids from the original antibody. In the germinal center reaction, B cells expressing antibodies with increased affinity due to favorable mutations can be selected. Somatic hypermutation may be involved in the generation of B cell lymphomas when non-Ig genes are targeted or

when chromosomal translocations happen as mistakes of the process.

▶ Diffuse Large B-Cell Lymphoma
▶ Hodgkin and Reed/Sternberg Cell

Somatic Recombination of V, D and J Segments

▶ V(D)J Recombination

Somatic Stem Cells

▶ Adult Stem Cells

Somatic Tissue

Definition

Most tissues in a multicellular organism. Cells in these tissues do not contribute to the production of gametes and thus mutations in these tissues are not heritable. In humans, many somatic tissues contain cells that are dividing or capable of dividing and thus are capable of renewal, repair, and sometimes regeneration.

▶ Aging

Somatostatin

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Synonyms

Somatotropin release inhibiting factor; SRIF

Definition

Somatostatin is a bioactive peptide that exists in the two isoforms SST-14 and SST-28, of 14 and 28 amino acids, (SST-14) and SST-28, respectively. It acts as a ▶ neuropeptide (neurotransmitter), is produced by neurons and endocrine-like cells, and is distributed throughout the central and peripheral nervous system, endocrine pancreas, gut, thyroid, adrenals, submandibular glands, kidneys, prostate, and placenta. Many tumor cells, immune cells, and inflammatory cells produce somatostatin.

Characteristics

Somatostatin was initially isolated as an inhibitor of growth hormone (GH) release, but is now best described as a multifunctional peptide, capable of inhibiting secretory processes and cell proliferation. Hypothalamic somatostatin inhibits the release of pituitary growth hormone (GH), thyroid-stimulating hormone (TSH), and corticotropin releasing hormone (CRH). As a neurotransmitter it affects several functions such as autonomous, sensory, locomotive, and cognitive. Locally produced somatostatin generally inhibits gut exocrine secretion (e.g., on insulin, glucagon, gastrin), suppresses stomach, small intestine, and gall bladder motility, and mediates vasoconstriction, especially of splanchnic vessels. In the adrenals, somatostatin inhibits angiotensin II-stimulated aldosterone secretion and in the kidneys hypovolemia-stimulated renin release. In the immune system it blocks the release of cytokines, including IFN-γ and IL-6, and limits the proliferation of lymphocytes, intestinal mucosa and inflammatory precursor cells. Furthermore, it blocks the action of growth factors such as ▶ IGF1, ▶ EGF, and ▶ PDGF. The plasma half-life of somatostatin is less than 3 min.

Somatostatin Secretion and Gene Expression

The human somatostatin gene is encoded on chromosome 3q28 in a prehormone form (preprosomatostatin) with an mRNA of 351 bp. The two bioactive forms SST-14 and the N-terminally extended SST-28 are produced by proteolytic cleavage of prosomatostatin. SST-14 is the peptide that is found predominantly. However, up to 30% of immunoreactive SST in the brain is SST-28. Somatostatin secretion is triggered by

membrane depolarization, certain ions, nutrients, and neurohormones/neuropeptides. Potent stimulators are glucagon, growth hormone releasing hormone (GHRH), neurotensin, corticotropin releasing hormone (CRH), calcitonin gene-related peptide (CGRP) and bombesin. Somatostatin gene expression is stimulated by multiple cytokines and growth factors including IGF1 and 2, GH, IL-1, -6, -10, TNF-α, IFN-γ, NMDA receptor ligands, and by steroid hormones such as testosterone, glucocorticoids, and estradiol. Insulin, leptin, ▶ TGF-β, and some glucocorticoids inhibit somatostatin expression. Transcription of the somatostatin gene is regulated by the intracellular second messengers cAMP, cGMP, NO, and Ca2+ and the associated pathways involving ▶ CREB and ▶ CBP (cyclic AMP response element binding protein and CREB binding protein).

Somatostatin Receptors (sst)

Somatostatin binds to five currently known G-protein-coupled seven transmembrane receptor subtypes (sst1–5) that were initially classified according to their differential binding of somatostatin analogues. The genes for sst1,3,4,5 lack introns, while sst2 contains at least three potential transcriptional start sites, one of which is located in exon 1, 50 kb upstream of the start site in exon 3. All cloned receptor subtypes contain recognition motifs for glycosylation and phosphorylation. Homo- and heterodimerization of the receptors as well as receptor internalization have been described. Upon ligand binding sst induce a multitude of intracellular effects mediated by varying G-proteins coupled to second messengers. All receptors block the formation of cAMP by inhibiting ▶ adenylyl cyclase and activate tyrosine phosphatases (Table 1).

Characteristics of the five cloned human ssts and information about their distribution in human cancer. Methods that rely on tissue homogenates are unreliable since ssts are found in immune cells and veins surrounding the tumor tissue. Subtype selectivity of ligands is indicated by bold italics for IC50. Subtype expression has only been studied in a limited number of tumor types.

Molecular Basis of Somatostatins Antiproliferative Effects

Somatostatin limits the proliferation of tumor cells in vitro and in vivo directly and indirectly. Direct regulation is by somatostatin receptors (ssts) that are localized on neoplastic cells; indirect regulation is exerted via ssts on non-neoplastic cells. Mechanistically, this is done by inhibiting the secretion of growth-promoting hormones and growth factors (e.g., IGF-1), by promoting vasoconstriction (which leads to a reduced blood flow to tumor tissue), by inhibiting angiogenesis and by influencing the function of immune cells. The block of secretion is due to inhibition of the second messengers cAMP and Ca2+ and the inhibition of exocytosis in a G-protein-dependent manner.

The direct antiproliferative effects of somatostatin appear to be largely due to the activation of protein phosphatases. Somatostatin-induced protein tyrosine phosphatases (PTP) dephosphorylate tyrosine kinases of receptors for growth promoters such as insulin and possibly EGF and IGF-1. Furthermore, somatostatin inactivates MAPK activity via PTP-dependent dephosphorylation (sst2), PTP-dependent Raf-1 inactivation (sst3), and inhibition of cGMP formation (sst5). Activation of PTP is also involved in somatostatin-induced apoptosis. While in CHO-K1 cells transfected with each individual sst, sst3 appears to induce apoptosis by activation of TP53, independent of G1 arrest, all other ssts prompt G1 arrest and induction of Rb.

A base pair change in the sst2 gene, found in a lung cancer cell line (COR-L103), lead some authors to the assumption of a tumor suppressor role for somatostatin receptors.

Clinical Relevance

Somatostatin Analogue Therapy

The demonstration of receptor subtype expression in malignancies has paralleled the creation of subtype selective receptor ligands. While the best characterized and oldest analogue, the octapeptide octreotide (plasma half-life 2 h) exhibits a preference for sst2 with lower affinities for sst5 and sst3, highly specific nonpeptide agonists for each of the five subtypes have been developed.

The longest clinical experience exists for the treatment of hormone-secreting tumors with octreotide and its microencapsulated long-acting release (LAR) form. The classic indication for somatostatin analogue therapy is a growth hormone-secreting pituitary adenoma in ▶ acromegaly. Furthermore octreotide and other somatostatin analogues have been used in the treatment of carcinoids, insulinomas, gastrinomas,

Somatostatin. Table 1 The somatostatin receptor family (sst) (Modified after Patel)

	sst1	sst2A	sst3	sst4	sst5
Chromosome	14q13	17q24	22q13.1	20p11.2	16p13.3
mRNA (kb)	4.8	8.5 (?)	5.0	4.0	4.0
Amino acids	391	369	418	388	363
Molecular weight (kD)	53–72	71–95	65–85	45	52–66
Ligand affinity (IC50 nM)					
SST-14	0.1–2.26	0.3–1.3	0.3–1.6	0.3–1.8	0.2–0.9
SST-28	0.1–2.2	0.2–4.1	0.3–6.1	0.3–7.9	0.05–0.4
Octreotide	290–1,140	0.4–2.1	4.4–34.5	>1,000	5.6–32
RC-160	>1,000	5.4	31	45	0.7
Seglitide	>1,000	0.1–1.5	27–36	127- > 1,000	2–23
CH275	3.2–4.3	>1,000	>1,000	4.3–874	>1,000
L-797,591	1.4	1,875	2,240	170	3,600
L-779,976	2,760	0.05	729	310	4,260
L-796,778	1,255	>10,000	24	8,650	1,200
L-803,087	199	4,720	1,280	0.7	3,880
L-817,818	3.3	52	64	82	0.4
Signal transduction					
Adenylyl cyclase	↓	↓	↓	↓	↓
Tyrosine phosphatase	↑	↑	↑	↑	↑
MAP kinase	↑	↓	↑↓	↑	↓
Ca2+ − influx	↓	↓			
Na+/H + exchange	↑				
Phospholipase C activity		↑			↑↓
Phospholipase A2 activity				↑	
Tumor expression	Gastroenteropancreatic tumors	Gastroenteropancreatic tumors	Gastroenteropancreatic tumors	Meningioma lipoma	Pituitary adenoma
	Medullary thyroid carcinoma	Growth hormone (GH) and thyroid-stimulating hormone (TSH) producing pituitary adenomas	Not in nonfunctioning pituitary adenoma		
	Ovarian cancer	Breast carcinoma	Ovarian cancer		
	Prostate cancer	Neuroblastoma			
	Pheochromocytoma	Pheochromocytoma			
		Medulloblastoma			
		Meningioma			
		Small cell lung cancer (SCLC)			
		Hodgkin lymphoma			
		Peritumoral vessels			

► VIPomas, glucagonomas, and somatostatinomas, producing symptomatic or subjective responses in 30–75%, and significant reduction in tumor size in 10–15% of patients. In clinical studies, octreotide and other analogues have been used as single agents or in combination with conventional cytostatic drugs with varying results in carcinomas of the breast, prostate, pancreas, colorectum, thyroid and lung, inmeningiomas, ► neuroblastomas, and non-Hodgkin lymphomas.

Somatostatin Receptor Imaging and Radiotherapy

The in vitro detection of somatostatin receptors on a multitude of tumor tissues has led to the development of ▶ somatostatin receptor scintigraphy (SRS). Apart from neuroendocrine tumors expressing ssts, SRS has successfully been used in the imaging of pheochromocytomas, non-small-cell lung cancers (NSCLC), meningiomas, ▶ breast cancer, ▶ gliomas, ▶ medulloblastomas, non-Hodgkin and Hodgkin lymphomas (▶ Hodgkin Disease), granulomatous disease, Sjögren syndrome, and rheumatoid arthritis. Binding of radioactive somatostatin analogues has been used in radioreceptor-guided surgery as an asset in the surgery of neuroendocrine gastroenteropancreatic tumors and neuroblastomas with occult metastases.

After ligand binding a fraction of receptors are internalized. This phenomenon has been used for clinical studies of in situ radiotherapy using 111Indium- or 90Yttrium-labeled somatostatin analogues in terminally ill patients with neuroendocrine tumors.

General Clinical Applications

In carcinoids and neuroblastomas, the level of somatostatin or somatostatin receptor expression (e.g., by SRS) has been reported to correlate with tumor differentiation and therapeutic outcome of the disease.

As a therapeutic adjuvant octreotide has been used in the treatment of infectious and secretory diarrhea, L-asparaginase-induced pancreatitis, symptomatic treatment of fistulas, and in the management of severe pain due to neoplasia.

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Somatostatin Receptor Scintigraphy

Definition

SRS; is a type of scan used for the diagnosis of neuroendocrine carcinomas. A radioactive form of octreotide (an analog of ▶ somatostatin) is injected into the patient; this will bind to the tumor cells with somatostatin receptors. A radioactivity-measuring device will detect radioactivity and make a picture showing where the tumor cells are localized.

▶ Neuroendocrine Carcinoma

Somatostatinoma

Definition

Is a functioning neuroendocrine tumor of the pancreas that produces high amounts of the hormone ▶ somatostatin that can result in the so-called somatostatinoma syndrome.

▶ Neuroendocrine Carcinoma

Somatostatinoma Syndrome

Definition

Is a clinical pentad of diabetes mellitus, cholelithiasis, weight loss, diarrhea, and hypochlorhydria/achlorhydria observed in association with tumors that overproduce the gastrointestinal hormone ▶ somatostatin.

▶ Neuroendocrine Carcinoma
▶ Somatostatinoma

Somatotropin

Synonyms

Growth hormone

Somatotropin Release Inhibiting Factor

► Somatostatin

Sonic

Definition

Gene in hedgehog signaling.

► Hedgehog Signaling

Sonic Hedgehog

Definition

One of three proteins in the mammalian hedgehog family that plays a key role in regulating vertebrate organogenesis, such as in the growth of digits on limbs and organization of the brain.

► Hedgehog Signaling

Sorafenib

Definition

An orally available multi-kinase inhibitor that has the chemical structure of *N*-(3-trifluoromethyl-4-chlorophenyl)-*N*ϒ-(4-(2-methylcarbamoylpyridin-4-yl)oxyphenyl)urea and is capable of simultaneously inhibiting ► VEGFR and ► PDGFRβ tyrosine kinases as well as the Raf serine/threonine kinase. Sorafenib is used to treat patients with ► renal carcinoma.

► Drug Design
► Raf Kinase

SOX18

Definition

A transcription factor in the SOX family, which regulates lymphatic vessel development. Missense mutations or deletions of this gene lead to primary ► lymphedema.

► Lymphangiogenesis

Soy Isoflavonoids

Definition

A group of ► phytoestrogens found in soybean which possess potent antioxidant and antiangiogenic properties. ► Isoflavones interact with animal and human estrogen receptors, causing effects in the body similar to hormone ► estrogen.

► Chemoprotectants

Soy Phytoestrogen

► Genistein

Soy Proteins

Definition

Are a mixture of proteins (α-, β-, and γ-conglycinins, β-amylase, lectin, the Kunitz inhibitor of trypsin, the Bowman-Birk inhibitor of chymotrypsin and trypsin, among others) differing in physicochemical and other properties. They are used in human foods in a variety of forms and their consumption is increasing because of reported beneficial effects on nutrition and health. These effects include lowering of plasma cholesterol, prevention of cancer, diabetes, obesity, and protection against bowel and kidney disease.

► Nutraceuticals

Sp

Definition

Specificity protein transcription factor.

► Betulinic Acid

SPARC

► Secreted Protein Acidic and Rich in Cysteine

SPB, in Yeast

► Centrosome

SPC1

► Furin

Specific Immunotherapy for Melanoma

► Melanoma Vaccines

Specificity

Definition

The specificity of a test indicates the proportion of patients without the target condition who have a negative result with the test.

► Molecular Pathology

Spectral Karyotyping

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Definition

Spectral karyotyping (SKY) is a multi-fluorochrome ► fluorescence in situ hybridization technique (FISH) in which all the chromosome pairs are simultaneously visualized in different colors in a single hybridization. SKY determines the unique spectral profile of each chromosome generated by specific combinations of different ► fluorochromes. At the present time, SKY can be used to analyze human, mouse, and rat chromosomes.

Characteristics

Structural and numerical chromosomal alterations (aberrations) are the hallmarks of malignant diseases. Routine cytogenetic analysis based on ► G-protein couple receptor (GPCR) techniques provides important information of diagnostic and prognostic relevance both in hematological malignancies and solid tumors. However, detection of chromosomal alterations by this method is complicated by the difficulty in routinely preparing metaphase spreads of sufficient quality and quantity, the clonal heterogeneity of the tumors, and the complexity of the many ► chromosomal aberrations. In addition, homogeneously staining regions or double minute chromosomes, results of oncogene amplification, are impossible to characterize using G-banding analysis alone. As a result a large number of chromosomal abnormalities are described as so-called marker and derivative chromosomes instead of being precisely defined. Fluorescence in situ hybridization, a highly sensitive and specific tool for the detection of chromosomal aberrations, provides additional information to G-banding analysis clarifying particular problems. However, the ► FISH technique is unable to screen the whole ► karyotype in one experiment and therefore analysis of complex unknown chromosomal alterations requires a large panel of painting probes and several hybridizations.

The fantastic advantage of Spectral Karyotyping (SKY) is the ability to visualize simultaneously all the chromosome pairs in different colors in one hybridization. In this way, karyotype rearrangements are easily detected as a transition from one color to another at the chromosomal breakpoint region.

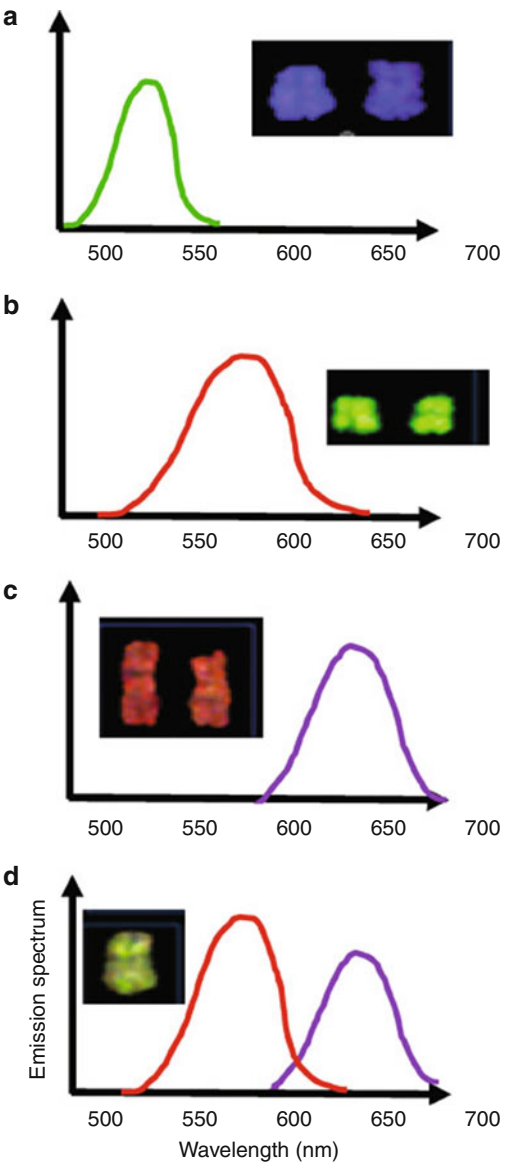
The SKY method involves several steps:

1. A probe cocktail (Applied Spectral Imaging Ltd, Migdal, Ha'Emek Israel) consisting of fluorescently labeled probes for each chromosome is made by

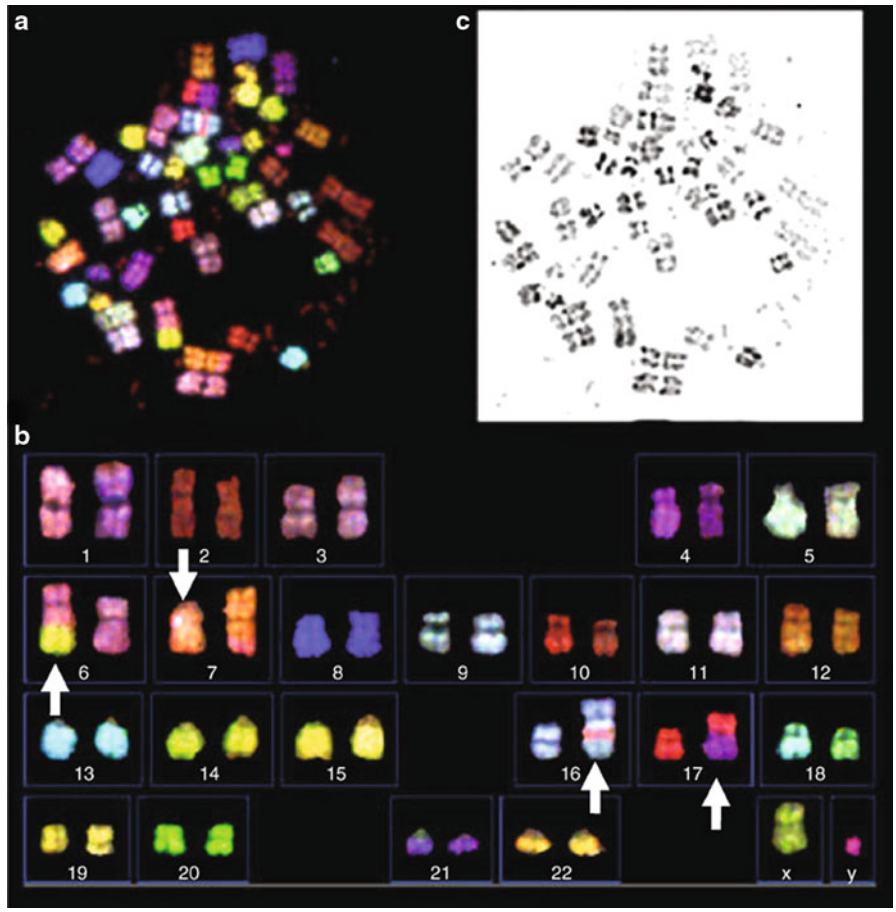
labeling chromosome-specific libraries generated by PCR from flow-sorted chromosomes with specific combinations of one or more of the five spectrally distinct fluorochromes (FITC, Rhodamine, Texas Red, Cy5 and Cy5.5).

2. Metaphase preparations are hybridized with this probe cocktail and then stained with 4,6-diamidino-2 phenylindole (DAPI) in antifade medium.
3. The SpectraCube® Imaging system (Applied Spectral Imaging Ltd, Migdal Ha'Emek, Israel) is used to discriminate between the different spectral characteristics of chromosomes. The system measures chromosome-specific emission spectra generated by the combinatorially labeled chromosome-specific painting probes.
4. The spectral signature of the fluorochrome combinations is analyzed using SKYView™ software. It classifies the chromosomes by comparing the acquired spectral characteristics to the combinatorial library containing the fluorochrome combinations for each chromosome. In the classified image the chromosomes appear in a red-green-blue (RGB) display in which FITC is seen as blue, Rhodamine and Texas Red are seen as different shades of green, and the infrared dyes not visible to the human eye, Cy5 and Cy5.5, are assigned different shades of red.

   Figure 1 schematically presents the principle of the chromosome classification according to the spectra characteristics. Each graph consist of fluorescence emission spectra of the distinct fluorochromes (or combination of fluorochromes) and a red-green-blue (RGB) display of the appropriate chromosome. Emission maximums for presented fluorochromes are: FITS – 525 nm, Rhodamine – 570 nm, Cy5.5–703 nm. Figure 1a shows chromosome 8 labeled with FITC, in the RGB display it is seen as blue color; Fig. 1b chromosome 20 labeled with Rhodamine, in the RGB display it is seen as green color; Fig. 1c chromosome 2 labeled with Cy5.5, in the RGB display it is seen as red color and Fig. 1d chromosome 2 labeled with combination of Rhodamine and Cy5.5, in the RGB display it is seen as specific color.
5. The chromosomes are then automatically sorted into a karyotype table according to the nomenclature rules for G-bands. Rearrangements, ▶ translocations between different chromosomes and components of marker chromosomes are all easily identified because of a change in color at the point of transition (Fig. 2a, b). Finally, the



Spectral Karyotyping. Fig. 1 Schematic presentation of chromosome identification according to the spectral characteristics. Each graph consists of a fluorescence emission spectra and a RGB display of the chromosome. The *horizontal axis* shows wavelength, the *vertical axis* shows relative intensity of fluorescence. (**a**–**d**) Chromosomes 8, 20, 2 and X, labeled with FITC, Rhodamine, Cy5.5 and a combination of Rhodamine and Cy5.5, respectively



Spectral Karyotyping.
Fig. 2 Spectral karyotyping of metaphase of a neuroblastoma tumor (**a**) Red-green-blue (RGB) display after hybridization with the SKY kit; (**b**) Karyotype table of spectrally classified chromosomes. Chromosomal aberrations are detected by the combination of two or more colors on the same chromosome (marked by *arrows*). (**c**) DAPI-stained separately and inverted to give a G-banding like pattern. (D. R. Betts, Department of Oncology, University Children's Hospital, Zürich, Switzerland; L.Trakhtenbrot, Institute of Hematology, Tel Hashomer, Israel)

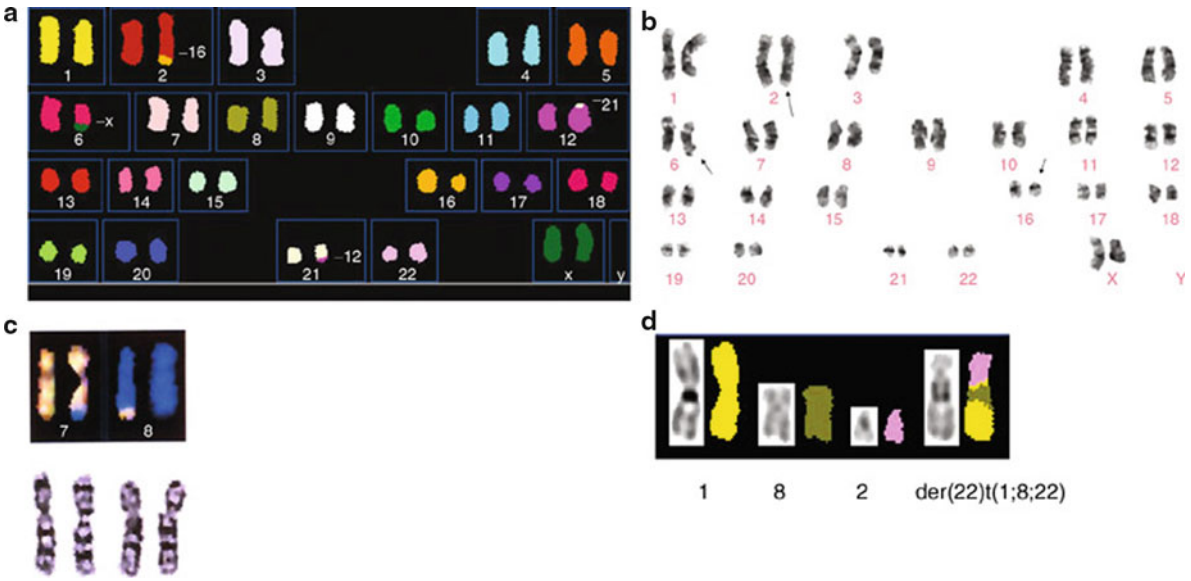
software assigns a specific classification pseudocolor to each chromosome allowing chromosomal aberrations to be even more easily visualized (Fig. 2b).

6. The DAPI image is captured separately and inverted to give a G-banding like pattern. This image may be used to compliment the SKY analysis with chromosome banding information (Fig. 2c).

Advantages of SKY

SKY analysis has revealed numerous marker and derivate chromosomes (Fig. 3a, b, d), hidden translocations (Fig. 3a, c), chromosomal insertions, homogeneous staining regions and double minutes unidentified or incorrectly identified by G-banding. The combination of SKY with FISH increases the accuracy of karyotype interpretation, permitting precise breakpoint mapping and the detection of small interstitial ▶ deletions and cryptic translocations. SKY is particularly useful in cancer cytogenetics and provides a much more detailed description of the highly abnormal karyotypes that characterize advanced tumors and cancer cell lines. The precise definition of markers by the SKY technique leads to the determination of an increased number of aberrations per tumor, identification of more chromosomal regions involved in the karyotype evolution, and the analysis of more metaphases, especially polyploid. SKY enables the discovery of a larger number of subclones and the revelation of different clonal evolution pathways of karyotype alterations. In general, SKY provides an opportunity to assess the level of tumor instability that is usually associated with advanced or aggressive disease.

The flexibility of SKY permits it to be used with any genome, provided the effective flow sorting of chromosomes can be accomplished. Recently SKY has been developed for murine and rat chromosomes, the



Spectral Karyotyping. Fig. 3 SKY detection of chromosomal aberrations incorrectly identified by G-banding. (**a**) SKY showing translocations t(12;21), t(2;16) and der(6)t(X;6), displayed in classification colors. (**b**) G-banding failed to recognized the cryptic translocation t(12;21), and the origin of the additional segments on chromosomes 2 and 6. (**c**) Translocation t(7;8)(q32;q34) is detected by SKY (RGB display), but not by G-banding. (**d**) SKY demonstrates a complex translocation involving three chromosomes – 1, 8, and 22. G-banding identifies this rearrangement only as a marker of chromosome 1 origin. (D. R. Betts, Department of Oncology, University Children's Hospital, Zürich, Switzerland; L.Trakhtenbrot, Institute of Hematology, Tel Hashomer, Israel)

most popular species used as model systems for the investigation of tumorgenesis.

Disadvantages

Due to the nature of painting probes, SKY alone cannot detect intrachromosomal rearrangements, such as paracentric or pericentric ▶ inversions, small duplications, and deletions. The resolution of SKY (1–3 Mb) depends on the level of chromosomal condensation and on the combination of the fluorochromes involved in structural rearrangements. Thus, SKY should be seen as a complement, and not as a replacement, of conventional G-banding analysis.

To overcome these limitations and to elevate the accuracy of cytogenetic analysis, a new method named "spectral color banding (SCAN)" was developed on the basis of the conventional SKY analysis. SCAN analyzes a single chromosome, allowing simultaneous visualization of all the chromosome bands in different colors in a single hybridization since each band is labeled with a unique combination of fluorochromes (similar to the SKY technique) with a specific spectral pattern. In this way different bands can be distinguished from each other by different colors. SCAN analysis employs a detection system and algorithm for spectral pattern recognition identical to that used in SKY analysis.

The drawback of SCAN is its inability to detect aberrations in the other chromosomes. To overcome this shortcoming, a combination of SKY and SCAN was developed based on a probe cocktail composed of the SCAN banding probes for an individual chromosome and the SKY probe kit without the painting probe for this particular chromosome.

Clinical Significance

Since its introduction in 1996, SKY has been extensively used to elucidate chromosomal aberrations in hematological malignancies as well as solid tumors, including sarcomas, carcinomas and brain tumors. The highly important input of SKY analysis to clinical research of malignant diseases is the identification of novel recurrent aberrations with pathogenic potential and the prediction of a specific phenotype or modified response to treatment or outcome. The results of SKY analyses contribute to diagnosis, therapy decisions, and follow-up studies.

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Spectral Unmixing

Definition

Mathematical algorithm-based technique, used in optical image analysis, to distinguish and quantitatively measure the light emission produced by multiple, differently colored fluorescent probes or genetic reporters following their simultaneous detection.

► Bioluminescence Imaging

Spermatocytes

Definition

Spermatocytes are the male germ cells before meiosis I.

► BORIS

S-Phase

Definition

Short for synthesis phase, S-phase is a period during the ► cell cycle where DNA synthesis or replication occurs. The cell cycle is divided into four phases: G1 (Gap1), S (synthesis), G2 (Gap 2), and M (mitosis). Origins of replication fire once per cell cycle allowing the entire genome to be replicated in S-phase.

► Flow Cytometry
► S-Phase Damage-Sensing Checkpoints

S-Phase Checkpoint

Definition

The ► cell cycle checkpoint that monitors cell cycle progression and decreases the rate of DNA synthesis following DNA damage.

► BACH1 Helicase

S-Phase Damage-Sensing Checkpoints

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Definition

Cell-cycle checkpoints function by sensing DNA damage and transmitting a signal that results in arrest at defined stages of the cycle. The ► S-phase "checkpoint" halts cells during the DNA synthetic period in response to DNA damage (► DNA Damage Responses) and is somewhat different from other checkpoints in two ways. First, in the classic paradigm, cell-cycle progression is halted at various *discrete* points in the cycle (e.g., in G_1, at the G_2/M transition, and during mitosis), and cells are usually arrested for many hours prior to reentry into the cycle. In contrast, the S-phase damage-sensing pathway arrests cell-cycle progression for only an hour or so and seemingly at any position within the S-phase, therefore, it is difficult to think of it as a checkpoint per se (although we will refer to it as such in this review). As more is learned about

the molecular basis of all of the checkpoints, it is becoming clear that each may have multiple targets. Thus, the underlying strategies for mobilizing these vital damage-sensing pathways may turn out to have many similarities.

A second possible difference is that the classic G_1, G_2/M, and mitotic checkpoints are thought to serve an anticipatory or surveillance function by arresting cells *prior to* critical processes such as DNA replication or mitosis, both of which are capable of converting potentially repairable damage into catastrophic lesions, such as double-strand breaks or chromosome nondisjunction. However, there is little evidence to suggest that the classic S-phase damage-sensing pathway has an anticipatory function, and it is formally possible that the arrest of DNA synthesis in response to damage results from a competition for *trans*-acting factors between multiple DNA-templated responses (repair, transcription, and replication).

Characteristics

At what point(s) in S-phase does the S-phase checkpoint operate? It has been known for decades that when exponentially growing cells are subjected to ionizing radiation, DNA synthesis is rapidly and significantly inhibited. Compelling evidence has been obtained that damage-induced inhibition of DNA synthesis represents a *bona fide* damage-sensing signal transduction pathway, as opposed to a simple arrest of replication forks by damage in the template (i.e., a *cis*-acting mechanism). For example, a dose of 5 Gy of gamma radiation results in a single-strand break every 25 replicons on average, yet inhibits overall DNA synthesis by ~50%. Therefore, it has been presumed for years that the S-phase checkpoint is a global cellular response that functions in trans and inhibits initiation of DNA replication as opposed to chain elongation. Indeed, there is direct experimental evidence from studies on mammalian viruses that doses of ionizing radiation too small to damage individual viral templates nevertheless downregulate viral replication, suggesting a *trans*-regulatory mechanism. Results from three different experimental systems also provide direct evidence that the S-phase checkpoint downregulates DNA synthesis by inhibiting initiation ► radiation sensitivity. Alkaline sucrose gradient analysis of pulse-labeled DNA showed that ionizing radiation inhibits the appearance and maturation of the smallest nascent fragments to a much greater extent than the maturation of larger fragments. Secondly, DNA fiber autoradiographic analysis of the changes in the pattern of replication after irradiation suggested that initiation is preferentially inhibited. Thirdly, a ► 2-dimensional gel replicon mapping approach that can distinguish between initiation and elongation of DNA synthesis, showed clearly that ionizing radiation preferentially downregulates initiation in a defined chromosomal replicon (the amplified, early-firing dihydrofolate reductase domain). To determine whether damage-induced inhibition of initiation also occurs at origins that fire later in the S-period, advantage was taken of the naturally amplified ribosomal gene cluster, in which a restriction site polymorphism distinguishes between early- and late-firing rDNA replicons. The 2-D gel replicon mapping approach showed that both the early- and late-firing origins were inhibited by ionizing radiation. By extension, it is likely that all origins in mammalian chromosomes will be inhibited in response to DNA damage, regardless of when they are activated.

An interesting recent observation suggests that there may be more than one checkpoint or pathway responsible for the overall inhibition of the rate of DNA synthesis that occurs when ionizing radiation is delivered to an asynchronous culture of growing cells. When cells are synchronized and replicate cultures are irradiated at hourly intervals during S-phase, the subsequent inhibition of DNA synthesis never exceeds more than about 25% at any time in the S-period, whereas DNA damage delivered to an asynchronous population results in 50% or greater inhibition of the overall rate of DNA replication. By careful fluorescence-activated flow sorter analysis of gamma-irradiated log cultures, it was possible to demonstrate the existence of a population of late G_1 cells whose entry into S-phase was prevented for several hours. Since this potentially new checkpoint was uncovered in CHO cells, which are deficient in p53 activity ► p53 Family, it is distinct from the well-known p53-mediated G_1 checkpoint. This pathway is therefore analogous to a G_1/S checkpoint that has been described in *Saccharomyces cerevisiae*, which acts between the cell-cycle steps affected by the DBF4 and CDC7 gene products.

It is possible that downregulation of entry into the S-phase in mammalian cells (i.e., at the G_1/S

transition) functions by inhibiting the earliest S-phase origins and is therefore mechanistically related to damage-induced inhibition of late-firing origins (i.e., does not differ from the S-phase checkpoint per se). Since irradiation delays the entire S-period for several hours (i.e., it effectively repositions the S-phase along the cell-cycle time axis), it would have to be argued that late-firing origins cannot fire until early-firing origins have done so. Interestingly, however, cells arrested near the G_1/S boundary in either mimosine or aphidicolin (both effective inhibitors of chain elongation) are resistant to radiation-induced inhibition of DNA synthesis, lending weight to the argument that there is a unique damage-sensing pathway that operates at the $G/_1S$ transition *prior to* origin firing. Since the events preceding initiation of DNA synthesis in mammalian cells are only now being characterized, it will be some time before the molecular nature of this potential new checkpoint is uncovered.

What proteins are involved in the S-phase damage-sensing checkpoint? The ataxia-telangiectasia mutated (► ATM) (► ATM Protein) and the ATM Rad3-related (ATR) protein kinases are critical checkpoint factors in human cells. Both are members of the phosphatidylinositol 3 kinase-related family of protein kinases (PI3K) that play a major role in sensing damage and triggering repair of DNA lesions in mammalian cells. ATM and ATR are preferentially activated by different forms of DNA damage. ATR is activated following induction of DNA adducts by UV or by chemical cross-linking agents, whereas ATM is activated in response to double-strand DNA breaks induced by irradiation, etc.

The ATM gene was cloned in 1995. Cells derived from patients with ► ataxia-telangiectasia (AT) fail to inhibit DNA synthesis in response to irradiation. This phenomenon was termed "► radiation-resistant DNA synthesis (RDS)," which reflects a failure of intra S-phase checkpoint control. ATM is thought to play a central role in the cellular responses (including the S-phase checkpoint) to DNA double-strand breaks (DSBs) and other genotoxic stresses. ATM exists as a catalytically inactive dimer in the absence of DNA damage. In response to DNA damage, especially DSBs, ATM undergoes rapid autophosphorylation on serine 1981, resulting in dissociation of the inactive dimers to yield active monomers. However, when the 1987 site was mutated (the mouse equivalent of serine 1981) and expressed in mice as the sole ATM species, the mutated ATM kinase was activated by radiation, suggesting that an alternative mode for stimulation of the ATM kinase must exist. The fact that several different serine/threonine phosphatases type 2 (PPP2) alpha isoform and type 5 (PPP5) regulate ATM activity, raises the question whether additional phosphorylation sites exist in the ATM protein that regulate its activity. In addition to DNA damage, chromatin-remodeling molecules are also able to induce rapid activation of ATM/ATR in the absence of detectable DNA strand breakage. The activation of ATM/ATR by DNA strand breakage might thus be mediated, at least in part, by a change in chromatin structure.

In 2000, the ► Seckel syndrome (an autosomal recessive disorder characterized by developmental abnormalities including growth retardation and microcephaly) gene was localized to human chromosome 3q22.1-q24 (SCKL1) by homozygosity mapping. This gene has subsequently been identified as ATR. ATR is thought to be activated by both stalled replication forks and bulky adducts produced by agents such as Mitomycin C. ATR function in cell-cycle checkpoint signaling pathways is dependent on an ATR-interacting protein known as ATRIP. ATRIP contains domains similar to the ATM-binding domain of NBS1, which is required for the interaction of ATRIP with ATR. ATR and ATRIP both localize to intranuclear foci after DNA damage or inhibition of replication. ATRIP is phosphorylated by and interacts with the single-stranded DNA (ssDNA) binding protein RPA. RPA coats ssDNA at replication forks to form an RPA–ssDNA complex in response to damage. Deletion of ATR in mice mediated by the Cre recombinase causes the loss of both ATR and ATRIP expression, the loss of DNA damage checkpoint responses, and cell death. Depletion of RPA inhibits ATR association with chromatin and abrogates the aphidicolin-induced DNA replication checkpoint. Moreover, RPA is also required for activation of ATR-mediated phosphorylation of Chk1 and Rad17.

► Nijmegan breakage syndrome (NBS) is a recessive genetic disorder characterized by elevated sensitivity to ionizing radiation, chromosome instability, and a high frequency of malignancies. Since cellular features partially overlap with those of ataxia-telangiectasia (A-T), NBS was long considered an A-T clinical variant. Nbs1, the product of the gene underlying the disease, contains three functional regions: a forkhead-associated (FHA) domain, a BRCA1

C-terminal (BRCT) domain at the N-terminus, an Mre11-binding region at the C-terminus, and several SQ motifs (consensus phosphorylation sites by ATM and ATR kinases) in the central region. Nbs1 forms a multimeric complex with hMre11/hRad50, which is recruited to the vicinity of DSBs sites by direct binding to histone H2AX via the FHA and BRCT domains of Nbs1. Recent studies suggest that the Mre11/Rad50/Nbs1 (MRN) complex, and possibly other proteins, play a role in the recruitment of ATM to the region of DNA strand breaks. In addition, BRCA1 (► BRCA1/BRCA2 germline mutations and Breast Cancer Risk) appears to be necessary for recruitment of ATM to damage foci. In response to IR, ATM fails to localize to damaged sites in the cells lacking full-length BRAC1 or full-length NBS1 despite the fact that ATM is activated. Once ATM is recruited to damage sites it can phosphorylate substrates at the break, including Nbs1, 53BP1, BRAC1, H2AX, and Smc1. Smc1 is a member of the ► Structural Maintenance of Chromosomes Protein (SMC) (family of proteins) and is thought to enhance the efficiency of DSB repair. Nbs1 therefore acts both as an upstream modulator of ATM as well as a downstream target of ATM.

In response to different genotoxic stresses, ATM and ATR transduce the damage signal to checkpoint control proteins to activate checkpoints. There are multiple parallel pathways underlying the intra S-phase checkpoint. The first pathway involves the ATM/ATR-Chk1/Chk2-Cdc25A-Cyclin E(A)/Cdk2-Cdc45 cascade, which links the upstream checkpoint kinases with the core cell-cycle machinery in S-phase cells. DNA-damage-activated ATM and/or ATR phosphorylate Chk1 (serines 317 and 345) and Chk2 (threonine 68). Chk1 and Chk2 inhibit Cdc25A activity by phosphorylation of its serine 123 residue followed by ubiquitin-mediated degradation. Cdc25A is a dual-specificity phosphatase, removing inhibitory phosphates from threonine 14 and tyrosine 15 from Cdk2. Thus, Cdk2 is inhibited, resulting in decreased phosphorylation of Cdc45. This prevents its association with chromatin and thereby decreases initiation of DNA replication at origins.

Another S-phase pathway involves the ATM-Nbs1-Smc1 cascade. Following IR, the MRN/Smc1 complex is recruited to sites of DSBs. Once ATM is recruited to the complex, it phosphorylates Nbs1 on serine 278 and serine 343. Mutation of these residues results in an S-phase checkpoint defect following ionizing radiation. In addition, phosphorylation of the protein Smc1 on Serines 957/966 by ATM is also necessary for activation of the S-phase checkpoint. The ATM-Nbs1-Smc1 axis is clearly distinct from the ATM/Chk2/Cdc25A pathway.

► Fanconi anemia (FA) is a recessive genetic disease characterized by cellular hypersensitivity to DNA interstrand cross-linking agents, mild sensitivity to other genotoxic agents, and clinical features that overlap with NBS, A-T, and ATR-Seckel syndrome. Phosphorylation of serine 222 of Fanconi anemia protein D2 (FANCD2) by ATM is dependent on Nbs1 and is necessary for the IR-activated S-phase checkpoint. Since the ATM-FANCD2 pathway apparently acts independently of SMC1 phosphorylation, its downstream effect is presently unknown.

What are the target(s) of the S-phase checkpoint? Precise regulation of the initiation of DNA synthesis is critical, since it ensures that the genome is replicated once and only once per cell cycle. Much of what we know about the regulation of initiation at origins comes from yeast. Autonomously replicating sequence (ARS) elements were first identified in a functional assay by virtue of their ability to support plasmid replication. In *Saccharomyces cerevisiae*, it is now known that a stepwise assembly of proteins onto origins precedes origin firing. In yeast, a multi-protein complex (the ► origin replication complex (ORC)) has been isolated and shown to interact with ARS elements. ORC is comprised of six proteins and remains bound to yeast origins throughout the cell cycle. During M-phase, the cdc6 protein is recruited to ORC ► Replication Licensing System, which, in turn, recruits ► minichromosome maintenance (MCM) proteins to form the pre-replicative complex (pre-RC) on origins. An S-phase cyclin is then thought to be necessary for the association of the cdc45 protein with the pre-RC just prior to the onset of DNA synthesis. Several other proteins such as Cdt1, Gins, and MCM10 play critical but poorly understood roles in effecting initiation at origins. Origin firing is likely downregulated through MCM2 phosphorylation, which itself is regulated by checkpoint pathways signaling through cdc45.

What is the biological significance of the S-phase checkpoint? Cell-cycle checkpoints by definition provide an adaptive cellular advantage following genotoxic stress. In the case of the S-phase checkpoint, radiation sensitivity has been uncoupled from

checkpoint function, arguing that the S-phase checkpoint may not always function to enhance survival following DNA damage. However, these experiments were performed on AT or AT-like cells in which it is not possible to exclude other mutations that are epistatic to the checkpoint defect. Furthermore, ATM likely directly influences DNA repair possibly *via* SMC1. Another possibility is that the S-phase checkpoint functions to maintain genomic stability. Irradiation produces single-strand DNA breaks, and replication through single-strand breaks has the potential to produce double-strand lesions that, if not correctly repaired, can lead to chromosomal rearrangements and carcinogenesis. An interesting further possibility is that the S-phase checkpoint functions in normal cell division to cope with endogenous oxidative or other genotoxic stresses. Future work will clarify these questions.

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S-Phase Kinase-associated Protein 2

► Ubiquitin Ligase SCF-Skp2

S-Phase Promoting Factor

Definition

SPF; has cyclin-dependent kinase activity that is capable of inducing the initiation of replication.

► Replication Licensing System

Spa-1

► Sipa-1

Spheroids

► Multicellular Spheroids

Sphinganine

Definition

Dihydrosphingosine, the basis of the minor dihydrosphingolipids, generally inactive in cell processes.

► Sphingolipid Metabolism

Sphingolipid Metabolism

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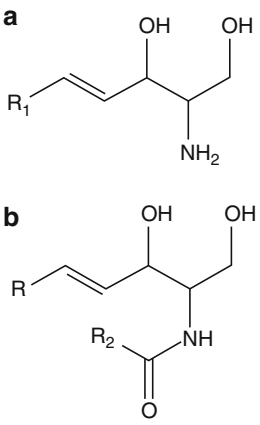
Definition

► Sphingolipids (► SLDs) contain ► sphingosine (Sph) or a similar moiety. Sphingosine (Fig. 1a) is D-erythro-*trans*-4-octadecene-2-amino-1,3-diol; R_1 is typically a C_{13} alkyl chain. ► Ceramide (Fig. 1b) is a fatty acid amide of sphingosine that plays a pivotal role in cell growth and death, in which R_2 symbolizes a saturated or monoenoic fatty acid containing 16–32 or more carbon atoms. Some fatty acids have an OH at carbon-2. Some ceramides contain phytosphingosine instead of Sph; here the double bond is replaced by an OH at C-4.

Hundreds of SLD species occur. The C-1 OH can be phosphorylated to form Sph phosphate or Cer



Sphingolipid Metabolism. Fig. 1 Sketch (**a**) shows sphinogosine and (**b**) shows aramide. R_1 represents a tridecyl alkyl chain of a saturated or monounsaturated fatty acid

phosphate, or esterified by a phosphocholine group to form ▶ sphingomyelin (▶ SM), the most common SLD. The OH can also be coupled to a sugar, usually galactose or glucose, to form GalCer or β-glucosylceramide (▶ GlcCer). GalCer is a major component of human brain; a portion of the lipid has a sulfate group attached to the sugar (▶ Sulfatide). GlcCer, the primary glucosphingolipid (▶ GSL), can add other sugar moieties to the glucose, particularly galactose, N-Ac-galactosamine, and N-acetylneuraminic acid (a characteristic moiety of the ganglioside series of GSLs).

Characteristics

Sphingolipids control the properties of cell membranes, the rate of cell growth–proliferation–destruction, apoptotic processes, the phosphorylation and dephosphorylation of proteins, the formation of reactive oxygen species (ROS), the hydrolysis of some proteins, the acetylation of nuclear histones, the binding of microbial pathogens and toxins to human cells, ▶ angiogenesis, telomerase, matrix metalloproteinase, cytosolic and mitochondrial ▶ glutathione level, and other cancer-relevant factors. Cancer cells appear to synthesize SLDs somewhat faster than normal cells and are more sensitive to SLD manipulation, even in multidrug-resistant cells. This difference in sensitivity bodes well for the design of a drug with a good therapeutic index.

Some SLDs (Cer, ▶ Gangliosides GM3 and GD3) induce ▶ apoptosis – especially in cancer cells – while other SLDs (Sph phosphate, Cer phosphate, GlcCer, GalGlcCer) promote growth and proliferation and *prevent* apoptosis. Since these two types of lipids can be interconverted by the enzymes that make and hydrolyze them, it is evident that cells must maintain a balance in their concentrations and activities. One or more controlling factors are missing from cancer cells, resulting in unrestrained growth and a high rate of DNA change. Cancer cells contain unusual assortments of SLDs, with dominance of the proliferative lipids. The essence of cancer chemotheraphy appears – from this viewpoint – to be the targeting of these lipids, their enzymes, and the factors that control their levels in tumors.

While SLDs occur almost everywhere in cells, there are special aggregates in plasma cell membranes, called ▶ rafts (GSL- and cholesterol-enriched lipoid/protein microdomains). The rafts function as signaling platforms for important cytokines and modification of their compositions can affect many of their functions. Depletion or augmentation of cellular SLDs leads to a remarkable number of important phenomena.

Poly-drug Chemotherapy and Multidrug Resistance

Because of the unusual sphingolipid Yin/Yang, Janus-faced metabolism, which promotes *and* blocks cell death, cancer therapy calls for the use of a poly-drug cocktail that stimulates the formation of Cer, ganglioside GM3, and GD3, while simultaneously inhibiting synthesis of the proliferative SLDs. The simultaneity is important because manipulation of just one of these lipid clusters leads the cancer cell to induce changes in the other cluster and proliferative factors. This response eventually leads to renewed tumor growth and resistance to the drugs that were used initially. A similar selection occurs when the initially attacked cancer cells mutate to form cells with a more resistant imbalance in SLD composition. Thus, treating tumors with a single, first-line drug can lead to clones that are resistant to the drug and the apparent cure turns out to be illusory. Evidently a cocktail is needed to cover all or most phases of SLD metabolism in an all-out attack, to kill all the cancer clones present in each individual patient. Preliminary trials of the poly-drug approach have indicated that the dose size of each component can be lower than the single-drug approach (i.e., synergistic), so side effects can be expected to be minimized.

Chemotherapy via Ceramide Control

Cer levels can be elevated by the following approaches. Some of the drugs listed are not – or need not be – approved by the FDA.

1. *Stimulate the first step in de novo synthesis of Cer* from serine and palmitic acid, formation of 3-keto ► sphinganine, via pyridoxal phosphate as cofactor. Exogenous palmitate and serine are effective and supplementary pyridoxine may also help. Dietary carnitine, which helps mitochondria destroy the fatty acid, should be avoided. The apoptogenic effect of palmitate is augmented by etomoxir, which inhibits transport of fatty acid by carnitine. Several anticancer drugs (fenretinide, camptothecin, etoposide, paclitaxel, retinoic acid, tetrahydrocannabinol, and valspodar) stimulate the de novo synthesis of Cer.

The next steps in Cer synthesis are reduction of the carbonyl oxygen, forming sphinganine, and acylation of the amine group, forming dihydroCer. This somewhat inert analog of Cer is next dehydrogenated to form Cer, producing a double bond between carbons 4 and 5. The crucial segment of Cer is the ► allylic structure, with the C-3 OH and the Δ^4 double bond. Fumonisin, a fungal toxin commonly found in significant concentrations in some foods, inhibits the acylation step and should be avoided via good food handling practices. It also inhibits acylation of free Sph (see below).

1. *Minimize the conversion of Cer to SM* (► Sphingomyelin), an exchange of the C-1 OH for the phosphocholine moiety of phosphatidylcholine (lecithin) with formation of diacylglycerol. A similar reaction utilizes phosphatidylethanolamine, followed by N-methylation. The enzyme, SM synthase, as well as lecithin, has been found in high concentrations in some tumors, suggesting that they protect themselves against Cer toxicity this way. It is possible to lower the body content of lecithin by restricting the intake of fatty foods. The anticancer phospholipid analog, hexadecylphosphocholine, which inhibits lecithin synthesis, also slows SM synthesis and speeds Cer synthesis. Thus, the various anticancer phospholipid analogs may owe some of their effectiveness to this mechanism. It follows from this also that drugs emulsified with the aid of lecithin should be reformulated. Tamoxifen, which inhibits diversion of Cer by glucosylation, also stimulates phospholipases C and D, and thus also lowers tumor lecithin.
2. *Stimulate SM hydrolysis*, which forms Cer + phosphocholine. The major SMases, Mg^{2+}-requiring neutral SMase and an acidic SMase, are normally somewhat inhibited by mitochondrial glutathione (GSH). Thus an agent that lowers GSH levels will greatly elevate Cer level. Radiation, oxidation by ROS, condensation catalysis by glutathionyl S-transferase, inhibition of GSH biosynthesis (buthionine sulfoximine), and Michael reaction of GSH with anticancer drugs that contain allylic ketone moieties (doxorubicin, camptothecin, tetracycline, a keto metabolite of fenretinide, manumycin, actinomycin D, curcumin, quercetin, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, illudin, gossypolone, ciprofloxacin, 17AAG (a geldanomycin analog), and others) act this way to produce much of their anticancer activity.

Other agents lower GSH levels in tumors. Cisplatin and dietary thiocyanates (sulforaphane in broccoli) react chemically with GSH. Sulforaphane also induces glutathione S-transferase activity. Cer and many antineoplastic drugs generate ROS in cells, thus raise the sulfur atom to more oxidized states. Even modest oxidation of GSH to form disulfides and mixed disulfides with the cysteine moieties of proteins unmasks the power of SM to form Cer. Many metabolites and nutrients generate ROS in mitochondria in the normal course of oxidative metabolism. Normally the ROS are kept under control by antioxidants. GSH is needed for important enzyme reactions, so its level should not be reduced too far.

The hydrolysis of SM is stimulated by arachidonic acid, a common dietary fatty acid that is converted to prostaglandins. Thus its use would have to be controlled by the use of COX inhibitors. Thalidomide inhibits angiogenesis by stimulating the synthesis of Cer from SM.

1. *Inhibit diversion of Cer to formation of GlcCer* by treatment with D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (► PDMP), N-cadherin, dietary glucose deprivation, chlorpromazine, tamoxifen, mifepristone, anti-androgens, ketoconazole, irinotecan, doxorubicin, dihydroxy vitamin D3, mitoxanthrone, dexamethasone, arabinofuranosylcytosine, and others. PDMP and its analogs PPMP

and PPPP produce Cer in more than one way. GlcCer and its anabolite, GalGlcCer, are growth stimulators and promoters of P-gp (MDR1), a major cause of ▶ multidrug resistance; thus inhibiting Cer glucosylation is important in several ways. It is possible that food intake reduction, which prolongs life, owes some of its effectiveness to depletion of glucose and UDP-glucose, the co-substrate in GlcCer synthesis.

In addition, GlcCer and GalGlcCer are the precursors of the gangliosides, which prevent the normal action of ▶ dendritic cells, the major first defense in immunological attack on cancer cells. Tumors shed a significant portion of their rapidly synthesized gangliosides into the extracellular fluid around the cancer cells. The mechanism by which these lipids inhibit dendritic cell development is not clear, possibly by inhibiting Cer glucosylation. The ▶ ganglioside secretion or shedding also promotes angiogenesis, a vital tool of growing tumors. Thus it seems essential to prevent GSL synthesis so that the patient's immune system can make a final clearing of cancer cells. The accumulated Cer prevents angiogenesis.

A further advantage of inhibiting GlcCer synthesis is that it depletes cell surfaces of the GSLs that act as binding sites for many microbial organisms, including viruses. Alternatively, mimics of the surface GSLs can be used to compete with the cells for binding to infective agents. Thus the complication of ▶ infection in cancer patients would be minimized.

Patients with ▶ Gaucher disease, who have a genetic deficiency in the amount of GlcCer hydrolase, are currently being treated by injection of a modified form of the human glucosidase. The enzyme lowers tissue GlcCer levels, forming Cer and glucose, thus should be helpful in cancer patients. A similar effect can be expected by injection of a small peptide that is needed for the glucosidase's activity, saposin C.

1. *Inhibit Cer hydrolysis*, which forms Sph + fatty acid, with D-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol (▶ D-MAPP), N-oleoyl ethanolamine, and N-octadecylSph. Blocking ceramidase seems to be an effective way of elevating Cer and inducing apoptosis. Reacylation of the Sph to regenerate Cer is performed by an acyltransferase produced from the longevity assurance gene.
2. *Inhibit the kinase that converts free Sph* to its 1-phosphate ester (▶ S1P). This ester competes with Cer in controlling a cell's fate, so the *ratio* of the two lipids is critical for rebalancing the SLDs. N,N-dimethyl sphingosine, trimethylSph, and glabridin (an allylic ether in licorice) are suitable inhibitors. DimethylSph is a normal metabolite of sphingosine, but its concentration seems to be too low in tumors.
3. *Chemotherapy with dietary Cer* has shown beneficial effects in intestinal cancer. However it has to be included as part of the total poly-drug approach in order to prevent conversion of the extra Cer to proproliferative SLDs. Dietary SM is converted to Cer in the intestine and also has a beneficial effect; it can be considered a pro-drug. Since both lipids and GSLs occur in food, it is likely that they constitute a natural cancer-preventing mixture. SM has been used as part of a liposomal cocktail for dispersing water-insoluble anticancer drugs. A solubilizing chain of poly(ethyleneglycol), attached to Cer by a dicarboxy acid bridge, may solve the problem of administering so insoluble a lipid. It may also be useful for forming a liposomal suspension with SM.

In recent years, analogs of Cer have been synthesized that are more active than Cer in the induction of apoptosis. Adding an additional double bond, conjugated with the allylic double bond of Cer, improved the lipid considerably. It is significant that some antineoplastic drugs also have an allylic alcohol group that is part of a chain of conjugated bonds. A Cer analog with a pyridinium group in the fatty acid moiety was found to concentrate in tumors and prevent their growth in vivo. Its effectiveness was greatly enhanced by including gemcitabine, an antineoplastic drug that produces Cer elevation.

Immunological Attack Against Cancer Cells

Some, perhaps many, tumors consist of cells with an unusually high concentration of rare GSLs. Indeed, the ratio of Cer to GSLs decreases as the tumor malignancy increases. Unfortunately, SLDs are not potent inducers of Ab induction (see dendritic cells above) and special techniques had to be developed. Tumors of neuroectodermal origin (e.g., melanoma and neuroblastoma) are being tested with a mouse/human chimeric mAb against ganglioside GD2, a prominent GSL in these cells. Promising results have also been found by active immunization with idiotypic peptides that mimic gangliosides. Pathogens containing

idiotypes that resemble GSLs sometimes evoke neuropathological autoantibodies, as in Guillain–Barré syndrome.

α-Linked galactose in GalCer (not the usual β-GalCer) is a potent stimulator of NKT cells and a killer of cancer cells. This finding is under active study.

Mechanisms of Sphingolipid Antineoplastic Action

1. Since addition of Cer to whole cells or mitochondria produces ROS and apoptosis, the proposal has been made that the allylic OH of Cer is oxidized to an allylic ketone (keto Cer). Presumably the latter is the therapeutically active form of Cer, since it can undergo adduct formation with GSH, other thiols, and active amines. Antineoplastic drugs that contain an allylic alcohol, ester, or ether moiety may mimic Cer, forming ROS, allylic ketones, and Michael adducts. Cer – and several anticancer drugs – interfere with the mitochondrial electron-transporting oxido/reduction cycle of ubiquinone/ubiquinol that normally generates ATP. Ubiquinone is an allylic ketone, thus is likely to oxidize allylic alcohols.
2. Cer can generate pores in mitochondrial membranes, allowing cytochrome *c* and other components to escape into the cytoplasm where they activate the ▶ caspases and initiate the apoptogenic death sequence. The pores, called barrels, seem to consist of assembled Cer molecules, lined up with their polar (OH) end facing the inside. A certain minimum amount of Cer must be accumulated in the mitochondria to generate one pore. DihydroCer (lacking the double bond) is inert.
3. Cer and Sph activate *kinases* that act on important proteins (e.g., PKC ζ involved in the control of apoptosis and many other phenomena). Cer also activates ▶ *phosphatases* (e.g., PP1 and PP2A) that remove phosphate groups from many proteins (e.g., ▶ Akt). These effects are seen with synthetic anticancer drugs too. Generally, sphinganine-based SLDs are inactive in these activations so the presence of the allylic polar moiety seems to be essential. The mechanism of activation could be ascribed to binding of the active lipid to an allosteric region of the enzymes. However, there is also the recently proposed idea that the SLD binds to the active site of the phosphate-transferring region, with the allylic alcohol moiety forming a transitional phosphate ester, functioning as an anion-transferring coenzyme for the catalytic processes.

These kinases and phosphatases control the activity of many phosphoproteins that control apoptosis and many other processes. For example, the Cer-activated phosphatase, PP2A, dephosphorylates and thereby inactivates μ- and m-calpains, leading to suppression of the migration and invasion properties of human lung cancer cells.

1. Cer is involved in other anion-transferring reactions of great importance to cancer therapy. It reduces N-acetylation of protein-linked lysine and is involved in ▶ histone acetylation. It binds to and activates ▶ cathepsin D, the acid aspartate protease that triggers the apoptotic program by activating Bid. The aspartate COOH in the enzyme's substrate may form a transient ester link with Cer during the peptide cleavage.

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Sphingolipids

Definition

Are a class of lipids derived from the aliphatic amino alcohol sphingosine. The sphingosine backbone is O-linked to a (usually) charged head group such as ethanolamine, serine, or choline. The backbone is also amide-linked to a fatty acid. Sphingolipids are often found in neural tissue and play an important role in both signal transmission and cell recognition. There are three main types of sphingolipids: ceramides, sphingomyelins, and glycosphingolipids, which differ in the substituents on their head group.

Ceramides are the simplest type of sphingolipid. They consist of a fatty acid chain attached through an amide linkage to sphingosine. Sphingomyelins have a phosphorylcholine or phosphoroethanolamine molecule esterified to the 1-hydroxy group of a ceramide. Glycosphingolipids are ceramides with one or more sugar residues and are called gangliosides when they carry three sugars, however, one of which must be sialic acid. Phosphorylated forms of sphingosine and ► ceramide, namely, sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P) are involved in cell survival, angiogenesis, and tumorigenesis.

► Lipid Mediators
► Sphingolipid Metabolism

Sphingomyelinase

Definition

Sphingomyelin-phosphodiesterase 1 (gene symbol: *Smpd1*) hydrolyses sphingomyelin to ► ceramide. Maximum enzyme activity is at an acidic pH.

Sphingomyelins

Definition

Are sphingolipids that possess a phosphorylcholine or phosphoroethanolamine molecule esterified to the 1-hydroxy group of a ► ceramide.

► Lipid Mediators

Sphingosine

Definition

Is an aliphatic amino alcohol that composes sphingolipids.

► Lipid Mediators

Sphingosine-1-Phosphate

Definition

S1P; is a phosphorylated form of sphingosine and possesses tumorigenic properties.

► Lipid Mediators

Spinal and Bulber Muscular Atrophy

Synonyms

SBMA

► Androgen Receptor

Spindle Apparatus

Synonyms

Spindle Pole Apparatus

Spindle Assembly Checkpoint

Synonyms

SAC

Definition

Regulates metaphase-to-anaphase transition of the ► cell cycle in order to ensure that chromosomes do not segregate until each is properly attached by microtubules to bilateral spindle poles during mitosis and meiosis.

► Checkpoint
► Mitotic Arrest-Deficient Protein 1

Spindle Pole Apparatus

Definition

Develops during the ► cell cycle. It is a highly organized structure that consists of kinetochore microtubules attached to segregating chromatids, and polar microtubules moving apart the spindle poles immediately prior to cell division. Independent changes during G_2/M culminate in the formation of the spindle pole apparatus (► G_2/M Transition). It consists of two opposed poles each arising from one of the two duplicated centrosomes. The bisecting line at which microtubules emanating from each pole of the spindle meet is known as the metaphase plate. Pairs of chromosomes align and attach themselves along the metaphase plate and are then pulled apart from one another by motors that push and pull the separated chromosomes toward opposite poles of the cell. Formation of a spindle pole apparatus requires a change in microtubule dynamics. This is brought about by changes in the phosphorylation states of structural and motor microtubule-associated proteins (MAPs); (not to be confused with mitogen-activated protein kinase, MAP kinase!). Cyclin-dependent kinase 1 (CDK1) is known to phosphorylate many MAPs.

► G_2/M Transition

Spindle Pole Body

► Centrosome

Spinocerebellar Ataxia Type 1

Definition

SCA1; is an autosomal dominant progressive neurodegenerative disorder characterized by ataxia, dysarthria, ophthalmoparesis, and variable degrees of amyotrophy and neuropathy. The disease causing mutation is an expansion of a CAG trinucleotide repeat which lies within the coding region of ataxin-1.

► Cajal Bodies

Spinophilin

Synonyms

Neurabin II

Definition

Is ubiquitously expressed and interacts with protein phosphatase 1 (PP1), an alternate reading frame (p14ARF, a 14 kDa polypeptide in humans, 19 kDa in mouse), doublecortin and actin, and synergistically suppress osteosarcoma tumor growth with p14ARF.

► Doublecortin

Spirometry

Definition

A physiologic test that measures inhaled and exhaled volumes of air independently and as a function of time.

► Chronic Obstructive Pulmonary Disease and Lung Cancer

Spleen

Definition

A lymphoid organ in the abdominal cavity that is an important center for immune system activities.

Spleen Tyrosine Kinase

► Syk Tyrosine Kinase

Splenic Marginal Zone Lymphoma

Definition

Indolent B-cell neoplasm, composed of small lymphocytes, that is marked by massive splenomegaly and

peripheral blood and bone marrow involvement, usually without adenopathy.

► B-Cell Tumors
► Rituximab

Splice Variant

Definition

Alternative ► splicing of introns and exons on pre-messenger RNA (mRNA) creates variants of mature mRNA from the same gene, allowing for different forms of the final protein produced.

Spliceosome

Definition

A large multi-protein and snRNA complex that mediates the catalytic steps of the splicing reaction.

► Pre-mRNA Splicing

Splicing

Definition

The process by which introns are removed from an RNA transcript.

► Pre-mRNA Splicing

Splicing Variants

Result from ► alternative splicing

Sp-Like Proteins

Definition

Proteins related to Sp-1 (specific protein-1), the first transcription factor identified. Sp-like proteins contain a C_2H_2-type zinc finger that binds to GC/GT-rich DNA elements. Sp1–Sp4 are four different full length members of the Sp-like protein family, while Sp5–Sp8 seem to be truncated forms of Sp1–Sp4.

► Parathyroid Hormone-Related Protein

SPM

► Second Primary Malignancy

Spontaneous Bacterial Peritonitis

Definition

Is characterized by the spontaneous infection of ascitic fluid in the absence of an intraabdominal source of infection involving the translocation from bacteria from the intestinal lumen to the lymph nodes with subsequent bacteremia and infection of ascitic fluid.

► Ascites

Sporadic

Definition

Referring to gene mutation; a term frequently used as synonym for non-hereditary.

SPRM

Definition

Selective ► progesterone receptor modulator.

► Mifepristone

Sprouty

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Synonyms

Spry

Definition

Sprouty (Spry) proteins are a family of endogenous proteins that negatively regulate the ▶ ERK/MAP kinase (ERK/MAPK) signaling pathway (Fig. 1) that is activated by ▶ Receptor Tyrosine Kinases (RTKs).

Characteristics

Sprouty was initially discovered as an inhibitor of ▶ fibroblast growth factor (FGF) signaling during tracheal development. It was involved in modulating branching in tracheal formation in the fly (Drosophila) and its absence led to excessive "sprouting" of tracheal tubules. As in the fly, mammalian Spry proteins work as feedback inhibitors of RTK signaling during ▶ branching morphogenesis of the lung, vascular system, kidney tubules, and breast ducts.

To date, four mammalian Sprys (Spry1–4) have been identified with sequence similarity to the *Drosophila* protein (Fig. 2). These are expressed in the brain, heart, lung, kidney, limbs, and skeletal muscle. The Spry proteins have a highly conserved cysteine-rich C-terminus. The N-terminal half of the Spry proteins are more divergent, however, except for the presence of an invariant tyrosine residue (Y) located in a short, conserved NxYxxxP motif. Many of the inhibitory functions of the Spry proteins are dependent on this residue. Of note, Spry1, 2, and 4 are tyrosine ▶ phosphorylated/phosphorylation in response to RTK stimulation while the ability of growth factors to induce tyrosine phosphorylation of Spry3 is unknown.

Mechanisms of Action

Various studies conducted in the mammalian system confirm Spry proteins as negative regulators of the ERK/MAPK pathway; they differ in their conclusions as to the specific point in the ERK/MAPK pathway at which Spry acts. Spry has been found to interact with regulators of MAP kinase or mainstream components of MAP kinase (Fig. 1) (a comprehensive description can be found in the review by Mason et al. [3]).

Regulation of the Activity of Sproutys

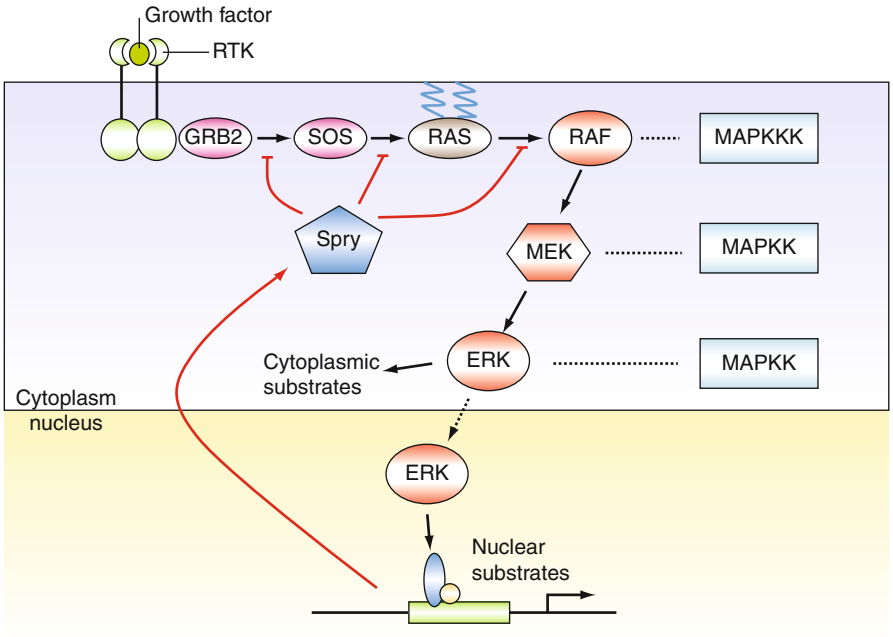
As a negative regulator, Spry is subject to tight control through posttranslational modifications and through regulation of the protein levels in the cell. The activity of Spry proteins is regulated by posttranslational modifications such as serine and tyrosine phosphorylation, palmitoylation, and ▶ ubiquitination.

In their N-terminal domain, Spry proteins contain a conserved tyrosine residue (Tyr55 in Spry2) that undergoes phosphorylation in response to growth factor (FGF) and ▶ Epidermal Growth Factor (EGF) stimulation. This tyrosine residue is necessary for Spry proteins to function as inhibitors of FGF signaling. While tyrosine phosphorylation on the conserved tyrosine on Spry is important for its functional activity, it also promotes the binding of the E3 ubiquitin ligase c-Cbl to Spry2. Tyrosine phosphorylation of Y55 on Spry2, or Y53 on Spry1, creates a consensus binding motif [NxY(S/T)xxP] which serves as a binding site for c-Cbl. The interaction of Spry proteins with c-Cbl promotes the ubiquitination and proteasome degradation of Sprys. This pathway has been postulated to serve as a mechanism to control the duration of Spry activity. In addition, several studies have found that serine phosphorylation of Sprys affect the activity of Sprys via influencing the stability and tertiary conformation of Spry proteins.

The activity of Spry proteins is also regulated by controlling the expression of Spry proteins in the cell. Spry proteins are induced by the same RTK signaling pathways they negatively regulate. During vertebrate development, *Spry* genes are often expressed at sites associated with FGF signaling activity. *Sprys* were also observed to be upregulated upon activation of RTK signaling in in vitro cell culture.

Deregulation of Spry Expression in Cancer

The central ERK/MAP kinase pathway is deregulated in many cancers. As Spry proteins are functional

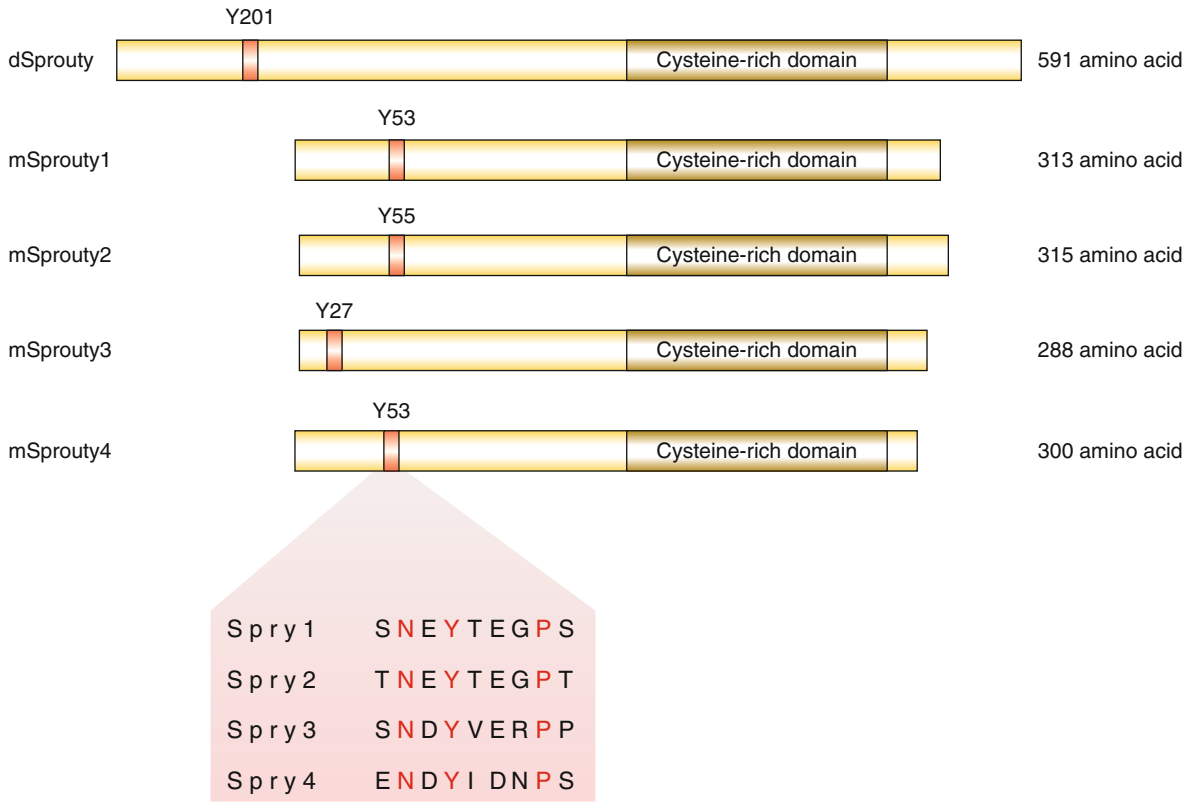


Sprouty. Fig. 1 The ERK/MAP kinase pathway. The ERK/MAPK cascade is known for its crucial role in mediating the transduction of signals from Receptor Tyrosine Kinases (RTKs). Following ligand binding, growth factor RTKs such as ▶ VEGFR and ▶ FGFR become activated. This induces the binding of adapter proteins such as growth-factor-receptor bound-2 (GrB2) that bind to the activated receptors. In cooperation with Grb2, the guanine-nucleotide exchange factor, son-of-sevenless (SOS) activates ▶ Ras. This initiates membrane recruitment and activation of ▶ MAP kinase ▶ Raf, which leads to the activation of the ▶ MAP kinase kinase (MEK) and subsequently, ▶ MAP kinase ERK. ERK phosphorylates several cytoplasmic targets or migrates to the nucleus, where it phosphorylates and activates transcription factors that control the expression of genes that are required for cell growth, differentiation and survival. Various studies conducted in the mammalian system confirm Spry proteins as negative regulators of the ERK/MAPK pathways. Spry proteins are induced by the same RTK signaling pathways they negatively regulate and are observed to be upregulated upon activation of RTK signaling

inhibitors of this pathway their own regulation is likely highly relevant to the status of some cancers. There is emerging evidence that the expression of the Sprouty family of proteins is deregulated in various cancers including breast, liver, prostate, ▶ melanoma, ▶ gastrointestinal stromal tumors (GISTs), and lung tumors. *Spry* genes have been shown to be downregulated in breast, liver, and prostate cancers and upregulated in melanoma, gastrointestinal stromal tumors, and Ras-induced lung tumors.

The upregulation of *Spry* expression in certain tumors (gastrointestinal tumors, melanoma, lung tumors) is often induced by the presence of ▶ Oncogenic lesions (mutation of ▶ c-Kit, ▶ Raf kinase, ▶ Ras) which cause the constitutive activation of the MAP kinase pathways in these tumors. Although numerous oncogenes are also activated in breast, liver, and prostate cancers, the same phenomenon of upregulation of *Spry* genes was not observed. Instead, downregulation of *Spry* genes was observed. The mechanisms of downregulation of Spry genes have not been completely elucidated in these cancers, but at least for prostate cancers, some genetic (loss of parts of chromosomes) and ▶ epigenetic gene silencing by ▶ methylation mechanisms have been identified to be responsible for silencing of Spry genes.

As the upregulation of Sprys in cancers is a reflection of an overactive MAP kinase signaling pathway in cancers, Sprys have the potential to be used as ▶ biomarkers to aid in cancer diagnosis and treatment. Upregulation of *Spry* genes can be used concurrently with other molecular markers to distinguish between different types of tumors. When gene expression patterns of different soft tissue tumors were analyzed, *Spry* genes were found to be specifically expressed in gastrointestinal stromal tumors (GISTs) but not in synovial sarcomas, neural tumors, and leiomyosarcomas. Similarly, *Spry2* expression was



Sprouty. Fig. 2 Structure of Sprouty proteins. Four mammalian Sprys (mSpry1–4) have been identified with sequence similarity to the fly (*Drosophila*) protein (dSpry). Spry proteins have highly conserved cysteine-rich C-termini and invariant tyrosine (Y) residues located in a short, conserved motif. Many of the inhibitory functions of Spry proteins are dependent on this residue

found to be upregulated in melanoma cells with *B-Raf (V599E)* or N-*Ras (Q61R)* mutations but not melanoma cells with wild-type *B-Raf*. Increased MAP kinase activity in these cells was found to contribute to the higher levels of Spry2.

Upregulation of *Spry* genes could also be potentially used concurrently with other biomarkers to aid ▶ prognosis or monitor the response of patients to drug treatment. However, depending on the particular type of cancer, upregulation of *Spry* genes can either be a marker for good or bad clinical prognosis.

Spry4 is shown to be induced by aberrant c-Kit activation in GISTs. Aberrant c-Kit activity derived from activating c-Kit mutations have been shown to be important for development of GISTs. In a study that involved treating GIST cells with an inhibitor of c-Kit, ▶ Imatinib (Gleevec, STI-571), *Spry4* was identified and confirmed as one of the most significant Imatinib-responsive genes that were consistently downregulated upon treatment with Imatinib. Imatinib has been shown to be an in vitro inhibitor of c-Kit phosphorylation and tumor cell ▶ proliferation while inducing ▶ apoptosis in a human GIST cell line. Treatment of GISTs cells with Imatinib resulted in a parallel loss of phosphorylated c-Kit, MAP kinase activation, and *Spry4* levels. *Spry4* was found to be a reliable marker with respect to the clinical response of the patients to Imatinib treatment. In patients responsive to the drug, *Spry4* levels were dramatically decreased. However, in non-responsive patients, *Spry4* levels did not decrease. In patients who initially responded but subsequently relapsed, *Spry4* levels decreased dramatically in the tumor biopsy taken during clinical response but returned to pretreatment levels upon clinical relapse.

Spry2 was also found to be upregulated in mouse lung tumors which are induced by oncogenic K-Ras. Individual lung tumors were isolated from the mice and examined for Spry expression. The degree of Spry2 upregulation correlated with the histological grade of the tumor.

While Sprouty is a potential marker of aberrant MAPK signaling in the above-mentioned cancers, in other cancers it is surprisingly a marker for good clinical prognosis. In a study of the gene expression profiles of ▶ renal carcinoma (clear cell Renal Cell Carcinoma), 51 genes that effectively discriminate between patients with good and poor outcome were isolated. *Spry1* was found to be upregulated exclusively in the good outcome group.

Ability of Sprys to Inhibit Cancer

Numerous in vitro cell-based assays demonstrated that Sprys inhibit cell proliferation, ▶ migration, and ▶ invasion as well as ▶ anchorage-independent cell growth by repressing RTK-induced MAP kinase activation.

Evidence from in vivo animal studies indicates that Spry proteins can act to suppress the tumorigenesis process. The first evidence detailing Sprys' ability to interfere with the tumorigenic process was a study where overexpression of Spry2 in an osteosarcoma cell line was found to inhibit tumor growth and metastasis, possibly via the inhibition of MAP kinase activation and cell migration. In a subsequent animal study, Spry2 was shown to inhibit K-Ras-induced lung tumors in mice. In K-Ras-induced lung tumorigenesis, mice develop lung tumors due to the presence of activated K-Ras in their lung tissue. Spry2 was found to be upregulated in the lung tissue and was found to inhibit the tumor development. Mice with the presence of activated K-Ras in their lung tissue developed greater amount of tumors when the Spry2 expression is abrogated (deletion of Spry2 gene; Spry2 null mice). Analysis of the tumors demonstrated mild increase of MAP kinase activation in tumors in *K-Ras; Spry* null mice compared to *K-Ras; and Spry2* wild-type control mice. This evidence indicates that Spry2 functions to inhibit K-Ras-induced tumor development and that the mechanism may involve antagonism of MAP kinase signaling.

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Spry

▶ Sprouty

Sputum Cytology

Definition

Microscopic examination of a mucus sample to determine if abnormal cells are present.

▶ Malignancy-Associated Changes

Squamous Cell Carcinoma

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Synonyms

Epidermoid carcinoma

Definition

Squamous cell carcinoma is a malignant tumor arising from non-glandular lining or covering epithelia.

Characteristics

Squamous cell carcinoma (SCC) is an invasive tumor which may present at numerous body sites, including skin, ocular epithelium, oral cavity, alimentary tract, anogenital region, larynx, and bronchial epithelium. Thus, the incidence of SCC lesions varies with site.

Estimates from the National Cancer Institute indicate that over one million new cases of skin SCC (▶ Skin Carcinogenesis) will be diagnosed in the USA in 2007, representing 16% of all types of skin cancer. Similarly, approximately 40,000 new cases of head and neck SCC (comprising oral cavity, pharynx, larynx) occur each year. Squamous cell carcinoma is also one variety of ▶ non-small cell lung cancer, the annual incidence of which is 170,000. Anogenital SCC, comprising lesions of cervix, vulva, vagina, penis, and anus, are less frequent. Approximately 23,600 new cases are predicted to occur in the USA each year, of which the majority (12,000) will be ▶ cervical cancers.

Etiology

As squamous cell carcinomas can occur at several body sites, multiple etiological factors have been implicated in development of these lesions. Skin cancer is largely associated with exposure to ultraviolet light, with the disease affecting those areas of the body exposed to sunlight. Additionally, persons with fair skin susceptible to sunburn are at higher risk. Immunosuppressed individuals, such as transplant recipients, patients suffering from ▶ epidermodysplasia verruciformis (EV), or those infected with human immunodeficiency virus (HIV) are prone to develop SCC of skin, which is related to infection with ▶ Human Papillomavirus (▶ HPV). For ▶ lung cancer, the major etiological agents are carcinogens present in ▶ tobacco smoke (around 90% of male cancer deaths and 80% of those in females are associated with smoking); additional factors include a family history of the disease, or exposure to radon gas. Similarly, head and neck SCC is primarily related to tobacco use, particularly lesions of the oral cavity (▶ Tongue Cancer), oropharynx, and larynx. In addition to cigarette smoking, intraoral lesions may also arise in users of chewing tobacco, as well as those who use pan (a combination of areca nut and slaked lime, rolled in a betel leaf to form a quid), with or without tobacco. This practice is popular in southern Asian cultures, and in immigrant populations from this region, resulting in large, exophytic tumors. Further etiological factors for head and neck cancer include alcohol use (also important for ▶ esophageal SCC) and HPV infection. Anogenital SCCs are primarily caused by infection with "high-risk" HPV (Human Papilloma Virus) types. Additional etiology includes tobacco usage and HIV infection.

Diagnosis and Clinical Features

Diagnosis of squamous cell carcinoma is based on a combination of clinical examination and histopathological assessment of a biopsy specimen. In some cases, such as ▶ lung and other internal tumors, clinical examination may be assisted by the use of imaging techniques, such as standard radiography or magnetic resonance imaging. SCCs of skin, oral, or genital mucosa may present as exophytic, verrucous (warty) lesions (verrucous carcinoma), or as persistent ulcers that fail to heal. Typically, such ulcers have a raised, rolled border, and larger lesions may show signs of necrosis in the center.

By definition, squamous cell carcinomas arise from squamous epithelium. Histopathologically, therefore, SCCs show signs of ▶ invasion through the basement membrane into the underlying stromal tissue – the hallmark of carcinomas. Lesions may show varying degrees of differentiation: well-differentiated tumors will be more recognizable as squamous epithelium, forming nests of tumor cells within the stroma, and may express an abundance of keratin, forming so-called keratin pearls. Some tumors can show fewer (or no) signs of squamous differentiation, and may be graded (▶ Tumor Grading) as moderately or poorly differentiated, or anaplastic. Highly aggressive lesions may invade adjacent muscle and bone, and enter into lymphatic vessels or blood vessels, thus aiding their spread to secondary sites of growth (▶ Metastasis).

Squamous cell carcinomas may develop through a series of well-defined premalignant stages, during which the epithelium shows increased disruption of normal growth and differentiation and loss of tissue architecture. The epithelium progresses through ever more advanced stages of ▶ dysplasia, in which some (or all, depending of the degree of dysplasia) of the following ▶ cellular atypia may be observed:

- Hyperchromatic nuclei
- More frequent mitoses
- Aberrant mitotic figures
- Mitoses in suprabasal cell layers (in stratified epithelia)
- Pleomorphic nuclei, altered nucleus/cytoplasmic ratio
- Loss of cellular polarity
- Dyskeratosis – keratinization deep within the epithelium
- Loss of ▶ adhesion
- De-differentiation and loss of tissue architecture

Typically, premalignant lesions are described as mildly, moderately, or severely dysplastic depending on the number of cellular atypia observed upon histopathological examination. The most severe cases (but where invasion has not yet occurred) may be referred to as carcinoma-in-situ (CIS). Cervical dysplasias are generally graded as cervical intraepithelial neoplasia (CIN) grade I, II or III, or as CIS, representing increasing severity.

Clinically, potential premalignant lesions may also be noted. ▶ Actinic keratosis is a well-recognized condition seen on areas of sun-exposed skin which presents as scaly, erythematous patches. Similar lesions on the lip are known as actinic cheilitis. These lesions may range from mild to severe, depending on the degree of cellular atypia present histologically. Full-thickness cellular atypia (CIS) is known as ▶ Bowen disease. Pre-neoplastic lesions are also recognized at other body sites. In the oral cavity, white patches (▶ Leukoplakia) may be dysplastic, although the majority does not progress to invasive cancer. Red, atrophic areas of ▶ erythroplakia should be regarded with much more suspicion, as they have a greater propensity for malignant change. A combination of these two lesions – "speckled leukoplakia" or leuko-erythroplakia – are also more likely to undergo malignant transformation. Less frequently, lichen planus (particularly the erosive form) may develop into squamous cell carcinoma. In cervical epithelia, suspicious areas are identified by application of a dilute solution of acetic acid to the area under investigation, producing acetowhite lesions, the histological condition of which is then determined by biopsy. In the development of lung SCC, one of the intermediate histological events is the onset of squamous metaplasia, where the normal respiratory epithelium assumes squamous characteristics. This change may be reversible if exposure to the initiating agent (most commonly tobacco smoke) ceases.

Disease Management

Therapeutic modalities for squamous cell carcinomas include one of (or more commonly a combination of) surgical excision, chemotherapy or radiotherapy. Typically, smaller well-localized lesions are excised with a wide surgical margin of normal tissue. The "normality" of the margin is generally based on clinical and histological assessment. However, this is complicated by the phenomenon known as ▶ field cancerization. This hypothesis proposes that histologically normal fields or patches of cells are present which have developed from genetically altered stem cells and, thus, are predisposed to undergo malignant ▶ progression. This underlies the propensity of squamous cell carcinomas to recur following excision or for patients to develop multiple second primary lesions. The likelihood of future tumor occurrence makes preventive strategies appealing. In this regard, the use of ▶ retinoids has been explored in several clinical trials. However, the success of this is still under debate as, in some trials, provision of beta-carotene actually increased the incidence of lung cancer. Novel chemotherapeutic drugs that target mutant epidermal growth factor receptors present on the tumor cells are being used for treatment of some ▶ lung cancers and head and neck SCC. The central role for HPV in cervical ▶ carcinogenesis has led to the development of a vaccine to prevent viral infection.

Molecular Aspects

A considerable body of research has documented the molecular genetics and biochemical features associated with squamous carcinogenesis. While distinct differences have been noted between tumors arising at specific body sites, there are many common genetic aberrations that are shared amongst SCCs. ▶ Loss of heterozygosity (LOH), indicative of inactivation of ▶ tumor suppressor genes, has been reported to occur at multiple chromosomal locations including chromosomes 1, 3p, 4, 5q, 6, 8, 9, 11q, 13q, 14q, 17p, and 19q. Two of the most actively studied are the ▶ *CDKN2A* gene on 9p21 and ▶ *TP53* on chromosome 17p13 which, respectively, encode p16/▶ INK4A, an inhibitor of ▶ cyclin D-dependent kinases, and ▶ p53, a multi-faceted regulator of cell cycle progression, genomic damage and programmed cell death (apoptosis). Loss of p16/INK4A has been shown to occur through chromosomal loss or deletion, as a result of promoter hypermethylation and, less commonly, through intragenic mutation. The net effect of such a loss is to deregulate the activity of ▶ cyclin dependent kinases (CDKs) 4 and 6, which are normally active in the G1 phase of the cell cycle. This leads to the hyperphosphorylation of pocket proteins, such as the ▶ Retinoblastoma Protein pRB, and more rapid progression through the cell cycle, thus contributing to the enhanced proliferation seen in cancer cells. Loss

or mutation of *RB1* is observed less frequently in SCC which may be ascribed, at least in part, to the loss of p16/INK4A, increased expression of cyclin D1 as a result of gene amplification of a locus on chromosome 11p, and the action of the HPV E7 (▶ Early Genes of Human Papilloma Viruses) oncoprotein.

Loss of expression of a functional ▶ p53 Protein is a common feature of SCC, in some cases through chromosomal loss or deletion. More commonly, however, intragenic mutations result in expression of ▶ p53 proteins harboring amino acid substitutions that disrupt normal function, or in expression of truncated proteins. The biological consequences of p53 loss can be wide-ranging, given the many functions of this protein, and include failure to activate cell cycle checkpoints in the event of genotoxic stress and failure to execute programmed cell death. C-to-T transition mutations are found commonly in skin cancer, with CC-to-TT changes present at high frequency. These are indicative of ultraviolet irradiation-induced damage, consistent with the known etiology of SCC at this site. Similarly, mutations found in head and neck and lung SCCs are frequently ascribable to the actions of carcinogens present in tobacco smoke, such as the occurrence of G-to-T transversions. The presence of mutation in the *TP53* gene may also be useful to identify clones of genetically altered cells that have arisen during the process of field cancerization.

Deregulation of cell growth by human papillomavirus contributes to the genesis of the majority of cervical cancers. Additionally, HPV has been linked with other anogenital tumors as well as head and neck (primarily pharyngeal), skin, and lung cancers. The E6 and E7 (▶ Early Genes of Human Papilloma Viruses) oncoproteins encoded by "high-risk" HPV types deregulate cell cycle progression by targeting p53- and pRB-dependent pathways. E6 (▶ Early Genes of Human Papilloma Viruses) targets p53 for degradation by the ubiquitin-proteasome pathway (▶ Ubiquitination). Additionally, E6 (▶ Early Genes of Human Papilloma Viruses) targets PDZ-motif-containing target proteins such as Dlg, MAGI-1, and MUPP1, each of which has also been shown to suppress cell growth. The E7 (▶ Early Genes of Human Papilloma Viruses) protein is well-recognized for its ability to bind the retinoblastoma protein and the related p107 and p130 pocket proteins, targeting them for degradation and, hence, also deregulating proliferation. E7 (▶ Early Genes of Human Papilloma Viruses) also inhibits the function of the transcriptional coactivator FLH2 which may further enhance cellular transformation, while E6 and E7 (▶ Early Genes of Human Papilloma Viruses) also upregulate expression of the cellular inhibitor of apoptosis protein cIAP2, leading to apoptosis resistance.

Inactivation of the fragile histidine triad gene, ▶ *FHIT*, located at chromosome 3p14 is another important ▶ tumor suppressor gene loss that is observed in squamous cell carcinomas. Studies have documented reduced expression in ▶ oral SCC and in oral premalignant lesions, as well as in esophageal, lung, and ▶ cervical carcinomas. In addition to gene loss, ▶ epigenetic gene silencing by promoter hypermethylation contributes to reduced transcript levels, and may be attributable to exposure to tobacco smoke. Decreased expression is also reported to be an early event in tumor progression and may be associated with a worse prognosis. Conversely, studies of skin SCCs (non-tobacco etiology) do not provide evidence of altered FHIT expression.

Mutations in the *HRAS* gene are reported in only 10–20% of skin tumors, in contrast to initial studies. However, *NRAS* mutations are found with higher prevalence in patients with xeroderma pigmentosum, an inherited condition in which there is a defect in DNA repair processes. Mutations in *KRAS* are reported in up to 30% of lung cancers. Genes encoding ▶ Ras proteins are infrequently mutated in head and neck cancers, except for lesions associated with a pan or betel quid chewing habit.

Disruption of growth factor signaling pathways is also common in many squamous cell carcinomas. Overexpression or mutation of the epidermal growth factor receptor (▶ Epidermal Growth Factor Receptor Inhibitors/Ligands) is seen with high frequency in head and neck, lung, alimentary, and anogenital tumors, and helps to drive proliferation as well as ▶ motility and ▶ metastatic spread of tumor cells. Loss of the negative regulatory effects of transforming growth factor beta also contributes to deregulated cell growth, as well as the transition from an epithelial to a mesenchymal (▶ Epithelial to Mesenchymal Transition) phenotype that is seen in some advanced cancers. Additionally, the role of ▶ inflammation is becoming increasingly recognized in squamous cell carcinogenesis, as well as in other tumor types. Notable players in this area include members of the ▶ chemokine network which can modulate immune function, induce

tumor neovascularization (► Angiogenesis), and stimulate proliferation and metastasis of tumor cells.

► Epithelial Tumors
► Lung Cancer

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SR Proteins

Definition

A family of RNA-binding proteins that are characterized by one or more amino-terminal RNA-recognition motifs (RRM), a glycine-rich region, and a carboxyl terminal region that is rich in the amino acids arginine and serine which are largely arranged as dipeptides. Individual members of the SR family of proteins are able to complement non-splicing competent cytoplasmic extracts to gain splicing function. SR proteins are important for constitutive ► splicing and have also been found to be important for the regulation of alternative splicing. SR proteins facilitate alternative splicing by binding to elements within the pre-mRNA called splicing enhancers and recruiting other components of the ► spliceosome.

Src

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Synonyms

c-Src; $pp60^{c\text{-}Src}$; $pp60^{v\text{-}Src}$; v-Src

Definition

v-Src (or viral Src) is a 60 kDa protein encoded by the oncogenic retrovirus, Rous sarcoma virus. The protein derives its name from its ability to induce sarcomas in experimental animals and malignantly transform cells in tissue culture. c-Src, or cellular Src, is the normal cellular progenitor of v-Src. c-Src is non- or weakly transforming when overexpressed in tissue culture cells. Both v- and c-Src are cytoplasmic tyrosine kinases that transfer phosphate from ATP to tyrosine residues within specific protein substrates. The resulting phosphotyrosine either conformationally activates the enzymatic activity of the recipient molecule or functions as a docking site for other molecules that transmit growth signals to the nucleus in a chain of events involving multiple phosphorylation and binding reactions. c-Src contains a carboxy-terminal region that maintains the molecule in a mostly inactive state. In v-Src, this 12 amino acid region is deleted, rendering the molecule constitutively active.

Characteristics

Domain Structure

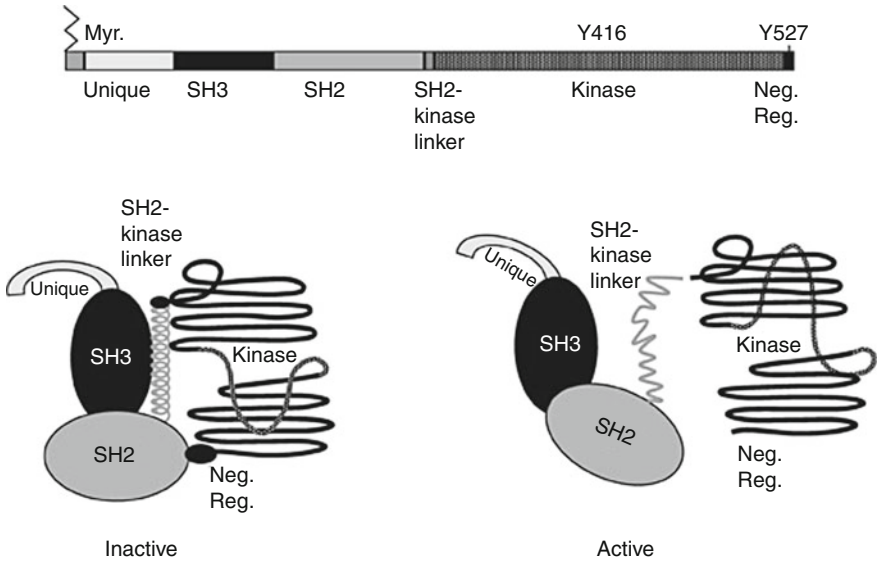
Each molecule of Src contains seven domains that are involved in targeting the protein to cellular membranes (the myristylation domain), in binding other proteins (the Unique, ► SH3, and SH2 domains), and in regulating the catalytic activity (Fig. 1). Src is one of a family of at least nine proteins that have a similar overall structure, including Fyn, Yes, Fgr, Hck, Lck, Blk, Yrk, and Lyn. Some of the family members are present only in certain cell types, such as cells of the hematopoietic lineage, while others are ubiquitously expressed. c-Src is one of the latter family members.

Subcellular Localization

The myristate fatty acid modification on the amino terminus of Src targets it to intracellular membranes, including the plasma membrane and membranes of intracellular organelles, especially those of the endocytic pathway. c-Src has also been found to associate with ► centrosomes in interphase cells.

Interacting Proteins

Src forms complexes with a variety of intracellular signaling molecules via its Unique SH2 and SH3 domains (Fig. 2). These proteins include, but are not



Src. Fig. 1 Structure of c-Src. As a linear molecule, c-Src consists of an N-terminal membrane association domain that contains the site of myrisylation, a Unique domain that exhibits the widest sequence divergence among family members, an SH3 domain, an SH2 domain, an SH2/kinase linker, the catalytic domain, and a negative regulatory domain that contains Tyr 527 (531 in human c-Src). When c-Src is activated, Tyr 416 (Tyr 419 in human c-Src) in the kinase domain becomes phosphorylated. How all the domains of c-Src relate to one another in a three-dimensional context to generate the inactive and active states of the enzyme is also shown and explained in the text

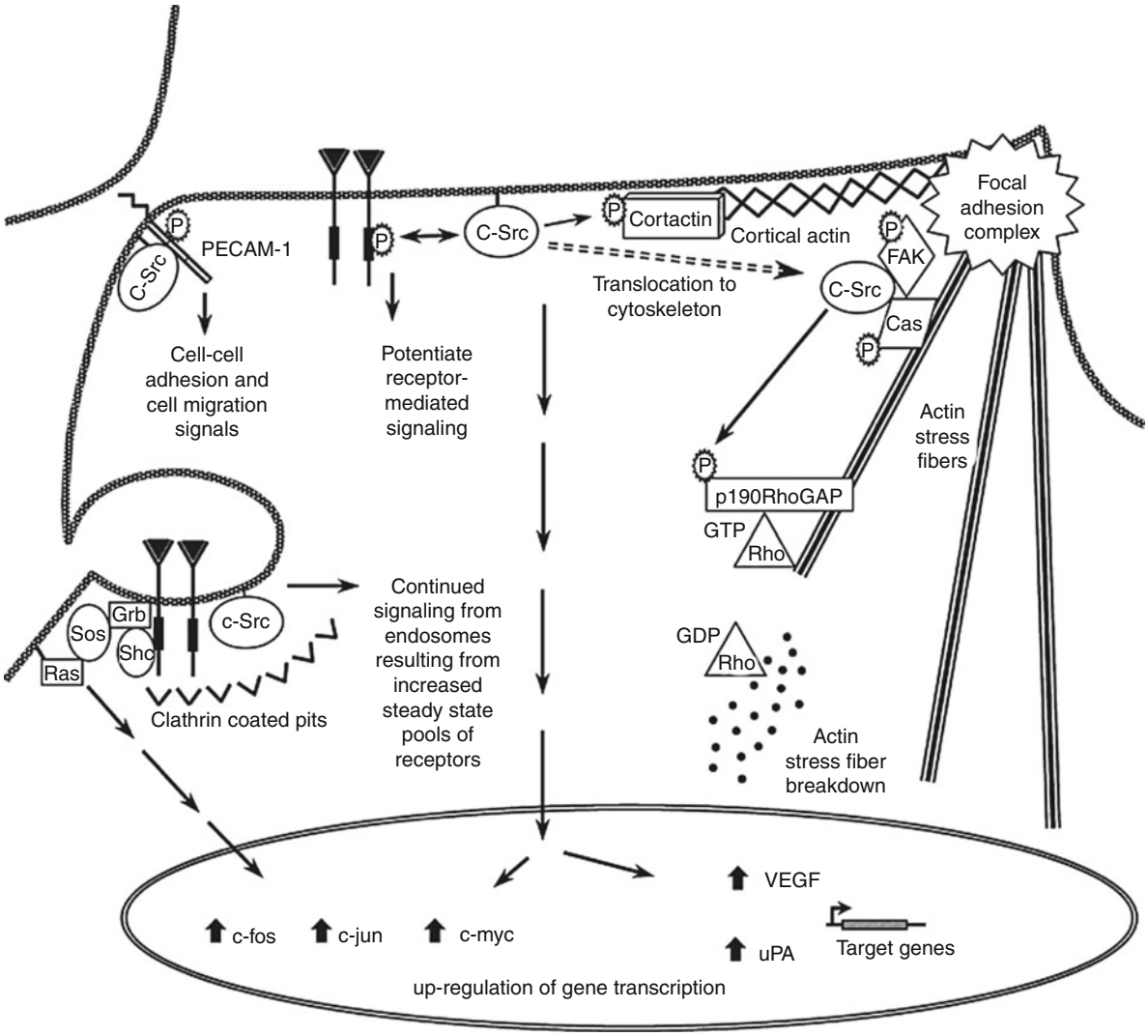
limited to, polypeptide growth factor receptors such as the ▶ epidermal growth factor (EGF) receptor, intercellular ▶ adhesion molecules such as PECAM and ▶ E cadherin, gap junction proteins such as connexin 43, and several proteins found in focal adhesions such as focal adhesion kinase (▶ FAK) and p130CAS. While most of these binding proteins are also substrates of Src, Src can phosphorylate proteins that do not form complexes stable enough to extract from the cell, such as the cortical actin binding protein, ▶ cortactin, p190RhoGAP (a GTPase activating protein for ▶ Rho Family GTPases), and clathrin, a component of endocytic vesicles.

Cellular and Molecular Aspects

The extreme C-terminal domain of c-Src contains a tyrosine residue (Tyr 527 in chicken c-Src and Tyr 531 in human c-Src) that when phosphorylated binds its own SH2 domain in an intramolecular fashion (Fig. 1). This binding, together with the coupling of the SH3 domain to a pseudo-polyproline sequence in the SH2 kinase linker, renders the protein inactive. c-Src becomes activated when these intramolecular interactions are disrupted by competition with other signaling molecules that contain either phosphotyrosine or polyproline regions that bind the SH2 and SH3 domains, respectively, of c-Src. Such events occur, for example, when the c-Src SH2 domain binds phosphotyrosine 397 of FAK or phosphotyrosines of activated and tyrosine phosphorylated polypeptide growth factor receptors. Full activity is achieved upon dephosphorylation of Tyr 527/531 and autophosphorylation of Tyr 416/419 in the activation loop of the catalytic domain. Antibodies specific for phosphorylated Tyr 416/419 are frequently used to assess the activation state of c-Src in human tumors. c-Src activity has been reported to increase following integrin engagement of extracellular matrix (and subsequent activation of FAK) or upon stimulation of cells with growth factors, such as EGF, PDGF, and FGF. v-Src is constitutively activated by deletion of the C-terminal phosphotyrosine 527/531 and mutations within the SH3 domain that reduce interactions with the pseudo-polyproline region.

Clinical Relevance

c-Src is overexpressed or activated in multiple human tumors, particularly in ▶ glioblastomas and ▶ carcinomas of the breast, lung, colon, prostate, cervix, stomach, and ovary. In ▶ breast cancers, the frequency of



Src. Fig. 2 Examples of c-Src targets and their potential roles in transformation

tumors overexpressing c-Src approaches 70%. Although analyses of other tumor types are not as extensive as those of breast cancers, existing data suggest the frequency of c-Src overexpression in lung and colon tumors may be similar to that in breast malignancies. Members of the EGF receptor family are also overexpressed in many of the same types of tumors that overexpress c-Src. Recent studies indicate that c-Src and EGFR synergistically promote tumor growth. This enhanced growth is accompanied by an EGF-induced association between c-Src and the EGFR, phosphorylation of EGFR by c-Src on several novel sites, and activation of signaling pathways that are required for EGF-induced mitogenesis and cell survival following genotoxic stress. These findings are providing the impetus for discovering novel therapeutics that disrupt both the physical and functional interactions between c-Src and EGFR family members.

c-Src's involvement in the formation and turnover of focal adhesions and cell–cell contacts also suggests a role for this protein in cell ▶ migration and ▶ metastases. Regulation of ▶ vascular endothelial growth factor (VEGF) expression in response to hypoxia implicates c-Src as a modulator of angiogenesis, further underscoring its potential importance as a target for antitumor therapy. To that end, numerous pharmaceutical companies have developed inhibitor compounds that target c-Src kinase activity, and several

of these are being tested for treatment of solid tumors in clinical trials, including AZD0530 (AstraZeneca), BMS-3554825 (Bristol Myers Squibb), and SKI-606 (Wyeth Research).

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Src Family Tyrosine Kinase

Definition

The ▶ *src* gene is the first discovered viral oncogene carried by the ▶ retrovirus Rous sarcoma virus and also is the first identified proto-oncogene. The Src family consists of nine family members encoding tyrosine kinases, which are anchored to the inner surface of cytoplasmic membrane with their N-terminal myristoylation. Some members such as Src and Fyn are also located within nuclei as well as within mitochondria.

▶ Membrane-Linked Docking Protein
▶ Transduction of Oncogenes

Src Homology 2

▶ SH2/SH3 Domains

Src Homology Domain

Definition

SH; a region of a protein whose tertiary structure allows it to specifically bind to phosphorylated tyrosine residues.

▶ SH2/SH3 Domains

Src Kinase

Definition

▶ Src was the first discovered tyrosine kinase. v-Src (viral sarcoma) was the first discovered oncogene, isolated from Rous sarcoma virus and is constitutively activated. Src comprises ▶ Src homology domains, which recruit proteins, expressing these domains to Src.

▶ Focal Adhesion Kinase

SRC-3

▶ Amplified in Breast Cancer 1

Src8

▶ Cortactin

SRC-Homology Domains

Definition

▶ SH2 Domain.

▶ SH3 Domain

SRCR Superfamily

Definition

The scavenger receptor cysteine-rich (SRCR) superfamily is a group of glycoproteins comprising cell surface molecules as well as secreted proteins that are

characterized by the presence of at least one highly conserved SRCR-domain.

SREBP

Definition

Sterol regulatory element-binding protein (SREBP); a transcription factor for the transcription of genes that encode the low-density lipoprotein receptors and enzymes in cholesterol synthesis.

► Fatty Acid Synthase

SRF

Definition

Serum response factor (SRF) is a transcription factor that recognizes the CArG box (CC-ATrich-GG) in cellular genes, such as immediate-early-genes. SRF interacts either with an ► Ras-regulated B-box-containing ► Ets protein (Elk-1, Sap, Net, or FLI-1) or with β-actin/Rho-GTPase (► Rho Family Proteins) -regulated Mal to mediate responses to a variety of extracellular stimuli.

► ETS Transcription Factors

SrGAPs

Definition

Family of ► GTPase activating proteins, srGAPs that facilitate hydrolysis of Cdc42 that ultimately leads to actin depolymerization and contributes to the repulsive effect of Slit in neurons.

► Slit

SRIF

► Somatostatin

St. John's Wort

Synonyms

Goatweed; Hypericum

Definition

The flowering tops of St. John's wort are used to teas and tablets with concentrated extracts. Although St. John's wort is not a proven therapy for depression, there is some evidence that St. John's wort is useful for treating mild to moderate depression. It is also used for anxiety and/or sleep disorders. St. John's wort can influence the clearance of specific drugs known to be metabolized by CYP3A (► Cytochrome P450) or excreted via ► ABC drug-transporters.

► Irinotecan

Stage

Definition

Referring to tumors; is an internationally agreed index which objectively measures the extent of tumor growth and progression. Common criteria include tumor size, extent of local spread, and presence of lymph node or visceral metastasis. Each tumor-type has its own staging system, with variations in the common criteria, which correlates with prognosis.

► Transcoelomic Metastasis

Staging of Tumors

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Synonyms

Determination of tumor extent and spread; Tumor staging

Definition

Tumor staging is a clinical procedure aimed at documenting the anatomic extent of a malignant tumor at a specific site, and the extent of its spread locally, regionally, or to distant sites. Upon completion of the clinical staging procedures, the tumor under consideration is assigned to a particular *stage* (stadium in Latin), i.e., given a semiquantitative designation summarizing the data about the size of the tumor and the extent of its spread.

Characteristics

Purpose

The staging of cancer is used to document the extent of the neoplastic disease in a standardized and consistent manner that will allow clinicians to compare the features of a particular tumor with other similar tumors. The main reasons for staging of tumors have been summarized by the American Joint Committee on Cancer as follows:

- Selection of the most appropriate therapy for each individual patient
- Formulation of prognosis for each cancer patient, i.e., prediction of the natural course of the neoplastic disease and its outcome with or without treatment
- Objective and measurable assessment of the effects of therapy
- Objective performance of clinical cancer studies within a single institution or geographically distant institutions

Clinical staging of tumors is of paramount importance for the proper selection of treatment of each particular cancer patient. Many univariate and multivariate studies have shown that the tumor stage at the time of diagnosis is in most instances the most powerful predictor of outcome of a neoplastic disease and cancer patients' survival with or without treatment. It is also essential for organizing multi-institutional cancer treatment studies and other forms of clinical cancer research.

Methods

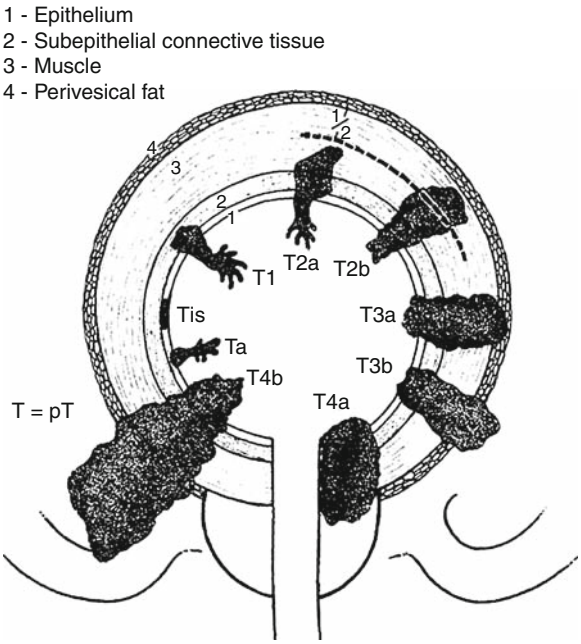
Tumor staging is typically based on a multidisciplinary effort including oncologists, surgeons, radiologists, pathologists, and even other clinicians. The data may be collected by biopsy, during surgical exploration, during definitive cancer surgery, laparoscopic surgery, and radiologic examination of the patient. The material collected during these procedures is usually submitted for macroscopic and microscopic pathologic examination. Additional studies, such as molecular biologic analysis of the tumor material, may be undertaken in research institutions, but it is not routinely included in the staging of most tumors.

The most widely used tumor staging system is called the ▶ TNM system. The TNM system is based on analysis of three parameters: the size or extent of the untreated primary tumor (T), the absence or presence of spread to the regional lymph nodes (N), and the presence or absence of distant metastasis (M). For the sake of uniformity and consistency the following standardized definitions are used:

Primary tumor (T)	
TX	Primary tumor cannot be evaluated
T0	No evidence of primary tumor
Tis	Carcinoma in situ (early cancer that has not invaded into the surrounding stroma)
T1, T2, T3, T4	Size and/or extent of the primary tumor
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be evaluated
N0	No regional lymph node involvement
N1, N2, N3	Involvement of regional lymph nodes (number and/or extent of spread)
Distant metastasis (M)	
MX	Distant metastasis cannot be evaluated
M0	No distant metastasis (cancer has not spread to other parts of the body)
M1	Distant metastasis (cancer has spread to distant parts of the body)

The staging based on the TNM data typically includes five categories, corresponding to stages 0 and stages I–IV. Each of these stages may be subdivided into subcategories labeled a, b, or c. Stage 0 denotes carcinoma in situ. Stage I, II, and III tumors are localized to the organ of their origin or have spread regionally. Stage IV tumors have metastasized to distant sites.

The criteria for T, N, and M and for stages 0–IV vary from one anatomic site to another. For example, bladder cancer T3N0M0 is stage III, while colon cancer T3N0M0 is stage II. For specific staging of tumors in various anatomic sites one must refer to specific staging manuals. Figure 1 illustrates the definition of T classifications for bladder cancer.



Staging of Tumors. Fig. 1 Tumor staging. Extent of primary bladder cancer (T classification) (Reproduced from Greene et al. [1])

Ta	Noninvasive papillary carcinoma
Tis	Carcinoma in situ: "flat tumor"
T1	Tumor invades subepithelial connective tissue
T2	Tumor invades muscle
pT2a	– Superficial muscle (inner half)
pT2b	– Deep muscle (outer half)
T3	Tumor invades perivesical tissue
pT3a	– Microscopically
pT3b	– Macroscopically (extravesical mass)
T4	Tumor invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall
T4a	– Prostate, uterus, vagina
T4b	– Pelvic wall, abdominal wall

Perspectives

Staging of tumors has been standardized to a great extent but still many problems remain to be solved. The older staging systems such as the staging of colonic carcinoma according to Dukes are not used anymore and are thus only of historical interest. New staging classifications are constantly being proposed and the merits of new approaches are discussed in medical literature. The same holds true for the new studies on the molecular biology techniques or new imaging techniques, which are used in experimental protocols in many leading research centers. Overall, one can predict with confidence that the current protocols for staging of tumors will be improved by the introduction of newer technology, but all these modifications will require extensive independent confirmation and validation before they become widely accepted.

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Standardization

Definition

A consistent set of procedures for designing, administering, and scoring an assessment. The purpose of standardization is to ensure that all individuals are assessed under the same conditions.

► Biomonitoring

STAR Trial

Definition

The Study of ► Tamoxifen and ► Raloxifene, known as STAR trial, has compared raloxifene with the drug tamoxifen in reducing the incidence of breast cancer in postmenopausal women who are at increased risk of the disease. Initial results of STAR in 2006 showed that raloxifene is as effective as tamoxifen in reducing the ► breast cancer risk of the women on the trial. In STAR, both drugs reduced the risk of developing invasive breast cancer by about 50%. In addition, a 4 year follow up suggested that women taking raloxifene had 36% fewer ► uterus cancer and 29% fewer blood clots than

the women who were on tamoxifen, reducing the serious side effects associated with tamoxifen treatment. Furthermore, the ▶ CYP2D6 enzyme is not needed to activate raloxifene. About 10% of people have an abnormal CYP2D6 enzyme, which alters patients' drug levels and keeps them from getting the full benefit of tamoxifen. Raloxifene is an alternative to tamoxifen for reducing the risk of invasive breast cancer in postmenopausal women with osteoporosis and in postmenopausal women at high risk for invasive breast cancer.

▶ Breast Cancer Rationally Designed Therapies

START

▶ Systemic Targeted Radionuclide Therapy

Start Codon

Definition

Either of two codons, AUG or GUG, that signal the initiation of ▶ translation and the first amino acid in a polypeptide chain; synonym initiation codon. The codon on a messenger RNA (▶ mRNA) molecule where protein synthesis begins.

STAT

Definition

Signal transducer and activator of transcription, proteins activated in response to interleukins and other cytokines that regulate function of immune system and proliferation, apoptosis, and angiogenesis in various cell lines.

▶ Signal Transducers and Activators of Transcription in Oncogenesis
▶ Suppressors of Cytokine Signaling

Stathmin

Definition

Op 18; is a ubiquitous cytosolic phosphoprotein with various regulatory functions in cell proliferation, differentiation signaling, and activation. In particular, stathmin is involved in the regulation of tubulin dynamics through inhibition of microtubule formation and/or microtubule depolymerization.

▶ Microtubule-Associated Proteins
▶ Oncoprotein 18

Statins

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Definition

Statins are synthetic agents that inhibit HMG-CoA reductase, the rate-limiting enzyme that controls cholesterol biosynthesis. Currently, there are several statins in use for the treatment of hypercholesterolemia in humans. It is well established that these agents induce their therapeutic effects by decreasing low-density lipoproteins (LDL). Such effects have been associated with a decrease in the rate of progression of atherosclerotic lesions in patients with coronary artery disease, and it is now well established that the use of statins has changed the natural history of coronary artery disease in humans. Beyond their ability to lower cholesterol, statins have important additional biological effects in vitro and in vivo, including anti-inflammatory and antitumor properties.

Characteristics

There has been substantial experimental evidence establishing that statins inhibit the growth of malignant

cells and induce programmed cell death in vitro. Moreover, statins exhibit chemopreventive effects toward certain types of tumors in vivo when administered to humans. The ability of statins to generate such responses in vitro and in vivo is of high interest and may prove to be of clinical value in the future.

Cellular Effects of Statins In Vitro

Statins induce apoptosis of different types of malignant cells in vitro. These include cells of leukemia origin as well as cells originating from a variety of solid tumors, including colon, prostate, breast, thyroid, pancreatic, small, and non-small cell lung cancers, as well as malignant melanoma, osteosarcoma, glioma, and medulloblastoma. Although the mechanisms by which statins induce apoptosis have not been fully elucidated, there is evidence that distinct cellular events are involved in the induction of statin-dependent pro-apoptotic responses. Blocking protein ▶ geranylgeranylation correlates with ▶ lovastatin-induced apoptosis of human leukemia cell lines. In addition, statins activate the pro-apoptotic JNK ▶ MAP kinase pathway in malignant cells, and the activation of this pathway is essential for statin-dependent programmed cell death. Beyond activation of the JNK pathway, statins inhibit the MEK/ERK signaling cascade, which is associated with increased cell proliferation, and this constitutes another major mechanism contributing to their antineoplastic properties. ▶ Atorvastatin and ▶ fluvastatin also induce differentiation of NB4 leukemic cells that are of acute promyelocytic leukemia (APL) origin. Such effects also occur in cell variants that are refractory to the differentiating effects of all-trans-retinoic acid (ATRA). Atorvastatin and fluvastatin exhibit also similar in vitro effects on primary leukemic blasts that have developed resistance to the differentiating effects of ATRA and appear to reverse such ATRA-resistance.

Statins in the Prevention and Treatment of Cancer

There is accumulating evidence from epidemiological studies indicating that statins exhibit chemopreventive effects against certain solid tumor types, including colorectal cancer, lung cancer, prostate cancer, and pancreatic cancer. On the other hand, statins do not seem to exert such chemopreventive effects against breast cancer. Extensive preclinical evidence has also suggested that statins may have therapeutic effects against certain malignancies. This has led to the recent initiation of clinical trials to examine the clinical activity of statins in certain malignancies. A recent phase I study assessed the combination of high doses of pravastatin with chemotherapy in the treatment of acute myelogenous leukemia patients. Such combination was found to be safe and well tolerated, while a high number of complete responses was seen. Altogether, there is a substantial amount of evidence raising the possibility that statins may eventually find a role in the prevention and/or treatment of certain tumors and hematologic malignancies. Several epidemiological and clinical studies are currently ongoing to address this issue.

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Staurosporine

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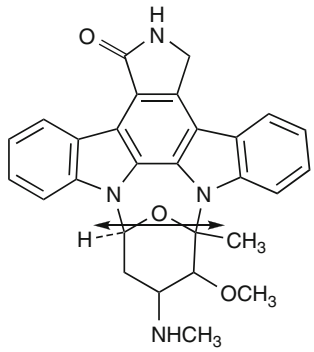
Definition

Staurosporine is a natural product originally isolated from the bacterium *Streptomyces staurosporeus* from a soil sample obtained in Japan (Iwate Prefecture) in 1977 during a search for new alkaloids present in actinomycetes and given the name AM-2282. The term alkaloid refers to a naturally occurring amine



Staurosporine. Fig. 1 Structure of staurosporine (AM-2282)

produced either by a plant, animal, or fungus. Actinomycetes are a group of Gram-positive bacteria that had previously been shown to produce the alkaloids pyrindicin, NA-337A, and TM-64.

Characteristics

The initial studies on AM-2282 focused on the taxonomy of the producing strain, fermentation, isolation, and physicochemical and biological properties of this new alkaloid. Besides being identified as a new species, they also discovered that AM-2282 had antimicrobial activity. The term antimicrobial is given to any type of chemical compound that can suppress the growth of, or aid in the death of microorganisms, such as bacteria, yeast, and mycoplasma. AM-2282 had antimicrobial activity against fungi and yeast, but no significant effects on bacteria.

The structure of AM-2282, which herein will be referred to as staurosporine, is shown in Fig. 1. Staurosporine was the first of over 50 alkaloids to be isolated with this type of chemical structure, which was elucidated through X-ray analysis. In Fig. 1, the portion of the structure above the arrow is known as an indole carbazole subunit, while the lower part of the structure is a sugar molecule. Although staurosporine was isolated in 1977 and its X-ray crystal structure was determined in 1978, it was not until 1996 that the first total chemical synthesis was achieved. Part of the challenge to the synthesis of staurosporine was the joining together of the sugar and indole carbazole groups and establishing the sugar stereochemistry. In 1992, the indole carbazole group known as staurosporine aglycon was isolated. Aglycon is the non-sugar compound remaining after replacement of the glycosyl group with a hydrogen atom.

Kinase Inhibition

The antimicrobial activities of staurosporine were initially thought to be via its role as a potent inhibitor of protein kinase C (PKC). These initial studies revealed that staurosporine was able to inhibit PKC from rat brain. Using other inhibitors such as triflouperazine, chloropromazine, and polymixin B, staurosporine was a more potent inhibitor of PKC than other known inhibitors. Furthermore, PKC inhibitors triflouperazine, chloropromazine, and polymixin B appeared to compete with phospholipids, whereas, the inhibition by staurosporine was not released by increasing the concentration of phosphatidylserine. Studies with cultured cells showed that staurosporine has very potent growth inhibitory activity.

In addition to being an inhibitor of PKC, staurosporine also inhibits several other kinases, such as tyrosine kinases. The in vitro activity of staurosporine was analyzed by investigating the autophosphorylation of $p60^{v\text{-}src}$ in chicken embryo fibroblasts (CEF) that were infected with the rous sarcoma virus (RSV), revealing staurosporine was able to inhibit the autophosphorylation. This was evidence as staurosporine could inhibit tyrosine specific protein kinases similarly to serine and threonine specific kinases such as PKC. Together, staurosporine is a more potent inhibitor of protein-tyrosine kinase than other known inhibitors.

Staurosporine can also inhibit the myosin light chain kinase. Myosin light chain kinase plays a critical role in smooth muscle contraction as well as in the activation of non-muscle cells, by catalyzing the transfer of the γ-phosphate from ATP to the myosin light chain, which is dependent upon Ca^{2+}/calmodulin. The phosphorylation of the light chain is necessary for the activation of actomyosin ATPase, which is a prerequisite for tension development in both smooth and non-muscle cells. Inhibition of the myosin light chain kinase is ATP-dependent.

Other kinases that can be inhibited by staurosporine include the insulin receptor tyrosine kinase activity non-competitive with ATP and the platelet-derived growth factor (PDGF) receptor tyrosine kinase.

Besides having inhibitory activity on these kinases involved in signal transduction pathways, staurosporine was also shown to block cells in different phases of the cell cycle. Staurosporine can block the G1 to S transition at low concentration (1–10 ng/ml) and block cells in late G2 phase at higher concentrations (100–200 ng/ml). There have been several reports showing that the retinoblastoma protein is important in the ability of staurosporine to arrest cells in the G_1 phase of the cell cycle. For example, staurosporine can be used to protect normal cells from the toxic effects of chemotherapy at subnanomolar concentrations, to allow for a reversible G1 arrest through inhibition of the retinoblastoma protein, followed by treatment with camptothecin. Such sequential treatment (with staurosporine first) results in a protection of cells against the toxic effects of chemotherapy. Tumor cells, however, appear unaffected by staurosporine, and are sensitive to low amounts of camptothecin.

The cell cycle associated kinase that is inhibited by staurosporine is Cdc2 (also known as CDK1) which is important in the G2/M transition of the cell cycle. Staurosporine also inhibits the cdk2 and cdk4 kinases which are involved in G1 and S phases.

Collectively, these findings suggest that staurosporine is a non-specific kinase inhibitor because it can inhibit kinases of several different functions with similar efficiencies. This is due to the fact that the serine/threonine kinases conserve the amino acid sequence and 3-D structure of the ATP binding domain. Additionally, staurosporine inhibits the activity of both the insulin receptor tyrosine kinase and of Ca^+/calmodulin-dependent protein (CaM) kinase II in a non-competitive manner with ATP. This suggests that staurosporine can interact with other catalytic domains distinct from the ATP binding site.

Staurosporine in Apoptosis

Staurosporine has been a useful tool in analyzing apoptosis because it has been able to induce apoptotic cell death in cell lines that are normally resistant to chemotherapeutic drugs and death-inducing ligands. One of the death-inducing ligands, TRAIL ((TNF)-related apoptosis-inducing ligand), can normally induce apoptosis in two-thirds of melanoma cell lines examined. Staurosporine, however, is able to induce apoptosis in all melanoma cell lines tested, including those resistant to TRAIL.

Although staurosporine was shown to have a strong cytotoxic effect on the growth of several mammalian cell lines, it did not show antitumor activity in any in vivo models tested. However, a naturally occurring analog of staurosporine, UCN-01 showed antitumor activity in vivo. UCN-01 was also isolated from the same culture broth *Streptomyces* sp. No. 126 that produced staurosporine. UCN-01 differs structurally from staurosporine with a hydoxyl group at the C-7 carbon. Adding this hydroxyl group causes UCN-01 to be specific for PKC inhibition. UCN-01 also shows apoptotic effects in vitro on the cell lines HeLa S3 and MCF-7 similar to what was observed with staurosporine.

Similar to staurosporine, UCN-01 can also inhibit cell proliferation by arresting cells in different phases of the cell cycle. The growth inhibitory affect of UCN-01 is often used to modulate the effects of radiation and chemotherapy. When cells are treated with radiation, which arrests cells in the G2 phase of the cell cycle, in the presence of UCN-01, the cells are no longer able to accumulate in G_2 leading to early mitosis and subsequent apoptosis. UCN-01 also abrogates the S phase of the cell cycle. For example, in p53 mutant cells, treatment with chemotherapeutic drugs such as camptothecin results in S and G2 arrest of the cells. When cells are subsequently treated with UCN-01, it leads to an abrogation of the camptothecin-mediated arrest resulting in mitotic catastrophe and cell death.

Antitumor Activity

Since UCN-01 shows antitumor activity in vivo with much less cytotoxicity than staurosporine, UCN-01 was used for further preclinical studies. Using a xenograft model system with several tumor cell lines (breast and renal carcinoma, and leukemia cells) and with either intravenous or intraperitoneal injections, UCN-01 was shown to have an antitumor effect.

The first case of using UCN-01 to treat patients was in 1996 with a 72 h infusion in the United States (Bethesda, MD) and in Japan as a 3 h infusion. These initial studies showed an unusual pharmacological effect that was not observed in the animal models. The concentrations of UCN-01 in the human plasma were considered abnormally high compared to animal models. Furthermore, the half-life in humans was over

500 h, which was 100 times longer than in the animal models. Eventually it was discovered that in humans, UCN-01 binds strongly to the plasma α_1-acidic glycoprotein resulting in its lack of plasma clearance. Although the initial schedule called for 72-h continuous infusion every 2 weeks, based on the low clearance and prolonged half-life of UCN-01, this was modified to 36-h continuous infusion every 4 weeks. This study has lead to several follow-up studies in which UCN-01 is given as 1–3 h infusions every 4 weeks along with several combinatorial trials. For example, a phase I/II trial of gemcitabine followed by UCN-01 for a 72-h infusion was recently initiated at the National Cancer Institute. Among some of the other agents that are being examined in combination with UCN-01 are cisplatin and 5-fluorouracil. Other ongoing studies with UCN-01 are to use this agent to protect normal cells against the toxic effects of chemotherapy. Since the concentration of UCN-01 required to reversibly arrest normal cells in the G1 phase of the cell cycle is much lower than those required to mediate a toxic affect in tumor cells, this rationale provides a promising alternative to chemotherapy alone. These preclinical and ongoing clinical studies provide a novel and potent target that can be used to biologically modify the mechanisms of deregulation of cancer cells and as such provide a novel class of anticancer agents.

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Steel Ligand Receptor

► Kit/Stem Cell Factor Receptor in Oncogenesis

Stefins

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Synonyms

Cystatins A, B; Type I cystatins

Definition

Stefins are members of the ► cystatin superfamily of cysteine proteinase inhibitors localized in the cytosol and thought to protect cytoskeletal proteins from degradation by ► cysteine proteases released from lysosomes.

Characteristics

Type-1 or Family-1 of the cystatin superfamily of cysteine protease inhibitors includes human stefins A and B and their homologues in other species such as ► cystatins α and β in rat, bovine thymus stefin C, porcine thymus stefins D1 and D2, mouse stefins A (1–4), and others (see MEROPS subfamily I25A). The genes for human stefins A and B have been mapped to chromosomes 3q21 and 21q22.3, respectively. The lack of a signal sequence and disulfide bonds makes stefins distinct from other members of the cystatin superfamily. Stefins are single chain proteins consisting of 98–103 amino acid residues, with a molecular mass of 11–12 kDa. Human stefin A is an acidic protein with pI values between 4.5 and 5.0, whereas stefin B is neutral, having pI values in the range of 5.9–6.5. Their tissue and cellular distribution are different, stefin A being localized mainly to epithelial and lymphoid tissue, while stefin B is evenly distributed in various cells and tissues.

Like other members of the cystatin superfamily, stefins are reversible and competitive inhibitors of cysteine proteases. The structural basis of the inhibition has been elucidated from the X-ray crystal structure of ► papain-stefin B complex and

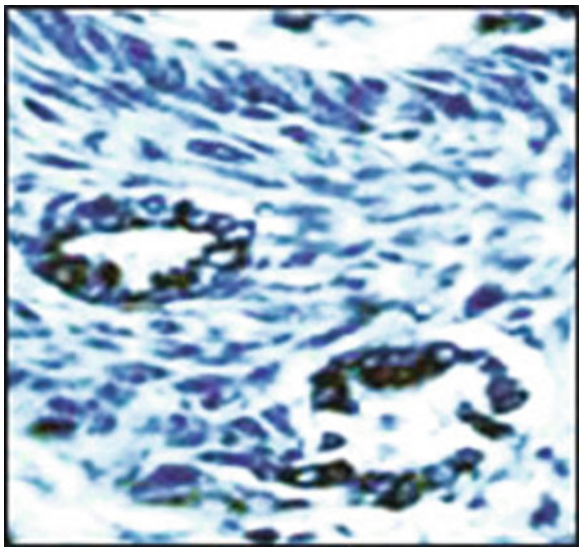
NMR structure of stefin A. Stefins are potent inhibitors of ▶ cathepsins L and S with Ki values in the picomolar range, whereas cathepsin B inhibition is weaker (Ki 10^{-8} M for stefin A and 10^{-7} M for stefin B).

Physiological and Pathological Roles

Besides protection of cytosolic and cytoskeleton proteins from degradation by cysteine proteases accidentally released from lysosomes, several other functions have been suggested for stefins. For example, stefin A may be important in the control of normal ▶ keratinocyte proliferation and differentiation. Also, it has been proposed that it plays a role in ▶ apoptosis, since apoptotic bodies consistently stain for inhibitor, which also correlates with p53 activation. Stefin A should also protect epithelial and lymphoid tissues from cysteine proteases produced by pathogens invading the body. Increased levels of stefin A were found in inflammatory skin samples, psoriatic epidermis, and in inflamed gingival tissue homogenates from patients with periodontal inflammatory diseases. Decreased levels of stefin B mRNA were detected in patients with ▶ progressive myoclonus epilepsy (PME) and associated with excessive activity of cathepsin B. Besides protease inactivation, stefin B could bind other proteins in a multiprotein complex that has a specific cerebellar function, which might contribute to the disease in patients with progressive myoclonus epilepsy. Recent genetic studies have also identified mouse stefin A to be involved in a control of ▶ ovarian follicular growth and maturation.

Stefins in Cancer

Cysteine proteases, the targets for stefin's inhibitory activity, have been implicated in multiple steps of tumor ▶ progression, including processes of cell transformation and differentiation, ▶ motility, ▶ adhesion, ▶ invasion, ▶ angiogenesis, and ▶ metastasis. In most tumor types their levels of expression, protein concentrations and enzymatic activities, in particular of cathepsins B and L, have been demonstrated to be significantly higher than in their control tissue counterparts. The increased levels of cathepsins are usually not accompanied by an equal increase of the cystatins, which was suggested as the main cause of harmful cysteine, cathepsins-dependent proteolytic activity in tumor tissue. However, the balance is selectively altered for type II cystatins, which are in most cases downregulated in tumors, whereas type I cystatins, that is, stefins A and B, have been found upregulated in a majority of cases when compared to control tissue counterparts. Higher levels of stefins A and B in tumors have been determined in lung, ▶ Brms1, head and neck, and ▶ prostate cancer, as well as in murine lymphosarcomas, ▶ hepatomas, and Lewis lung carcinomas (Fig. 1). These higher levels, up to a certain level, may counterbalance the excessive activity of cysteine cathepsins, associated with matrix remodeling, resulting in the progression of the disease. On the other hand, high cytosolic levels of stefins may be relevant for regulation of apoptosis, when initiated via lysosomal cell death pathway inhibiting cathepsin B, which was proposed as a dominant execution protease in the lysosomal apoptotic pathways, induced in a variety of tumor cells by tumor necrosis factor alpha (▶ TNF-α). In some studies, lower levels of stefins in tumors have been reported. For example, stefin A immunoreactivity was lower in lymphomas and in tumors of squamous epithelial cell origin, as well as in prostate and brain tumors. Lower mRNA levels of stefin A or B have been reported in breast and ▶ esophagus tumors as compared to adjacent control tissues.



Stefins. Fig. 1 Stefin A immunostaining in lung adenocarcinoma (Kindly provided by B. Werle)

Although stefins are cytosolic proteins, they have also been detected in body fluids of cancer patients.

Stefin A and stefin B have been detected in ascitic fluid from patients with ovarian carcinoma and in bronchoalveolar fluid of lung cancer patients. Increased serum levels of stefin A in patients with ▶ hepatocellular carcinoma and liver cirrhosis have been correlated with tumor size and with a number of neoplastic lesions. Stefin A, but not stefin B, levels were moderately increased also in patients with ▶ colorectal or lung cancer.

Increased levels of cysteine protease activity, not being balanced by a corresponding increase of cysteine protease inhibitors, are associated with the progression of malignant disease and patients' poor prognosis. Enhanced expression of stefins is expected to diminish the tumor-associated proteolytic activity and indeed, there is evidence of a suppressive role of stefins in various cancer types. Moreover, higher levels of stefin A and stefin B in tumor tissues have been shown to correlate with a favorable prognosis for cancer patients. A significant prognostic value of stefin A and stefin B was determined in patients with lung and head and neck cancer. In the latter, high stefin A tumor levels were found as a strong factor for prediction of prognosis also in multivariant analysis when correlated with established clinical parameters. In prostate tumors higher cathepsin B/stefin A ratios were associated with a more aggressive behavior of prostate cancer.

Animal models with excluded expression of particular cystatin did not support a suppressive function for cysteine protease inhibitors in cancer. In stefin B, as well as cystatin C ▶ knockout mouse, a significantly lower metastatic spread was detected than in wild-type animals. Similarly, higher levels of stefin A and stefin B in body fluids have been associated with a poor prognosis in cancer patients. Alterations in secretion may result in higher extracellular and lower intracellular levels of stefins and, therefore, a reverse correlation with patient survival is to be expected. However, cysteine proteases, and consequently their inhibitors, are involved in various physiological processes, including those which may act in a manner opposing tumor progression, such as apoptosis, activation of T-cell immune response, cell ▶ migration, seeding, and drug resistance processes. Thus, besides their concentration, cell and tissue localization of stefins, and a number of extrinsic and intrinsic factors, could make a critical switch between harmless and harmful.

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Stem Cell Factor

Definition

SCF; is a glycoprotein that acts as both a positive and negative regulator of hematopoiesis. The cell surface receptor for SCF is KIT, a cancer stem cell marker. SCF is also involved in mast cell development, gametogenesis, and melanogenesis.

▶ Kit/Stem Cell Factor Receptor in Oncogenesis
▶ Mastocytosis
▶ Stem Cell Markers

Stem Cell Hypothesis in Cancer

Definition

Tumors arise from cells termed cancer ▶ stem cells that have properties of ▶ adult stem cells, particularly the abilities to self-renew and differentiate into multiple cell types, and that these cells persist in tumors as a distinct population that likely causes disease relapse and metastasis. They are the only cells capable of, by themselves, giving rise to new tumors.

Stem Cell Markers

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Definition

Stem cell markers are molecules used for the identification of unspecialized, undifferentiated cells, and in the case of malignancy, presumptive ▶ cancer stem cells.

Characteristics

There is increasing evidence that subpopulations of neoplastic cells demonstrate heterogeneity with respect to proliferation, differentiation, and expression of cellular proteins characteristic of ▶ stem cells. Subpopulations of cells that express stem cell markers that can also be shown to contribute to tumor progression and resistance to chemotherapy are termed cancer stem cells. Cancer stem cells are found in very small subpopulations within tumors, in the range of 0.1–1% of the total cell number. Microscopically, cancer stem cells outwardly appear the same as any other tumor cell. Therefore, in order to identify these rare subpopulations, a number of stem cell markers have been identified and developed as a means of distinguishing stem-like cells from other cells within a cancer population. Unique expression patterns of stem cell markers provide a means for scientists to identify as well as isolate stem-like cells from heterogeneous tumor populations.

Stem cell markers are generally cell surface proteins with the ability to selectively bind to, or activate other signaling molecules. In some cases, stem cell markers are transcription factors that maintain stem cell properties of ▶ pluripotency and/or ▶ self-renewal. Stem cell markers are often designated by short-hand terms, based on their cellular function and/or the molecules to which they bind. As yet, there is no unanimously agreed-upon universal stem cell marker. Therefore, multiple markers are used in combination for verification of stem cell identity.

Stem cell markers have been assigned to embryonic, hematopoietic, and neural categories based upon the original location(s) in which they were discovered. These categories work fairly well for embryonic and adult stem cells; distinctions blur in cancer, where, for example, embryonic and/or hematopoietic stem cell markers can be found in tumors of neural origin. Some examples of commonly used stem cell markers with applications in cancer biology include the following:

ABCG2 (72,300 kDa) (also known as ATP-binding cassette superfamily G member 2, BCRP, BCRP1, BMDP, MXR, MXR1) defines a ▶ Hoechst-33342-negative phenotype of side population (SP) cells. ABCG2 is expressed in cancers of the blood, ▶ Brms1, ▶ prostate, ▶ lung, germ cell, and retina. ABCG2 may be a potential marker for positive selection of cancer stem cells from a wide variety of tissues.

Bmi-1 (44–46 kDa, murine viral ▶ oncogene homolog) is a ▶ polycomb group gene. In addition to its role in development, Bmi is also a tumor-associated antigen, expressed in cancers of the blood, brain, lung, oral mucosa, and gastrointestinal tract.

CD44 (80–95 kDa) is a ▶ cluster of differentiation (CD) molecule that functions as a receptor for hyaluronic acid. It is involved in cell/cell and cell/matrix interactions. Overexpression of CD44 has been associated with the development and spread of a range of different types of malignancies. CD44 is found in cancers of the blood, breast, prostate, germ cell, and lung.

CD133 (120 kDa), also a cluster of differentiation molecule, was first identified as a hematopoietic stem cell marker. It is a glycosylated protein which recognizes a CD34+subset of human hematopoietic stem cells. CD133 expression has been demonstrated in human leukemias, prostate cancer, germ cell tumors, as well as in ▶ brain tumors.

CD164 (80–90 kDa), also known as sialomucin, is a cluster of differentiation molecule expressed on CD34+hematopoietic progenitor cells. CD164 has been detected in prostate cancer.

▶ c-kit (145 kDa, CD117) is the membrane receptor for ▶ stem cell factor (SCF). It is an oncogene expressed in cancers of the blood, prostate, germ cell, lung, and gastrointestinal tract.

Musashi-1, 2 (39 kDa) are RNA binding proteins associated with ▶ asymmetric cell division (asymmetric

Stem Cell Markers. Table 1 Stem cell markers with applications in cancer biology for each marker; an "X" indicates localization to cancers of various tissues

Marker	Blood	Breast	Brain	Prostate	Retina	Germ cell	Lung
BCRP/ABCG2	X	X		X	X	X	X
CD133	X		X	X	X	X	X
CD34	X					X	
CD44	X	X		X		X	X
CD164	X			X			
Bmi-1	X		X				X
Sca-1 (mouse)	X			X	X		
Nestin			X				
Musashi-1,2	X	X	X		X		
Sox2			X				X
Oct3/4					X	X	
Nanog		X			X	X	
▶ c-kit	X			X		X	X

cytokinesis) in neural stem cells. Musashi-1 has been detected in cancers of the breast, brain, and retina, while Musashi-2 is rearranged in chronic myeloid leukemia.

Nanog (34 kDa) is a human embryonic stem cell marker and transcription factor also found in osteosarcomas, breast cancers, ▶ retinoblastomas, and germ cell tumors.

Nestin (177 kDa) is categorized as a neural stem cell marker. It is a class VI intermediate filament protein primarily expressed in stem cells of the central nervous system, including brain tumors.

Oct-4 (also termed Oct-3 or Oct3/4) is an embryonic stem cell marker. It is a ▶ POU transcription factor that confers self-renewal and pluripotency to embryonic stem cells. Oct 4 is expressed in both embryonic stem and germ cells, as well as in germ cell neoplasias, ▶ bone tumors, and retinoblastoma.

Sca-1 (18 kDa) is a mouse-specific stem cell marker (stem cell antigen 1, Ly-6A/E), and a member of the ▶ lymph node metastasis. Sca-1 is expressed in cancers of the blood, prostate, lung, breast, and retina.

Sox2 (34 kDa) is a transcription factor important in maintaining self-renewal properties of neural progenitor cells. Sox2 is an acronym for "SRY-related HMG-box gene 2." SRY refers to "sex-determining region Y," as the first Sox gene was found to be important in sex determination of developing gametes. HMG refers to ▶ "high mobility group (HMG group)," which is the DNA binding domain of Sox2. Sox2 has been localized to cancers of the brain, lung, and gastrointestinal tract.

A summary of stem cell markers with applications in cancer biology is shown in Table 1. Note that the field is changing rapidly, as new markers and tissue locations are added constantly. Therefore, this is not an exhaustive list, but rather a representative list of the most common stem cell markers to date that are applicable to cancer.

Methods Used to Detect Stem Cell Markers in Cancer Biology

Antibodies directed against stem cell markers can be used to identify and isolate stem cells. Fluorescently-labeled secondary antibodies directed against primary antibodies bound to stem cell proteins identify specific populations of stem-like cells.

Fluorescence-activated cell sorting can isolate rare stem cells from a heterogeneous tumor population. In this technique, a suspension of labeled cells passes by a laser, one at a time, and then through an electric field. The fluorescent cells become negatively charged and can be directed, based on charge, to a separate collecting tube for further analysis. In this way, a very small population of cells expressing stem cell markers can be isolated from a heterogeneous tumor population.

Magnetic beads linked to secondary antibodies also allow for the isolation of rare cell populations based on

expression of stem cell markers on the cell surface. Magnetically-beaded cells expressing stem cell markers can be separated based on magnetic attraction while the non-expressing cells are washed away. Once the beaded cells are isolated, the cells of interest can be freed from the magnetic beads for further analysis.

Fluorescent antibodies can also be used to visually assess cells as they exist within the tissue of origin or within mixed cell cultures. Fluorescently-labeled stem-like cells will emit light at specific wavelengths that can be visualized by fluorescence microscopy.

Polymerase chain reaction (PCR) can be used to detect the presence of genes coding for stem cell markers that are expressed in putative stem cells. This method is not as useful for isolating stem cells, but can be used to screen populations for stem cell markers and test isolated cell populations for expression of stem cell genes.

Clinical Applications

The hypothesis that a subpopulation of cancer stem cells drives tumorigenesis and chemo-resistance may lead to new approaches for cancer prognosis and therapy. Expression patterns of stem cell markers may indicate the differentiated or undifferentiated state of a tumor, and may correlate with a favorable or an unfavorable prognosis in the clinical setting. Once cancer stem cells are identified and characterized using stem cell markers, they can be targeted, using specific stem cell markers for immunologically-based therapies. New stem cell markers will continue to be discovered and are certain to play an important role in the rapidly emerging field of cancer stem cell biology.

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Stem Cell Niche

Definition

The local ▶ microenvironment that houses the stem cell population in a tissue or organ. Made up of cells, blood vessels, and extracellular matrix; ▶ stem cells adhere to the niche through ▶ cell adhesion molecules and receive signals, e.g., Wnt glycoproteins, that regulate their behavior.

▶ Stem Cell Plasticity

Stem Cell Plasticity

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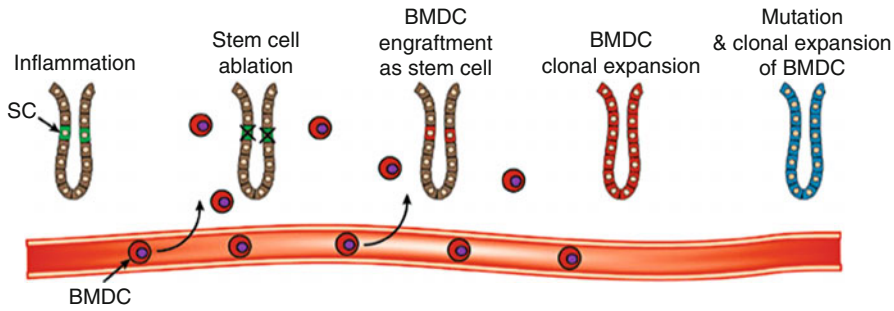
Synonyms

Metaplasia; Transdifferentiation

Definition

Stem cell plasticity refers to the ability of some ▶ stem cells to give rise to cell types, formerly considered outside their normal repertoire of differentiation for the location where they are found. Included under this umbrella title is often the process of "transdifferentiation" – the conversion of one differentiated cell type into another, and ▶ metaplasia – the conversion of one tissue type into another. From the point of view of this entry, some metaplasias have a clinical significance because they predispose individuals to the development of cancer. Circulating bone marrow-derived cells (BMDCs) that usually generate all blood cell lineages, can switch cell lineage commitment and contribute to the regeneration of several damaged non-hematopoietic tissues, and some carcinomas may even have their origins in BMDCs. The bone marrow origin of some tumor stromal cells and vasculature is now widely acknowledged, but since this pathway was not formerly



Stem Cell Plasticity. Fig. 1 Stem cell plasticity could provide an alternative pathway for cancer development as seen in experimental gastric carcinogenesis. Continued inflammation and tissue damage leads to eradication of the indigenous stem cell compartment and its replacement by bone marrow derived cells (BMDCs), whose progeny subsequently repopulate the whole gland. Mutation in a BMDC engrafted as a stem cell can then lead to a dysplastic gland and subsequent gastric cancer. Key: indigenous normal epithelial cells are *brown*, indigenous stem cells are *green*, BMDCs are *red*, and mutated BMDCs are *blue*

recognized, it too can be considered a further example of plasticity, which, importantly, has significant biological and therapeutic implications for cancer.

Characteristics

Most adult tissues have *multipotential* stem cells (▶ Adult Stem Cells), cells capable of producing a limited range of differentiated cell lineages appropriate to their location, for example, small intestinal stem cells can produce all four indigenous lineages (lysozyme-secreting Paneth cells, mucin-producing goblet cells, absorptive columnar cells, and enteroendocrine cells). However, tissue-based stem cells may be more versatile than previously thought, particularly those of bone marrow, and these cells may generate unexpected cell types when engrafted in a damaged non-hematopoietic tissue or organ. This so-called plasticity is being exploited in the field of regenerative medicine where it is hoped to produce new cell therapies for currently intractable diseases such as diabetes and congestive heart failure. Other sources of malleable stem cells include ▶ umbilical cord blood, ▶ mesenchymal stem cells (MSCs) from many sources including liposuction waste (fat), skin fibroblasts, and spermatogonia.

Stem cell plasticity has been questioned by some investigators who have been unable to reproduce some of the claims: "blood to brain," "brain to blood," "bone marrow to oocytes," and "bone marrow to cardiomyocytes." Other instances of plasticity have now been attributed to cell fusion between bone marrow cells (or their macrophage descendants) and cells of the recipient organ. Cell fusion may have implications for tumorigenesis. Such a process could endow differentiated cells with stem cell properties such as infinite self-renewal, while at the same time result in genetic instability with obvious tumorigenic potential.

From the viewpoint of cancer, we should note that while a scattering of engrafted cells of hematopoietic origin (but with a phenotype appropriate to their new location) is often observed in damaged parenchymal organs, these cells appear to have engrafted not as stem cells but either as ▶ transit amplifying cells or ▶ terminally differentiated cells, thus their long-term significance for cancer development is highly questionable. If BMDCs did engraft as stem cells in a new location, it is not inconceivable that they could be the founder cells of tumors at these sites. Indeed in a murine model of gastric cancer, BMDCs repopulate the gastric mucosa and over time contribute to metaplasia, dysplasia, and cancer in response to chronic infection with *Helicobacterfelis* (▶ Gastric Cancer, ▶ *Helicobacter pylori*). BM-derived gastric glands are seen after 20 weeks of chronic infection, leading to a final replacement of 90% of the gastric mucosa with BMDCs after 1 year. Upon progression to epithelial dysplasia and gastric adenocarcinoma, the majority of the dysplastic glands were of BM-origin, most likely from MSCs. Chronic inflammation led to atrophic gastritis, probably ablating the ▶ stem cell niche of numerous indigenous gastric glands, and the vacant niches were then occupied by BMDCs that subsequently behaved as gastric gland stem cells (Fig. 1) (▶ Stem Cells and Cancer).

An origin of carcinoma from BMDCs has also been suggested for one case of skin basal cell carcinoma arising in a female recipient of a male kidney transplant. In this case, most of the cytokeratin-positive tumor cells were male, and since BCC rarely if ever metastasizes (so no occult metastasis in the transplanted organ), it is likely that donor BMCs in the graft had migrated to the skin, either fusing with or differentiating into keratinocytes, before undergoing malignant transformation. On a cautionary note, it has been observed that in some murine and human cancers there are occasions when BMDCs incorporate into the tumors, phenotypically mimicking the cancer cells, but of course not actually initiating them.

Metaplasia

Metaplasias are major switches in tissue phenotype, invariably they occur in tissues subjected to chronic trauma, and are likely to represent epigenetic reprogramming of tissue stem cells – *in-situ* stem cell plasticity. Cancers can arise in areas of metaplasia, and we often have a metaplasia – dysplasia – carcinoma sequence. For example, the esophagus is normally lined by squamous epithelia, but due to acid reflux it can become lined by glandular tissue (Barrett's esophagus) and adenocarcinomas usually arise in areas of dysplastic Barrett's esophagus. In the airways of the lung, chronic irritation by tobacco smoke can lead to a switch from columnar to squamous epithelia, and squamous carcinoma of the lung usually arises from patches of squamous epithelia. Metaplasias may well be caused by misexpression of homeotic genes, a good example being intestinal metaplasia in the stomach caused by overexpression of the intestine-specifying *CDX* genes; here too, metaplasia predisposes to gastric cancer.

The Bone Marrow and Tumor Stroma: Clinical Relevance

BMDCs may indirectly influence tumor behavior by contributing to the ▶ desmoplastic response and to the tumor vasculature, lineages not formerly considered to have arisen from bone marrow (▶ Desmoplasia, ▶ Angiogenesis). ▶ Myofibroblasts are a distinguishing feature of pathological fibrosis, normally regarded as having originated by activation of local organ fibroblasts, but there is clear evidence that many are generated from bone marrow cells. These BMDCs can contribute to organ fibrosis, including the fibrosis surrounding many cancers – the desmoplastic response. Thus, BMDCs may be useful for the local delivery of anticancer agents such as interferon.

▶ Endothelial progenitor cells (EPCs) are mobilized endogenously in response to tissue ischemia or exogenously by cytokine therapy to augment neovascularization. The development of a vascular supply to a tumor is a prerequisite for tumor survival – allowing for the provision of oxygen and nutrients as well as the disposal of waste products. New vessel formation is also required for tumor metastasis. Previously, tumor vasculature was thought to develop exclusively *via* endothelial cell migration and proliferation – *angiogenesis*. However, the creation of new blood vessels by EPCs is known as vasculogenesis, and such a process is a significant event in tumorigenesis. This opens the possibility of using bone marrow cells as a vehicle for transporting antiangiogenesis molecules directly to the tumor vascular bed, in effect using BMDCs as Trojan horses.

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Stem Cell Telomeres

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Synonyms

Guanine-rich tandem DNA repeats of chromosomal ends; Telomeric repeats of stem cells

Definition

► Telomeres are guanine-rich tandem DNA repeats that cap the ends of eukaryotic chromosomes. Their primary function is to prevent chromosomal degradation, fusions, and instability. During cell division, telomeres shorten as a result of the incomplete replication of linear chromosomes. The slow rate of cell turnover in stem cell compartments means a longer period of ► telomere length stability in stem cells versus somatic cells. Nevertheless, stem cell telomeres do gradually shorten with age.

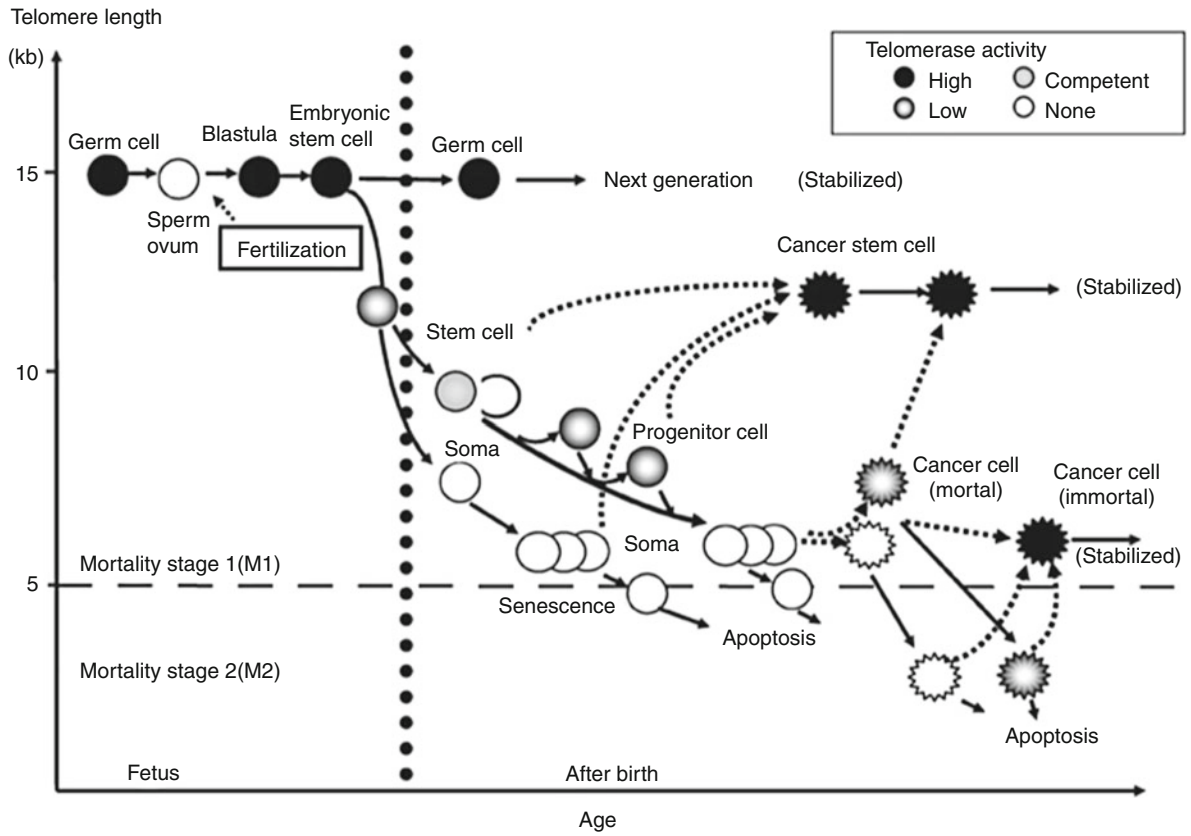
Characteristics

Human ► telomeres consist of tandem repetitive arrays of the hexameric sequence TTAGGG, with overall telomere sizes ranging from ~15 kb at birth down to <5 kb in ► senescence cells or in some chronic disease states. The end of telomere forms a 3´ overhang of the G-rich strand, which is generated by the postreplicative processing of the C-rich strand, folds back into the duplex telomeric DNA to form a protective t-loop. During the process of cell division, telomeres shorten as a result of the incomplete replication of linear chromosomes, the so-called ► end-replication problem. This progressive telomere shortening is one of the molecular mechanisms underlying aging, since critically short telomeres trigger chromosome senescence and ► apoptosis. A critical length of telomere repeats is required to ensure proper telomere function and avoid activation of the DNA damage pathways that result in replicative senescence or cell death.

The ends of telomeres are protected and regulated by telomere-binding proteins and form a special lariat-like structure called the ► t-loop that prevents degradation by exonucleases or processing as DNA damage signal caused by exposure of DNA ends. A human telomere needs >5 kb of its length to form the t-loop. When the length shortens to <5 kb, cells fall into senescence (► Mortality Stage 1 (M1)). In immortal cells, telomere length is maintained by ► telomerase, a reverse transcriptase, or another mechanism such as ► alternative lengthening of telomere (ALT). In most human somatic cells, except for stem cells and lymphocytes, telomerase activity is diminished after birth, such that telomere length shortens with each cell division (Fig. 1). Most malignant tumors must have a mechanism for bypassing M1, a cell-cycle checkpoint. The overcome of this checkpoint leads to an extended lifespan but continued telomere losses, eventually producing a crisis or the ► mortality stage 2 (M2) of replicative senescence caused by ► chromosomal instability, unless some mechanisms that escape this stage are activated. To escape M2, the telomeres in immortalized cancer cells must be stabilized by reactivation or upregulation of telomerase activity.

Germ cells and ► embryonic stem cells maintain telomere length between each generation with high levels of telomerase activity during rapid proliferation. In the developmental stage, telomerase activity gradually decreases and diminishes in most somatic cells after birth and falls in senescence (M1 stage). Thus, the telomere length of somatic cells is one of the limiting factors of cell division. In ► adult tissue stem cells, the level of telomerase activity is low or undetectable. Activity is upregulated in committed progenitor cells which have high reproducible activity in each tissue, but it is insufficient to maintain telomere length. On the other hand, most cancer cells bypass M1, which leads to an extended lifespan but continued telomere losses. Eventually, the consequence is crisis or entry into the M2 stage of replicative senescence. ► Cancer stem like cells and cancer cells are immortalized with stabilized telomeres by escaping M2.

Stem cells are capable of karyotypically stable, prolonged self-renewal. They are also characterized by their potential to generate a very large number of committed progenitors and descendants during a small number of self-renewal divisions. Since stem cells have elongated proliferative capacity, they must have a mechanism that maintains telomere length through many cell divisions. In human stem cells, telomere length is maintained for many cell divisions and telomere length remains in longer than somatic cells by telomerase or ALT. The former mechanism is at work in ► hematopoietic stem cells (HSCs) while the latter functions in ► mesenchymal stem cells (MHCs). The mechanism of telomere maintenance in human stem cells is different in each type of stem cell, but telomeres are not completely maintained in human stem cells except for embryonic stem cells. Thus, human ► adult stem cells have an intermediate existence between somatic cells and immortal cells, showing aging-related changes concomitant with telomere



Stem Cell Telomeres. Fig. 1 Telomere dynamics in human stem cells

shortening. The temporary activation or low expression of telomerase in adult stem cells may partially account fot the resistance to telomere erosion of stem cells in general but the telomere lengths of these cells were gradually eroded, consequently yielding the ageing of stem cells. The gradual loss of telomeres is interpreted as a regulator for cell life span and is considered as a cancer prevention mechanism, because prolonged replications and longevity would subject cells to the accumulation of mutations leading to transformation of stem cells to cancerous cells. If these stem cells are immortalized, they are no longer human stem cells but may become "cancer stem like cells." Recently, the important role of telomeres in human illness has been highlighted by studies of the rare genetic disorder "▶ dyskeratosis congenita (DKC)." This essay shows the role of telomeres and telomerase in the function and regulation of the stem cell compartment, and their importance in stem cell failure diseases.

In the hematopoietic system, low telomerase is detectable in stem cells with self-renewal potential and their early descendants, but age-dependent telomere loss is observed in both lymphocytes and neutrophils. In bone marrow transplant patients, the length of telomeres in blood leukocytes was shorter than that in germline cells from the same donor. A subset of stem cells isolated from adult bone marrow showed shorter telomeres than in fetal cord blood, suggesting that a progressive decline in telomere length with age occurs in hematopoietic stem cells. Thus, transplanted HSCs derived from aged donors may reach their proliferative limit during the lifetime of the recipient, resulting in graft failure after HSC transplant. The regulation of telomere length and telomerase activity is a complex and dynamic process that is tightly linked to cell-cycle regulation. Therefore, individual stem cell turnover at any given point would be minimal and the upregulation of telomerase activity in stem cells could be minimal. It is, however, upregulated in

lineage-committed progenitor cells that undergo rapid expansion, such as committed hematopoietic progenitor cells, and activated lymphocytes. In more mature cells, repression of telomerase activity is independent of proliferation. These findings suggest that one important function of telomerase in stem cells is to reduce the rate of telomere loss during cell division, preventing premature critical shortening of telomeres and loss of telomere function. Such a role of telomerase would be critically important in the case of increased proliferative demand, such as infection and regeneration.

In T and B lymphocytes, telomerase activity appears to be upregulated in response to mitogenically activated proliferation and progressively downregulated in more mature subsets. Telomerase is also essential for the lifelong maintenance of telomeres in normal memory lymphocytes that have a high rate of turnover in clonal expansion. Loss of the ability to upregulate telomerase in an antigen-specific highly differentiated memory cell leads to replicative senescence, resulting in a reduced response to reinfection. Low levels of telomerase activity have been found in some non-hematopoietic stem cells, including neuronal, skin keratinocytes, intestinal crypt, mammary epithelial, pancreas, adrenal cortex, and kidney stem cells (Fig. 1). However, some controversial data remain in telomerase activity and TERT (telomerase reverse transcriptase) expression in non-hematopoietic stem cells. The difference of the levels of telomerase activity in the components of various organs is likely derived from the difference of their cellular turnover among organs. It has been shown that hepatocytes enter senescence due to telomere shortening in the cirrhotic stage of a wide variety of chronic human liver diseases. The replicative senescence in hepatocytes is probably in part the result of continued proliferation during 20–30 years of chronic liver disease. Chronic inflammation, the presence of growth factors, and DNA-damaging agents such as reactive oxygen and nitrogen species may also play a role in this process.

Recently, the critical importance of telomerase activity in some human stem cells has been highlighted by the discovery of the etiology of dyskeratosis congenita (DKC), stem cell failure disease. In this disease, dysfunction of the telomerase RNA template gene causes the absence of telomerase activity and premature telomere shortening, resulting in bone marrow failure, intestinal disorder, or malignancy, at <50 years of age. Positional cloning associated the affected gene in many patients with X-linked DKC, termed *DKC1*, that encodes the dyskerin protein. The association of the telomerase complex with dyskerin suggested that the DKC phenotype may be the result of altered telomerase activity. The subsequent discovery of a 3′ deletion in the gene encoding TERC in a single large family with autosomal dominant DKC confirmed that telomerase deficiency is important to the etiology of DKC, and that telomerase is important for the maintenance of telomeric cellular lifespan and replicative potential in the stem cells of human organs.

In *Terc* knockout mice complete absence of telomerase activity is tolerated without abnormality in the first few generations; however, after the fourth generation, they begin to exhibit abnormalities similar to the phenotype of human DKC. Wild-type mice derived from late generations of *Terc* +/− mice with short telomeres and positive telomerase activity also displayed a similar phonotype of DKC. These findings indicated that short telomeres are the cause of stem cell failure. In addition, the observation that the *Cdkn1a* deletion improves the stem cell function and lifespan of mice with telomere dysfunction indicates that upregulation of ▶ p21 (WAF1/CIP1/SDI1) in response to shortened telomeres impairs repopulation capacity of stem cells in age-related diseases and senescence.

Aplastic anemia is generally thought to be the result of HSC damage or loss resulting in the failure of bone marrow stem cells to produce sufficient quantities of all hematopoietic lineages. Telomere length was significantly shorter in the peripheral blood granulocytes and monocytes of patients with aplastic anemia or related disorders. An inverse correlation between age-adjusted telomere length and peripheral blood counts was also observed in aplastic anemia. As in DKC-associated aplastic anemia, a report found mutations in the *TERC* gene of patients with aplastic anemia. Data from congenital disorders, like DKC and aplastic anemia, suggest that disturbed telomere maintenance may play a role in replicative exhaustion of the stem cell pool in vivo, again highlighting that the disturbance of stem cell telomere maintenance is an important etiology of "stem cell failure diseases," which are age-related diseases and premature senility syndromes.

Another mechanism of telomere maintenance in human stem cells is ALT. Mesenchymal stem cells (MSCs), which can be derived from bone marrow, can differentiate into multiple mesoderm-type cell

lineages including fibroblasts, adipocytes, osteoblasts, chondrocytes, and endothelial cells, as well as into non-mesoderm-type lineages including neuronal-like cells. In human MSCs (hMSCs), replicative senescence reportedly sets in rather early. Without growth factors, cells cease dividing around 40–50 population doublings, telomere length gradually shortens by 30–120 bp/PD, and telomerase activity is undetectable. However, hMSCs maintain long telomeres without the upregulation of telomerase activity for more than 100 population doublings in culture with basic FGF. Even using highly sensitive assays, no telomerase activity has been detected in hMSCs so far, and there may be a mechanism of telomere maintenance other than telomerase, such as ALT, in hMSCs. A recent observation of subtelomeric DNA hypomethylation facilitating telomere elongation in mammalian cells suggests that such epigenetic modification of chromatin may occur in hMSCs. However, in hMSCs, overexpression of telomerase indeed resulted in the elongation of telomeres, and *TERT*-transfected cells continued proliferating when untransfected control cells ceased growth. Furthermore, the potential of telomerase-overexpressing cells to form bone in vivo was greatly enhanced.

Embryonic stem (ES) cells, primitive stem cells, are likely immortal and are characterized by indefinite self-renewal and the potential to differentiate and contribute to the germ line. To maintain telomere length completely, ES cells display high levels of telomerase activity and hTERT expression, both of which are rapidly downregulated during differentiation and are much reduced or absent in somatic cells, including stem cells, in self-renewal tissues (Fig. 1). The downregulation of telomerase activity in differentiating ES cells is reportedly tightly correlated with histone deacetylation and DNA methylation of the *TERT* gene. High telomerase activity or the expression of TERT can, therefore, be regarded as a marker of undifferentiated ES cells.

In cloned animals originating from adult nuclei with shortened telomeres, telomere length in somatic cells has been found to be comparable with that in age-matched normal animals originating from a fertilized egg with long telomeres. This finding indicates that the enucleated oocyte has the ability to reset the telomere length of the nucleus derived from a donor adult somatic cell by the elongation of telomeres. How this mechanism is "reset" remains unknown in oocytes in cloned animals. Solving this mystery would be a technical breakthrough in developing cloned animals.

In summary, because of lifelong cell turnover of stem cells, telomere length in these cells is maintained longer than in somatic cells but gradually shortens due to aging. Insufficiency of telomere maintenance mechanisms in stem cells causes "stem cell failure diseases" such as DKC.

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Stem Cells

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Definition

With the exception of hemopoietic stem cells, morphological criteria do not exist to identify stem cells in most tissues, and therefore, they are generally defined in terms of their properties. *Stemness* is not a single property but a number of properties that a cell has the capability to perform depending upon circumstances. In adult steady state renewing tissues, a stem cell is a relatively undifferentiated cell capable of proliferation and self-maintenance, producing a variety of cell lineages and capable of tissue regeneration following injury. Probably the most important property of stem cells is that of *self-renewal*.

Stem Cell is a founder cell that exists in all multicellular organisms and shows self-renewal ability

through mitotic cell division and can differentiate into a wide range of lineage-committed cells. Upon division, each new cell has the potential to either remain as a stem cell or become another type of cell with a more specialized function. There are three kinds of stem cells: embryonic stem cells, germinal stem cells, and adults stem cells that have different developmental potentials: totipotent, pluripotent, multipotent, or unipotent.

Characteristics

Tissue Renewal and Models of Cellular Hierarchy

Many tissues in the adult undergo self-renewal, and accordingly, establish a lifelong population of relatively pliable stem cells – the ▶ adult stem cell. The different capacity of tissues to proliferate is the basis for conventionally categorizing tissues in adulthood into three categories:

- Those that are constantly renewing (e.g., bone marrow, intestine)
- Those that proliferate slowly but may renew their population in response to injury (e.g., lung, liver)
- Those that are more static (e.g., nerve, muscle)

Tissues are renewed by cell division and differentiation from a small number of stem cells, which have a high capacity for cell proliferation, but their actual rate of cell division is usually slow in the absence of injury or demand.

Between the stem cell and the mature cell of a particular tissue, a number of different stages of differentiation may be recognized, of which some retain a degree of stemness. Potten and Loeffler classify cells within a system into three types on the basis of their replicative potential as *actual stem cells, potential stem cells*, or *committed cells*. Actual stem cells are defined as undifferentiated cells capable of (1) proliferation; (2) self-maintenance; (3) production of large numbers of differentiated progeny; (4) regeneration of the tissue after injury; and (5) flexibility in the use of their options. The potential stem cells are latent or reserve counterparts of actual stem cells, which may be reactivated to become functioning stem cells. The essential properties of *stemness* may be retained by some proliferating cells located distally in a lineage. This is referred to as the *compartment model of cellular hierarchy* and is illustrated for the intestinal crypt in Fig. 1.

Stem Cell Division

It has been proposed that a specific type of mitosis occurs in the functioning stem cells, which is responsible for the self-maintenance of this cell type. In general, normal stem cell mitosis will proceed by *asymmetrical division* to produce one daughter stem cell and one daughter that continues to divide, mature, and differentiate. However, mathematical modeling suggests that about 5% of the time, a stem cell may undergo *symmetrical division* to produce either two stem cells or two maturing cells. In the former case, a stem cell is lost from its niche by differentiation, displacement, or apoptosis to ensure constancy in numbers. Promoting this form of division could be an approach to deplete mutated stem cells (probably the earliest step in carcinogenesis – see below) and may constitute an alternative strategy to inducing cell death to treat early neoplastic lesions.

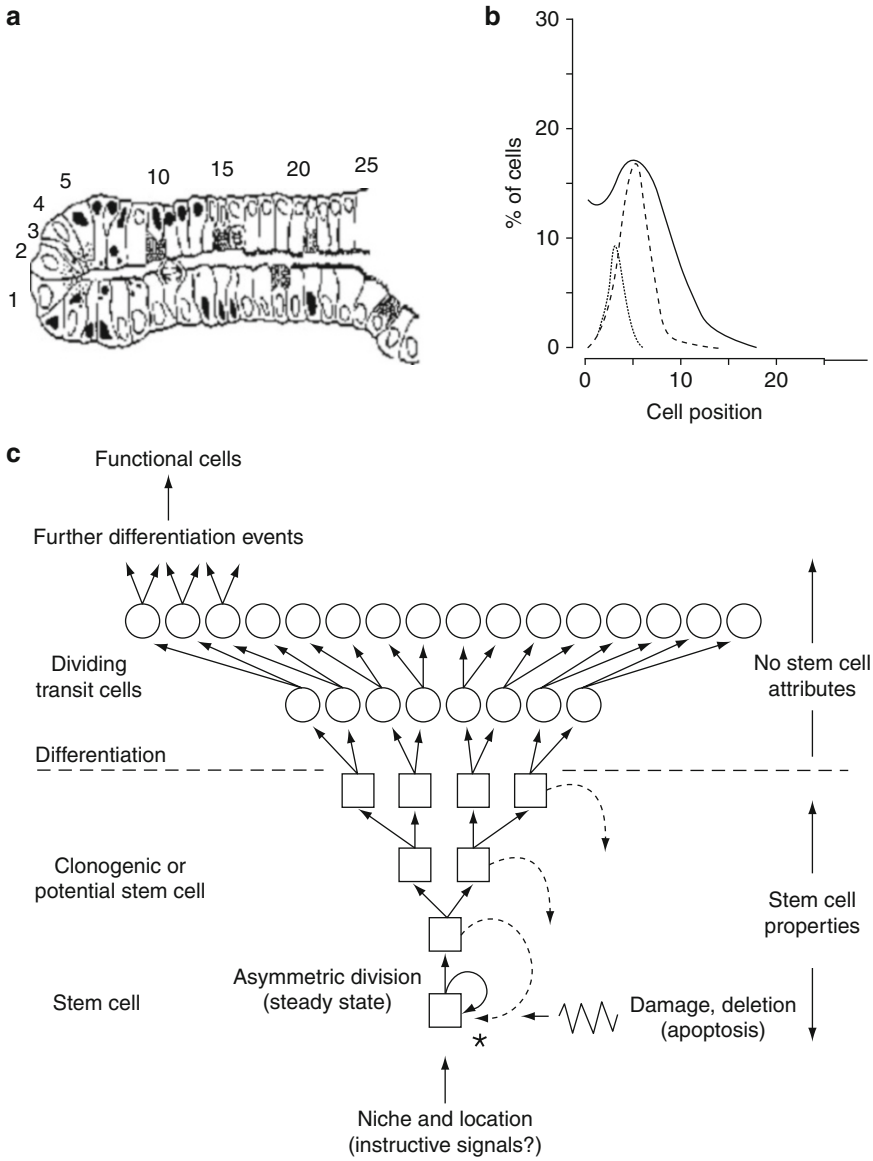
Stem Cell Markers and Identification Problems

The development of a stem cell specific marker for each tissue of origin is the "holy grail" of stem cell research. Several markers and approaches have been suggested; examples are listed in Table 1, but to date (with the exception of hemopoeitic stem cells), there is no ideal stem cell marker for adult stem cells.

In attempting to measure stem cells, one may find oneself in a circular argument. In order to answer the question whether a cell is a stem cell, we have to alter its circumstances, and in doing so inevitably lose the original cell, and one may only see a limited spectrum of responses. This situation has a marked analogy with *Heisenberg's uncertainty principle* in quantum physics – which states that the very act of measuring the properties of a certain body inevitably alters the characteristics of that body.

Stem Cell Location and the Niche

Adult stem cells are often localized to specific environments or *niches*. For a number of systems, these niches correspond to specific histologically identifiable locations, but these have not been determined for all tissue. Within each niche, the stem cells are influenced by neighboring cells and extracellular matrix. For each tissue, the stem cells in specific niches are *pluripotent* for the cell population of that tissue. Thus, for example, five different cell types (Paneth cell, goblet cell, entero-endocrine cell, M cell, enterocyte) are derived from the intestinal stem cell.



Stem Cells. Fig. 1 (**a**) Diagram of a longitudinal crypt (large intestine) section showing the cell positions. (**b**) The apoptosis frequency plot (radiation-induced: *solid line*) can be compared with the theoretical distribution of actual stem cells (*dotted line*), clonogenic or potential stem cells (*dashed line*) based on mathematical modeling. All these are centered round cell position 4. The *continuous gray line* represents the distribution of rapidly proliferating cells which are predominantly determined by the committed dividing transit cell. (**c**) The current model for a three-tiered hierarchical stem cell compartment is also illustrated. There are from four to six actual stem cells per crypt, but many more cells (potential stem cells) that are capable of stem cell function. When a stem cell undergoes a commitment to differentiation, it often first enters a transient state of rapid proliferation. Upon exhaustion of its proliferative potential, the transiently amplifying cell withdraws from the cell cycle, and executes its terminal differentiation.
*Approximately 5% are symmetrical divisions

Some stem cells give rise to a particular phenotype but there is heterogeneity in the cellular characteristics depending on the microenvironment – for example, blood vessels present in renal carcinoma metastases to the brain display the typical fenestrated morphology of renal vessels and not the continuous morphology of endothelial cells in invading brain vessels. Further studies demonstrated that transplanted bone-marrow cells (in sex-discordant transplant patients) may give rise to new hepatocytes in the livers of the recipients raising the possibility that human stem cells may be reprogrammed to express dormant areas of the genetic code, and thereby regenerate physically distinct phenotypes.

What remains unclear is whether stem cells are intrinsically different from daughter cells or whether they are instructed to be different by their microenvironment. In other words, could any cell behave as a stem cell if given the appropriate niche and signals – a phenomenon referred to as *plasticity*? This has implications for tumorigenesis and clinical situations such as tumor seeding and implantation. It is conceptually possible to manipulate any cell in vitro to behave like a stem cell but this is an unlikely scenario in vivo. The very early mammalian embryo (less than four cells) – the ultimate stem cells – is strong evidence favoring the argument that stem cells are intrinsically different.

Stem Cells. Table 1 Examples of potential stem cell markers and techniques for stem cell isolation

Marker/techniques	Tissue example	Comments
CD34	Bone marrow	A $CD34^+/CD38^-$ cell surface phenotype is found in acute myeloid leukemia stem cells and in normal primitive hematopoietic progenitors
CD44	Breast	$CD44^+/CD24^-$ cell phenotype isolated from human breast tumors cause breast cancer in SCID mice
CD133 (prominin)	Liver	A transmembrane protein on stem cells from several organ sites
	Bone marrow	
	Neural	
EpCAM (CD326)	Liver	Present on hepatic stem cells but also hepatoblasts and committed progenitors. Overexpressed in many cell lines
NCAM	Liver	Present on hepatic stem cells but not any progenitor cells thereafter
	Neural	
Musashi-1 (Msi-1)	Intestinal crypts	Musashi-1 gene encodes an RNA-bind protein that is required for asymmetric divisions. It may mark intestinal stem cells but also has broader expression in the crypt
Hes-1	Intestinal crypts	Like Msi-1, Hes1 may be expressed outside the stem cell region of the crypt
SOX2	Neural stem cells	SOX2 is expressed in multipotent neural stem cells at all stages of development
Stem cell antigen (Sca-1)	Breast	Sca-1 positive cells demonstrate enhanced regenerative potential
^{3}H-thymidine retention	Breast Intestine	Stem cells retain DNA synthesis incorporated label over a long period (see Fig. 2)
Electron microscopy	Breast	Identification of large light cells (ULLCs) and small undifferentiated light cells (SLCs) juxtaposed suggest they result from an asymmetric single mitotic event
Hoechst dye side population (SP)	Breast	SP mammary cells are similar to SLC cells and are highly enriched for Sca-1 expression
Laser capture microdissection (LCM)	Intestinal crypts	LCM was followed by DNA microarray analyses, which demonstrated increased expression of cell proliferating genes
Gene profiling	Skin	Examples include Wnt inhibitors (sFrp1, Dkk2, Wif1), cell cycle inhibitors (Gas1, Ak1, Inhbb), and TGF-β signaling components

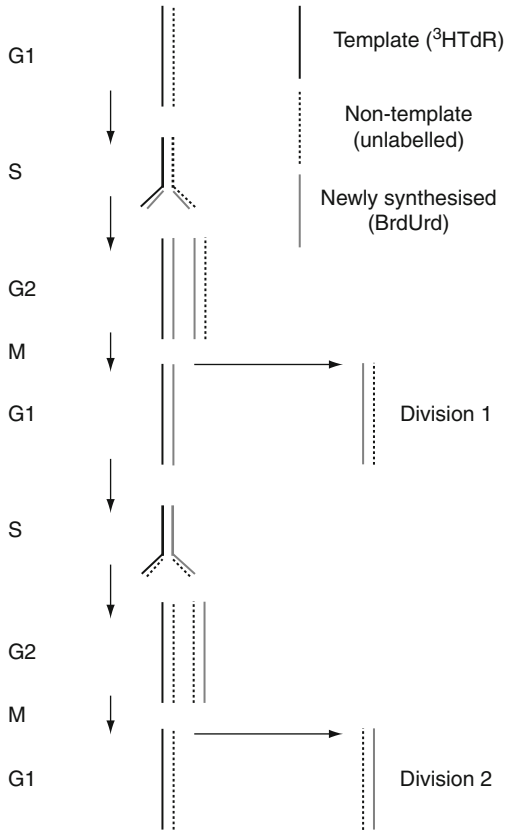
EpCAM epithelial adhesion molecule, *NCAM* neural cell adhesion molecule, *Hes-1* a homeobox gene expressed by murine embryonic stem cells, *SOX2* a homeobox gene transcription factor derived from murine embryonic stem cells

The Concept of Stemness and "Immortal" DNA Strands

A recurrent theme in stem cell biology is whether stem cells are long-lived progenitors with the intrinsic capability to self-perpetuate their pluripotency, or whether "stemness" is not an intrinsic property, but rather a nonautonomous feature. The latter model suggests that in every adult tissue with renewal capabilities there must be a niche that determines the stem potential of cells within. Owing to the general lack of specific markers, epithelial stem cells have been traditionally identified by their ability to retain radiolabeled thymidine for long periods of time. More than 25 years ago, John Cairns proposed that stem cells selectively retain old (i.e., labeled) replication error-free DNA strands while donating newly synthesized strands to their descendents which will be lost from the tissue after a short time. Although this has long been controversial, recent work demonstrates asymmetric segregation of chromatids in stem cells of small intestine using specific labels for new and old chromatids (Fig. 2). Retention of old chromatids by stem cells has also been demonstrated using in vitro models and in in vivo breast stem cell systems. The existence of "immortal" DNA strands has implications for understanding the lifespan and mutagenesis dynamics of stem cells, but it also implies that certain stem cell properties are maintained or inherited autonomously throughout adulthood.

Regulation of Stem Cell Numbers

Until recently, little was understood about the molecular regulation of stem cells. Over the past 5 years, several genes and signaling pathways have been shown to have important regulatory functions for some stem cells. Three key gene systems are ▶ *Wnt* (Wingless), *Shh* (Sonic ▶ Hedgehog), and ▶ *Notch*, and their associated tissue stem cells are listed in Table 2. However, as these genes frequently operate in other cell types, they cannot be called "stemness" genes.



Stem Cells. Fig. 2 Diagram showing the segregation of template and newly synthesized DNA strands in one chromosome. The Cairns' hypothesis proposed that all the chromosomes would behave in this way. The template strands are selectively retained by the stem cell daughter of a cell division, whereas the newly synthesized strands are segregated to the daughter cell destined to enter the dividing transit compartment and be shed from the tissue after a few days, thus removing any replication-induced errors. The label introduced into the newly synthesized strands takes two divisions to be removed from the stem cells. The label in the template strand would persist in the stem cell line

Properties of Stem Cells Which Favor Tumorigenesis

There is a general hypothesis in cancer biology that most cancers arise from a mutated stem cell, and there are a number of characteristics of stem cells which support this hypothesis. These are as follows:

- Capacity for self-renewal and progenitor production via asymmetric cell division
- Regulated by a niche – conceivably, disruption of this host control may lead to aberrant expansion of the stem/progenitor cells and cancer initiation
- Long-lived allowing time to accumulate multiple mutations

Stem Cells. Table 2 Signaling pathways that regulate stem cells

Signaling pathway	Stem/progenitor cell self-renewal	Implicated in tumorigenesis
Wnt	Hematopoietic	Colon carcinoma
	Epidermal	Epidermal tumors
	Intestinal	
Shh	Hematopoietic	Medullablastoma
	Neural	Basal cell carcinoma
	Germ line	
Notch	Hematopoietic	Leukemia
	Neural	Mammary tumors
	Germ line	

Wnt wingless, *Shh* Sonic hedgehog

- Ability to generate multiple lineages via downstream differentiation
- Active ▶ telomerase expression
- Activation of anti-apoptotic pathways (high ▶ Bcl2 and inhibitors of ▶ apoptosis (IAPs) proteins)
- ▶ Anoikis resistance allowing survival during dissemination
- Ability to undergo ▶ migration
- Increased membrane transporter activity, which may in turn be a mechanism of ▶ drug resistance
- Distinct signaling pathway patterns including Wnt, Notch, Snail, Twist, and Hedgehog

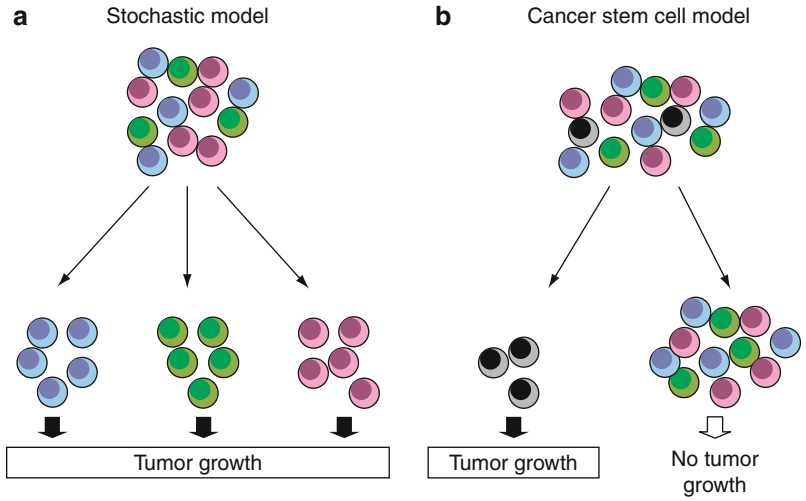
Evidence That Human Tumors Arise from Stem Cells

Additional evidence that most tumors arise from stem cells comes from the following:

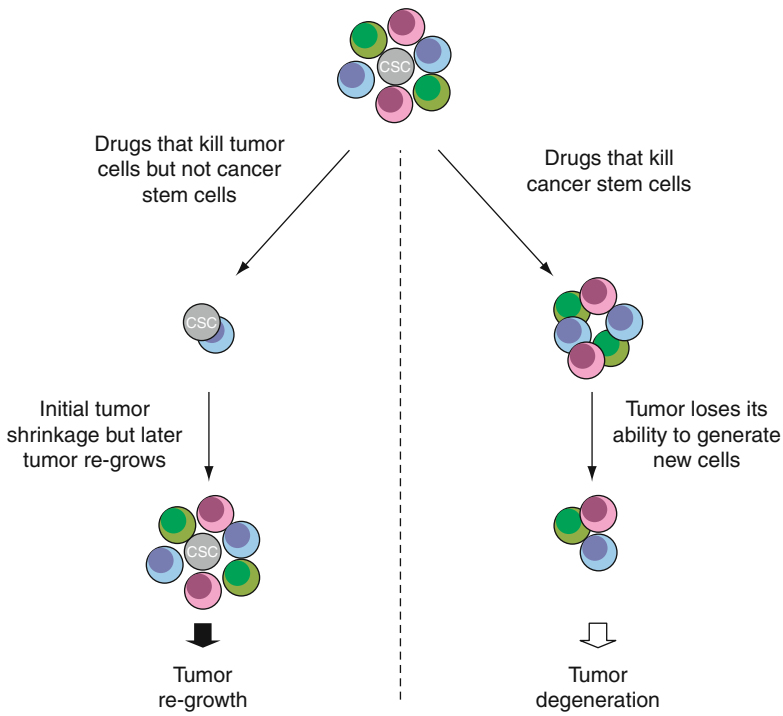
- Many cancers arise in tissues where self-renewal is essential (e.g., skin, gut, bone marrow).
- For the most part, human tumors are monoclonal, suggesting that they arise from a single transformed cell.
- Tissue-specific differentiation – most human tumors contain cell types consistent with an origin from the stem cells of that tissue.
- Changes in stem cell regulation mechanisms occur commonly and early during tumorigenesis.
- Biomathematical modeling supports the concept that tumors arise from mutated stem cells.

Cancer Stem Cells

Through increased understanding of the molecular biology of embryonic organ development and self-renewing adult tissues, a clearer idea is emerging that



Stem Cells. Fig. 3 Cartoon of two common models of tumor growth. In the stochastic model (**a**), tumor cells are heterogeneous with every cell having a low but equal probability with time of proliferating and forming new tumors. According to this model, the genetic changes leading to cancer development and progression are operative in all cells within the tumor. In the cancer stem cell model (**b**), tumor cells are heterogeneous but most cells have only limited potential to proliferate and only a small subset – the cancer stem cells (*gray*/*black shaded* cells) – have the ability to initiate new tumors



Stem Cells. Fig. 4 Conventional therapies (*left-hand side of panel*) may shrink tumors by killing mainly cells with limited proliferation potential. If the putative cancer stem cells (CSCs) are less sensitive to these therapies, then they will remain viable after therapy and reestablish the tumor. By contrast, if therapies can be targeted against cancer stem cells (*right-hand side of panel*), then they might more effectively kill the CSC, rendering the tumor unable to maintain themselves or grow

a unique population of stem cells – cancer stem cells (CSCs) – may be responsible for the maintenance of tumor growth (Fig. 3), and that these cells are inherently resistant to standard treatments. These concepts are not new, being first promulgated by Cohnheim in 1875. However, recently, a number of investigative teams have been able to successfully isolate subpopulations of human cancer cells with dramatically enhanced tumorigenic capacity when transplanted into immunodeficient mice. This has now been

successfully demonstrated for ▶ acute myeloid leukemia, ▶ breast cancer, ▶ colon cancer, ▶ brain tumors, and ▶ Pancreatic Cancer. However, we do not know with certainty whether or not CSCs are derived from normal stem cells.

As an extension of the CSC model, recent studies have postulated that migrating non-proliferating stem cells, located at the tumor invasion front, exhibit ▶ epithelial to mesenchymal transition, which may contribute to tumor ▶ metastases.

Clinical Implications of Cancer Stem Cells

The emergence of a molecular understanding of CSCs will have major implications for how we view conventional anticancer treatments, and how we develop novel anticancer strategies (Fig. 4). Conventional therapies may shrink tumors by killing mainly cells with limited proliferation potential. If the putative CSCs are less sensitive to these therapies, then they will remain viable after therapy and reestablish the tumor. By contrast, if therapies can be targeted against CSCs, then these might more effectively kill the CSCs, rendering the tumor unable to maintain themselves or grow. Thus, even if cancer stem cell-directed therapies do not shrink tumors initially, they may eventually lead to cure.

A fascinating question, at present, is whether or not stem cells express the molecules that clinicians are currently targeting such as estrogen receptors, ▶ HER-2, and ▶ Epidermal Growth Factor Receptor (EGFR). The major signaling pathways so far identified in stem cells are often mutated in cancers but they are not, by and large, the focus of current molecular treatments. Stem cell regulatory pathways (as outlined above) are thus the potential unexploited anticancer targets of the future.

▶ Adult Stem Cells
▶ Stem Cell Markers
▶ Stem Cells

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Stem-Cell Harvest and Purging

Definition

Following stimulation with growth factors, chemotherapy or both, CD34+ stem cells can be mobilized to the blood and harvested via a central vein catheter. In case of malignant bone marrow involvement, tumor cells may be mobilized as well. By in vitro ▶ purging the stem cells may be selected (e.g., CD34+ cell selection) or the tumor-cells depleted. By in vivo purging of the patient with monoclonal antibodies (e.g., CD20 antibodies in B-cell lymophomas), the amount of tumor cells in the stem cell product may be reduced to undetectable (by ▶ PCR) levels.

▶ Mantle Cell Lymphoma

Stem-like Cancer Cells

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Synonyms

Cancer stem cells; Cancer stem-like cells; CSC; TIL; Tumor progenitors; Tumor-initiating cells; Tumor-reinitiating cells; Tumor-repopulating cells

Definition

Cancer stem cells (CSC) are functional rather than having a fixed definition. The least stringent definition would be that the prospectively purified CSC

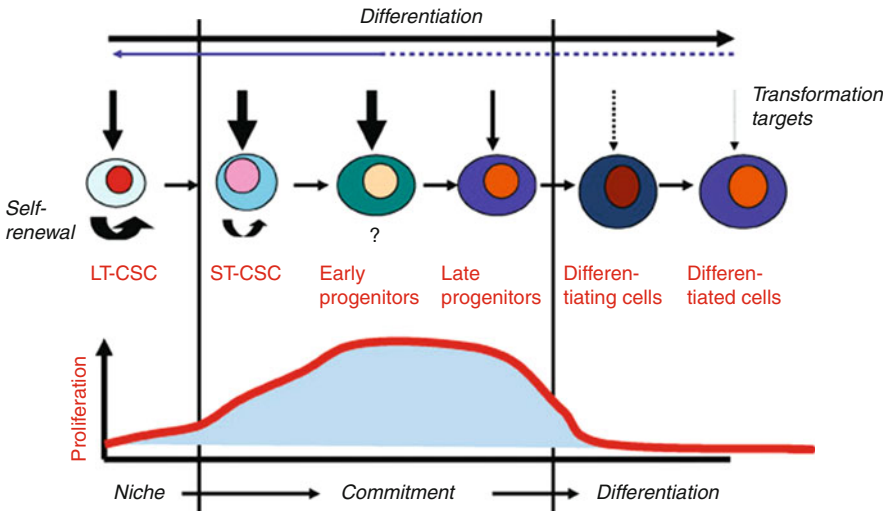
population is more tumorigenic than the bulk or the marker-negative tumor cell population(s) in a suitable tumor development assay. Using the most stringent definition, a cancer stem cell should be a cell that, at the single-cell level, can reconstitute, in a recipient animal, a tumor that is identical to the parental patient tumor and that can be serially xenotransplanted indefinitely. Therefore, in a strict sense, none of the CSC thus far reported can be truly classified as CSC and should more appropriately be called tumor-reinitiating cells. In reality, it will be very difficult to identify a tumorigenic cell that can fulfill the most stringent definition of a cancer stem cell mentioned above. Firstly, a tumor, especially a solid tumor, is made of numerous cell types. To expect one cell or even a population of cells, when transplanted into a foreign host (i.e., mice) in an exotic environment, to fully reconstitute an original patient tumor in its complete composition is very difficult and essentially impossible to prove. Secondly, when such experiments are actually done, the best one can do is to coinject the putative tumorigenic cell population with stromal components (e.g., fibroblasts, carcinoma-associated fibroblasts or CAFs, urogenital sinus mesenchyme or UGM, etc.) in an extracellular matrix (e.g., Matrigel or collagen) into an "orthotopic" animal site such as brain, mammary fat pad, or prostate lobes. These so-called orthotopic sites are considerably different from their human counterparts and tumor establishment would inevitably require the recruitment of various host (i.e., mouse) cells by the tumor-reinitiating cells. Such "reconstituted" tumors could never be identical to the original patient tumors. Thirdly, during the purification process, the majority of cells are often discarded to obtain marker-positive and marker-negative populations. These discarded cells would be important in the original tumor composition but they would be very difficult to reconstitute in tumor development assays. Altogether, one can say, at the very best, that the experimental tumor reconstituted from the presumptive CSC histologically "resembles" the (patient) primary tumor.

Therefore, we probably have to seek a middle ground when making the claim to a cancer stem cell. A functional definition of CSC is as follows. First, the presumptive CSC (i.e., the cell population enriched in putative CSC) must be prospectively purified from, e.g., cell cultures, xenografts, and/or primary tumors. When purifying candidate populations of CSC from tumors, lineage selection must be performed to remove "irrelevant" cells such as stromal and blood cells that may contain other ▶ stem cells (SC) including mesenchymal and hematopoietic SC, which might have the ability to undergo transdifferentiation. Second, in vivo tumorigenicity experiments must be done to show that such cell populations are enriched in tumor-reinitiating cells. When feasible, serial tumor xenotransplantation should be carried out to determine whether the tumors derived from the putative CSC can be transplanted for multiple generations. Histologically, the reconstituted as well as serially xenotransplanted tumors should resemble the original tumor. Third, importantly, the presumptive CSC population, or a subpopulation within, has to be studied to show that they possess certain intrinsic biological properties normally associated with SC elaborated below. Only when these conditions are fulfilled can one confidently claim that the candidate population of tumor cells under investigation is enriched in potential CSC or tumor-reinitiating cells. Importantly, even such tumorigenic populations are likely heterogeneous with true CSC representing perhaps only a very small fraction.

Characteristics

CSC identified in different types of tumors or the same type of tumor but from different patients may manifest very different biological and functional properties. On the other hand, all CSC are expected to share certain common properties such as relative dormancy but with extended proliferative capacity, significant clonogenic potential, preferential expression of stem cell–related genes (e.g., self-renewal genes), the ability to undergo asymmetric cell division, and enhanced in vivo tumorigenic and metastatic potentials.

Theoretically, tumor cells in any cancer, depending on the differentiation status, may be organized as a hierarchy that can be roughly classified as long-term CSC (LT-CSC), short-term CSC (ST-CSC), early and late tumor progenitors, and differentiating and differentiated tumor cells (Fig. 1). The LT-CSC should possess indefinite self-renewal and ST-CSC some self-renewal properties. The early tumor progenitors may or may not possess any self-renewal activities. The LT-CSC, localized in putative CSC niche, likely proliferate slowly although these cells possess the highest proliferative potential. By contrast, the cell



Stem-like Cancer Cells.
Fig. 1 This figure presents a hypothetical model of tumor cell hierarchy. LT-CSC localized in putative niches make the commitment to develop into ST-CSC and tumor progenitors, which in turn differentiate into "functional" cell types such as PSA-producing prostate cancer cells. In most tumors, tumor progenitors and ST-CSC may constitute the bulk of proliferating cell compartment and their normal counterparts may represent the major transformation targets. See text for more descriptions

types that constitute the bulk of the proliferating cell compartment in a tumor are mostly tumor progenitors and ST-CSC (demarcated by two vertical lines, Fig. 1). Differentiating and differentiated tumor cells, which should express tissue-specific differentiation markers, are hypothesized to lack proliferative capacity and self-renewal in vivo. In theory, both CSC (LT- and ST-CSC) and tumor progenitors should have the ability to regenerate tumors in tumor development assays. In principle, however, tumors initiated by CSC should be able to be passaged indefinitely whereas tumors initiated by tumor progenitors can only be propagated for a limited number of times.

Putative CSC are often thought to derive from normal SC, which may or may not be the case. In fact, because normal progenitor cells are the major proliferating cells in a tissue or an organ and they may possess certain self-renewal abilities or they may acquire self-renewal abilities by the transforming events (e.g., silencing of p16, mutations of ▶ PTEN, etc.), these cells are more likely the targets of initial tumor transformation (Fig. 1). In other words, transformed tissue progenitor cells may actually be the real CSC. Alternatively, the initial tumor transformation may occur in normal SC but further genetic mutations and/or epigenetic alterations take place in more mature tumor progenitor cells, which thus represent the real CSC that drive tumor formation, ▶ progression, and recurrence. Both of these concepts have been recently born out in acute and chronic myelogenous leukemia (AML and CML). Finally, although most differentiated cells are thought not to be able to "dedifferentiate" (i.e., going back along the lineage development; Fig. 1), some somatic cells such as hepatocytes and endothelial cells are known to be able to replicate themselves. Therefore, some differentiated cells may also become transformation targets and subsequently become CSC.

Identification

Putative CSC have now been reported in multiple human tumors including AML, CML, multiple myeloma (MM), ▶ brain tumors (glioblastoma, medulloblastoma, etc.), breast tumors, ▶ melanoma, and ▶ prostate cancer, ▶ colon cancer, and ▶ gastric cancer. In principle, CSC can be identified by several experimental strategies.

1. *Marker-Based Analysis*. A variety of adult tissue SC are found to express relatively specific markers, which can be cell surface or intracellular such as nuclear. Interestingly, many reported CSC also seem to express the cell surface markers that identify their normal counterparts. This observation provides a relatively simple enrichment procedure utilizing either flow cytometry-based cell sorting or microbeads-based affinity purification. For nuclear markers, a marker promoter-driving reporter construct such as GFP-tagged retroviral or lentiviral vector system can be developed to track down putative CSC and then purified by flow cytometry. The disadvantages associated with using predetermined marker(s) to identify CSC include that the marker proteins frequently change during cell development in vivo and cell

preparation in vitro and that in most cases the functions of "stem-cell" markers in both normal SC and stem-like cancer cells are unclear.

2. *Side Population (SP) Analysis*. Mouse hematopoietic SC are found to preferentially express multidrug resistance (MDR) family proteins such as MDR1 and other membrane transporters such as ABCG2 (also called BCRP for breast cancer resistance protein). This property allows the HSC, in an experimental setting, to pump out the Hoechst 33342 dye. Therefore, on dual-wavelength flow cytometry, the HSC-enriched cell population is identified as a "side" or tail Hoechst^{dim} population at the lower left quadrant of the histogram. By contrast, the major population of cells, devoid of HSC, is displayed as Hoechst^{hi} cells called as non-SP or main population (MP). Recent work reveals that multiple adult tissue SC can also be enriched by the SP protocol and that the SP from several cancer types are also enriched in stem-like cancer cells. The major advantage of this technique when used to identify putative CSC is its simplicity. The potential problems associated with the technique are that chronic accumulation of Hoechst dye in non-SP cells may be cytotoxic (thus invalidating suitable controls) and that SP cells isolated from some normal tissues seem to be enriched in progenitor cells rather than SC.
3. *Sphere-Formation Assays*. Many normal SC such as neural, hematopoietic, and mammary SC, when maintained under special culture conditions, can form three-dimensional spheres, which are like miniorgans that can differentiate into multiple cell types. Putative CSC identified in brain and prostate tumors as well as in melanoma also have the ability to form anchorage-independent spheres. The advantage of using sphere-forming assays to enrich for CSC is its initial independence of specific markers. The disadvantages include the empirical nature of finding culture conditions suitable for sphere formation and the necessity of finding ways later to identify and purify the real CSC from the spheres.
4. *Label-Retaining Properties*. Mammary SC and normal keratinocyte SC in interfollicular epidermis and hair follicle bulges are quiescent and can be identified with a pulse label with the thymidine analog BrdU (bromodeoxyuridine) followed by a long-term "chase" (i.e., removal of the label). Fast-proliferating progenitor cells dilute out the BrdU label after several cell divisions whereas the slow-dividing SC retain BrdU and thus identified as "label-retaining cells" or LRCs by either immunohistochemistry or flow cytometry analysis. Recently, the LRCs purified from the bulge regions in transgenic mice indeed are found to be enriched in SC based on both gene expression profiling and functional assays. Interestingly, the LRCs in human breast tumors coexpress mammary epithelial SC markers and seem to have certain SC properties. Human prostate cancer cell spheres and xenograft tumors also possess slow-cycling LRCs. These tumor LRCs are yet to be prospectively purified out to show that they indeed represent slow-cycling CSC.

Clinical Implications

CSC are thought to have high levels of prosurvival mechanisms such as heightened expression of antiapoptotic BCL-2 family proteins (including Bcl-2, Bcl-xL, and Mcl-1), ▶ telomerase, and antioxidant and detoxifying enzymes. In addition, they preferentially express cell surface pumps such as ABCG2 and MDR1. Furthermore, they generally proliferate much slower than the progenitor cells. Most current anticancer therapeutics targets fast-proliferating tumor progenitor cells. All these together lead to relatively transient therapeutic efficacy as CSC will most likely survive therapeutics due to their relative dormancy, increased survivability, and enhanced capacity to efflux therapeutic agents. CSC, once mobilized to the cell cycle, may regenerate a less differentiated tumor with expanded CSC and tumor progenitor cell compartments and result in drug-resistant tumor progression and ▶ metastasis. There is emerging evidence that CSC are more resistant to therapeutic regimens.

CSC, due to their different genetic makeups and signaling requirements than in their normal counterparts, might be preferentially targeted. For instance, leukemia SC seem to show some differences in the expression of surface marker repertoire compared to normal hematopoietic SC. Therefore, these different markers may be taken advantage of for the antibody-based therapeutics. CSC with mutant ▶ p53 and PTEN tumor suppressors may be particularly more sensitive to apoptosis induction induced by restoration of p53 and PTEN expression/functions. Leukemic SC are recently shown to be extremely sensitive to cell death

triggered by BH3 mimetics, presumably because of their heavy reliance on Bcl-2 and Mcl-1 for their survival. Finally, normal neural and mesenchymal SC have been employed alone or as vehicles to carry cytotoxic cytokines or gene products to specifically target CSC in certain tumors such as ► glioblastoma multiforme.

Stent

Definition

A synthetic (usually plastic or alloy mesh) tube inserted into a blood vessel, ureter, or segment of intestine to prevent or treat blockage.

► Desmoid Tumor

Steradian

Definition

The SI unit (international system of units) for solid angle.

► Bioluminescence Imaging

Stereocenter

Definition

A carbon atom with four distinct functional groups.

► Small Molecule Screens

Stereotactic Radiosurgery

Synonyms

Radiation surgery; Radiosurgery; Stereotactic external-beam radiation; Stereotactic radiation therapy

Definition

A radiation therapy procedure that uses special equipment to position the patient and precisely deliver a large radiation dose to a tumor and not to normal tissue.

► Glioblastoma Multiforme

Sterile-α-Motif

SAM domains are a class of protein modules characterized by a globular structure consisting of five a-helices and can mediate interactions with other proteins, RNA, and lipids.

Steroid Hormone Receptor Coactivators

Definition

Are defined as cellular factors that do not bind DNA directly but are recruited to the promoters by steroid hormone receptors. These promoters enhance agonist-dependent transcriptional activation by facilitating transcription initiation through interaction with components of the basal transcription machinery. Coactivators also enhance transcriptional activity of SHR by other mechanisms such as modulating alternative RNA ► splicing.

► Progestin

Steroid Hormone Receptors

Synonyms

SHR

Definition

Members of a superfamily of nuclear receptors that function as ligand- or hormone-dependent transcription factors. The term superfamily defines a set of

genes derived from a single progenitor gene that has diverged to produce the unique functions of its members.

► Progestin

Steroid Receptor Coactivator-3

► Amplified in Breast Cancer 1

Steroid Sulfatase

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Synonyms

Aryl sulfatase C; Steroid sulfohydrolase; Steryl sulfohydrolase; STS

Definition

Steroid sulfatase (STS) is an enzyme which is virtually ubiquitous throughout the human body and its activity is implicated in a range of physiological processes and pathological conditions. Its prime responsibility is for the hydrolysis of alkyl (e.g., dehydroepiandrosterone sulfate (DHEAS)) and aryl steroid sulfates (e.g., estrone sulfate (E1S)) and therefore plays an essential role in regulating the formation of steroids such as DHEA and E1 which can be converted to biologically active steroids (e.g., androstenediol (Adiol) and ► estradiol (E2)).

Characteristics

The activity of STS was first discovered in rat liver microsomes. Now it is known to be active in a plethora of tissue types, including testis, ovary, placenta, skin, lung, brain, and bone. Therefore it is thought to be found in small quantities throughout the body. It is a member of a superfamily of 12 different mammalian sulfatases. The gene for the human STS is located on the distal short arm of the X-chromosome. The actions of this enzyme make a large contribution to the in situ estrogen production in hormone-dependent malignant tissues. Little is known about the regulation of STS gene expression or enzyme activity. However, the expression levels of mRNA and enzyme activity are increased in cancerous breast and endometrial tissue compared to normal tissues. During the last decade there has been a significant amount of research carried out to elucidate the role that STS plays in the conversion of steroid sulfates, such as DHEAS and E1S, and the consequent formation of biologically active steroids, such as androstenediol and E2.

The highest incidence of breast cancer occurs in postmenopausal women after the cessation of the production of ovarian estrogens. However, estrogens are still produced by the local conversion of androstenedione to E1, a reaction that is catalyzed by the ► aromatase enzyme. A major proportion of the estrogens that are formed are available for conversion by estrone ► sulfotransferase into E1S which is biologically inactive because it is unable to bind to the ► estrogen receptor (ER). However, steroid conjugates, because they bind to albumin, have a much longer half-life in the plasma (about 9 h) compared to the unconjugated estrogens and are also found in circulating blood at greater concentrations. This acts as a potential reservoir for the formation of biologically active estrogens, e.g., E1, via the actions of STS (see STS figure). The E1 thus formed is further metabolized by 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1 to E2 which is the most biologically active natural steroid that can interact with the estrogen receptor (ER) and stimulate tumor cell growth. In postmenopausal women, the production rates of E1 and E2 are ~ 40 μg/day and 6 μg/day, respectively.

There is another route via which STS may influence the growth of some tumors. There is evidence that DHEAS and its unconjugated metabolite DHEA, stimulate cancer development. These steroids are able to act as precursors for the formation of steroids with estrogenic properties, such as Adiol. Furthermore, various studies have shown that DHEAS, DHEA, and Adiol all have the ability to increase proliferation of

breast cancer cells in vitro and are capable of stimulating the growth of carcinogen-induced mammary tumors in vivo. The most abundant of these steroids is DHEAS which is secreted by the adrenal cortex. Removal of the sulfate group on DHEAS by STS results in the formation of DHEA which can undergo a further reduction to make Adiol (see STS figure). In postmenopausal women, a significant proportion of Adiol produced comes from DHEAS and DHEA in the peripheral tissues. The importance of Adiol becomes evident when it is known that it can bind to the ER, albeit with a slightly lower affinity than E2. However, as Adiol plasma concentrations are reported to be about 100-fold higher than those of E2 in postmenopausal women, its significance in the development of cancer should not be underestimated.

Regulation of STS

There has been little research carried out as to how the STS enzyme is regulated in situ. It is thought that the cytokines ▶ interleukin (IL)-6 and tumor necrosis factor (TNF)-α can act synergistically to increase STS activity in some breast tissue and cancer cells. Conversely, IL-1β, an inflammatory cytokine, is capable of reducing STS activity and mRNA levels in human endometrial stromal cells, vascular smooth muscle, and human aortas. The presence of these cytokines in breast cyst fluid may explain the differential regulation of STS in breast cancer cell lines by breast cyst fluid. Also, basic ▶ fibroblast growth factor and ▶ insulin-like growth factor type I (IGF-I) are known to increase STS activity in some breast cancer cells. This indicates that these growth factors, which are released by malignant breast tissue, can elevate local estrogen levels.

Other steroids are also thought to influence the expression of STS mRNA and subsequent activity. The use of exogenous testosterone treatment in the male mouse causes an induction of STS. The uteri of pregnant guinea pigs have greater levels of STS activity compared to fetal or mature females which implies an estrogenic regulation. The substrate for STS, E1S, is thought to increase STS activity in the liver of ovariectomized rats. Conversely, a reduction in STS mRNA levels was found when breast cancer cells were treated with the ▶ progestagen, Promegestone. However, exposure to the same cells with a different progestagen stimulates STS activity in these cells. Therefore, it seems likely that STS expression and activity can be regulated by other steroids suggesting various feedback mechanisms, which are still poorly understood, are active.

STS in Hormone-Dependent Breast Cancer (HDBC)

There is increasing evidence to support the importance of STS in human breast cancers. Breast tumor tissue of postmenopausal women can have as much as ten times the estrogen levels than are seen in the plasma of the same patients. Furthermore, STS activity has been shown to be at least 50 times greater in both pre- and postmenopausal breast tumors compared with normal breast tissue. STS expression is detected in 90% of breast tumors, whereas aromatase expression is only found in 60–70%, and activity of STS in breast tumors is much higher than that of the aromatase complex. This increased STS activity could account for as much as a tenfold greater amount of E1 originating via the STS route than via the aromatase pathway.

Real time RT-PCR experiments have demonstrated that STS mRNA expression in malignant breast tissue is significantly higher than that in normal tissue. Clinical studies have now shown that STS mRNA expression may be a predictor of recurrence in breast cancer patients and that this association and prognosis only applies to ER + ve tumors. Significantly, elevated STS mRNA expression is associated with a poor prognosis in both pre- and postmenopausal women. This indicates that even in premenopausal women intra-tumoral estrogen synthesis could play a major role in the growth of breast tumors. The overexpression of the aromatase enzyme has also been examined as a potential prognostic marker as it is also supposed that its actions are pivotal in regulating tumor estrogen synthesis. However, it was found to have no prognostic value. Therefore, it is possible that the STS pathway may be more important than the aromatase pathway for the production of biologically active estrogens in the tumor. STS mRNA levels have been shown to correlate with tumor size and to be elevated in some tumors exhibiting metastasis compared to nonmetastatic cancers.

STS Inhibitors

The importance of STS in the development of various cancers has led to the design and synthesis of a range of STS inhibitors. The main therapeutic focus for these compounds has been targeted on breast cancer in

postmenopausal women. These patients are initially treated with standard endocrine therapy, such as antiestrogens, or more recently, aromatase inhibitors. Unfortunately, many breast tumors will fail to respond to these therapies making the use of STS inhibitors a potential option for therapeutic intervention. STS inhibitors come under three main categories:

1. *Alternative substrates*. These compounds (e.g., DHEAS, pNPS, MUS, flavone, and isoflavone sulfates), which contain at least one sulfate group in the structure, are designed to compete with E1S for binding to the active site of STS and, as a consequence, impede the hydrolysis of the natural substrate to E1. However, they are in principle alternative substrates for STS and hence the value of using these agents clinically in the treatment of HDBC is limited.
2. *Reversible inhibitors*. Reversible STS inhibitors are mainly E1 derivatives that are designed to compete with E1S for the enzymes active site but remain metabolically stable by not acting as potential substrates.
3. *Irreversible inhibitors*. The majority of STS inhibitors reported to date belong to this class of inhibitor. EMATE (estrone-3-*O*-sulfamate), the very first highly potent STS inhibitor, inhibits STS in a time- and concentration-dependent fashion. Its sulfamate group (OSO_2NH_2) was originally designed to mimic the sulfate group of E1S. However, despite EMATE being orally active and highly potent, it is not considered as a suitable agent for HDBC therapy because it was found to be strongly estrogenic in rodents. As a result, nonsteroidal STS inhibitors were developed, of which STX64 (BN83495, 667COUMATE) has shown excellent efficacy in various in vivo tumor models and also, promising results in a recent Phase I trial in patients with advanced breast cancer. This "proof of concept" provided by STX64 strengthens the role of STS inhibitors in the treatment of HDBC.

There is still a considerable amount of research to be done on establishing the roles of STS in hormone-dependent cancer, mainly in the breast and prostate. The availability of many structurally diverse STS inhibitors may further assist in this work. While STX64 is the first and most advanced development STS inhibitor to date, the considerable therapeutic benefit of STS inhibitors on patients with hormone-dependent malignant tumors should further encourage the development of second-generation inhibitors.

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Steroid Sulfohydrolase

► Steroid Sulfatase

Steroidogenesis

Definition

Refers to the synthesis of steroid hormones such as estradiol, testosterone, and mineralcorticoids.

► Estradiol

Steroid-Refractory Graft-Versus-Host Disease

Definition

A potentially fatal bodily condition that results when T cells from a tissue or organ transplant, especially a bone marrow transplant, react immunologically against the recipient's antigens, attacking cells and tissues (refractory to steroids).

► Rituximab

Steryl Sulfohydrolase

► Steroid Sulfatase

Stevens-Johnson Syndrome

Definition

► Erythema Multiforme Major.

Stewart-Treves Syndrome

Definition

Is a rare, deadly cutaneous ► angiosarcoma that develops in long-standing chronic ► lymphedema. Most commonly, this tumor is a result of lymphedema induced by radical ► mastectomy to treat ► breast cancer. Unfortunately, although the breast cancer may be cured with such radical surgery, this second primary cancer may be responsible for the patient's worsening course.

STI-571

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Synonyms

CGP57148; Gleevec; Glivic; Imatinib; Signal transduction inhibitor-571

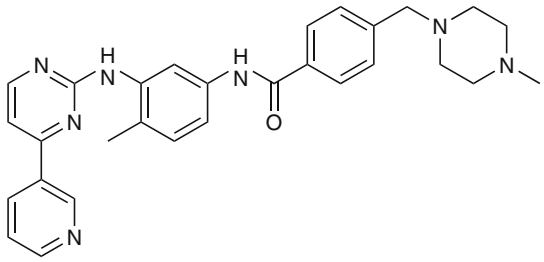
Definition

STI-571 is an oral drug marketed by Novartis, and is currently used for the treatment of certain types of ► cancer(s), including ► chronic myeloid leukemia (CML) and ► gastrointestinal stromal tumor (GIST).

Characteristics

Physicochemical Properties

STI-571 is a small compound belonging to the phenylaminopyrimidine class of compounds (Fig. 1).



STI-571. Fig. 1 Structure of STI-571

It is chemically designed as 4-[(4-methyl-1-piperazinyl)methyl]-N-[[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamidine methanesulfonate, with a molecular formula of $C_{29}H_{31}N_7O{\cdot}CH_4SO_3$. Its molecular weight is 589.72.

Pharmacology and Pharmacokinetics

► Protein kinases are an important group of ► enzymes that activate a range of substrates through phosphorylation. According to the nature of the phosphorylated –OH group in their substrates, they are classified as protein-serine/threonine kinases, or protein-► tyrosine kinases; in addition, a small group of dual specificity kinases catalyze phosphorylation of both threonine and ► tyrosine. ► Mutation or dysregulation of these enzymes is a frequent cause of the excess proliferation and/or reduced ► apoptosis of cancer cells. Therefore, protein kinases have become important therapeutic targets.

In humans, at least 518 protein kinase genes have been identified, with 385 coding serine/threonine kinases, 90 coding tyrosine kinases, and 43 coding tyrosine kinase-like proteins.

STI-571 selectively inhibits certain types of protein-tyrosine kinases, including stem cell factor receptor (KIT), ► platelet-derived growth factor receptors (PDGFRs) (PDGFRα and PDGFRβ), ABL and its oncogenic form ► BCR-ABL1. These tyrosine kinases catalyze the following reaction by binding adenosine triphosphate (ATP) and transferring phosphate from ATP to tyrosine residues on various substrates:

$$MgATP^{-1} + \text{protein–OH} \rightarrow \text{Protein–}OPO_3^{2-} + MgADP + H^+$$

STI-571 functions by competitively blocking the ATP binding site in these tyrosine kinases, thereby inhibiting their activities. The drug has either no or minimal effects on more than 30 other intensively studied protein kinases.

Data from ▶ pharmacokinetics studies in healthy subjects and over 900 patients have shown that STI-571 is well absorbed after oral administration. Following a single 400 mg dose of STI-571, a peak plasma concentration of 1.9±0.4 μg/ml is reached within 3 h. After multiple dose administrations on a once-daily schedule, the plasma peak concentration reaches a level of 4.4 μM (2.6±0.8 μg/ml). The steady-state plasma trough concentration 24 h after administration of 400 mg daily is over 1 μM. This trough concentration exceeds that required for inhibition of the cellular tyrosine phosphorylation activities of ▶ KIT, ▶ platelet-derived growth factor (PDGF), and ▶ BCR-ABL1 in in vitro assays (50% inhibitory concentration of STI-571 ranges from 0.1 to 0.25 μM). When the daily dose is increased to 600–800 mg, a steady-state plasma peak concentration as high as 13 μM can be reached. Mean absolute bioavailability is 98%.

Following oral administration in healthy volunteers, the half-lives of STI-571 and its major active metabolite (the N-desmethyl derivative) are ~18 and 40 h respectively. At clinically relevant concentrations, ~95% of STI-571 binds to plasma proteins (mainly albumin and α_1-acid glycoprotein).

CYP3A4, a ▶ cytochrome P450, is the major enzyme responsible for metabolism of STI-571. The main circulating active metabolite in humans is the N-demethylated piperazine derivative which has an in vitro potency similar to that of the parent STI-571. Elimination is predominately via bile, mostly as metabolites (75%). After an oral ^{14}C-labeled dose of STI-571, ~81% is eliminated within 7 days, with a fecal to urinary excretion ratio of 5:1. Of the unchanged STI-571, 20% is excreted in urine, and 80% in feces.

Clinical Applications

Chronic Myeloid Leukemia (CML)

▶ Chronic myeloid leukemia (CML) is a ▶ clonal hematopoietic malignancy, with an annual incidence of 1–1.5/100,000. The median age of onset is 45–55 years. The disease typically has three phases. Most patients present in chronic phase but, in an average of 3–5 years, the disease progresses to an accelerated phase with increased tumor loads and additional ▶ chromosomal aberrations. This usually progresses relatively quickly to the ▶ blast crisis phase where the disease comes to resemble acute leukemia. Treatments, including chemotherapy and stem cell transplantation, are usually effective only in the chronic phase of the disease.

More than 90% of cases of CML are caused by the ▶ BCR-ABL1 fusion protein, resulting from a translocation between the bcr gene on chromosome 9 and the abl gene on chromosome 22 leading to a ▶ fusion gene. This ▶ cytogenetic abnormality causes the non-receptor tyrosine kinase, ABL, to become constitutively active, and is necessary and sufficient to induce the malignancy via increased ▶ proliferation, reduced ▶ apoptosis, and perturbed interactions between CML cells and bone marrow stroma. Therefore, this oncogenic ABL tyrosine kinase has become an ideal therapeutic target in CML.

In 1992, STI-571 was synthesized at Ciba-Geigy Pharmaceuticals (now Novartis) as an effective tyrosine kinase inhibitor. Four years later, the inhibitory effects of STI-571 on in vitro growth of ▶ BCR-ABL1-positive leukemic cells was confirmed and reported. Phase I clinical trial studies with STI-571 started in June 1998 and phase II trials were initiated in 1999. These trials confirmed that STI-571 is safe and effective in the treatment of chronic-phase CML. In 2001, STI-571 was approved in both the USA and Europe for the treatment of CML.

Large scale, phase-III multicenter trials have now shown that STI-571 is superior to conventional interferon-α-based therapy in previously untreated chronic-phase CML, with an initial complete hematological response (CHR) rate of 95% versus 55% and a complete cytogenetic response (CCgR) rate of 76% versus 15%. The expected 5-year overall and progression-free survival for STI-571-treated patients has reached ≥90%. Therefore, STI-571 is now used as first-line treatment for CML.

In addition, STI-571 has some activity in the accelerated and ▶ blast crisis phases of the disease. Thus, around 40% and 20% of patients in accelerated phase can achieve a CHR and a CCgR respectively. The 3-year progression-free survival has been reported to be as high as 40%. For patients in blast crisis, the rate of response to STI-571 (including CHR, partial hematological response, and hematological improvement) is also higher than that in historical controls treated with cytarabine-containing regimens (52% vs 29%). However, the response of patients with blast crisis is usually temporary, with a median progression-free survival of ≤10 months and a 3-year survival of only 7%.

The standard dose of STI-571 for CML is 400 mg daily. A higher dose, 600–800 mg daily, has also been used, particularly for cases previously treated with conventional chemotherapy. However, whether such increased doses of STI-571 will achieve better responses remains to be determine in prospective studies.

Gastrointestinal Stromal Tumors (GISTs)

► Gastrointestinal stromal tumor is a rare type of soft tissue sarcoma, with an annual incidence of 0.68–1.45/100,000 according to epidemiological data from North America and Sweden. More than 90% of GISTs occur in the stomach or small intestine, but they can occur anywhere along the length of the digestive tract. Although the exact causes of GISTs are unknown, most have a constitutively activated ► Receptor tyrosine kinase (RTK) pathway. This occurs through ► mutation in either the ► KIT (in more than 80% cases) or the PDGFRα (in about 5–7% cases) gene. The abnormally increased activity of either of these two receptor tyrosine kinases is responsible for mediating the signals which stimulate the proliferation and survival of the tumor cells.

The most common treatment for GISTs is surgery to remove the solid tumor. However, GISTs have an extraordinarily high rate of recurrence after surgical resection and are very resistant to radiation and standard chemotherapy. Since STI-571 targets both of these oncogenic tyrosine kinases (as well as ABL), its effects were soon tested in GISTs. The agent was found to have activity, and is now standard therapy for advanced tumors that cannot be removed by surgery alone and for metastatic and recurrent disease.

Since 2000, thousands of patients worldwide with metastatic or recurrent GISTs have been treated with STI-571, and it has been found that a partial response or stable disease is achieved in 80% of cases. Furthermore, survival is prolonged with improvement of quality of life. Seventy percent cases with metastasis now have an expected survival of over 24 months compared to 15 months before the introduction of STI-571. Studies of STI-571 in both the ► neoadjuvant (STI-571 used both prior to and post surgical resection) and ► adjuvant (STI-571 used post surgery) settings are now being conducted to evaluate whether the low rates of cure obtained with surgical resection alone can be improved.

A daily dose of 400 mg with or without surgery is the recommended first-line treatment for recurrent or metastatic GIST; a higher dose (600–800 mg) may be considered in patients who progress, develop secondary resistance, or present with specific genotypic characteristics. Treatment should be continued until there is progression or adverse effects become intolerable.

Other Diseases

STI-571 is now being used as experimental treatment of other diseases, including BCR-ABL-positive ► acute lymphoblastic leukemia, KIT-positive small-cell lung cancer, and PDGFR-positive ► prostate cancer.

Side Effects

Common side effects include edema, rash, nausea, diarrhea, and fatigue. Anemia, neutropenia, and/or thrombocytopenia can develop in some patients, especially those with advanced disease. Almost all of these side effects are dose dependent and controllable.

Resistance to STI-571

In CML, resistance to STI-571 more frequently develops in advanced disease, although it can be seen in chronic phase, usually after initial response. This resistance can be caused by multiple mechanisms. Among these mechanisms, mutations in the BCR-ABL kinase domain are the most important. They are detected in more than 90% of cases with resistance to STI-571, and may be acquired during treatment, or may exist in a mutated clone present before therapy. Other mechanisms include BCR-ABL overexpression or amplification, reduced intracellular drug concentrations (e.g., caused by low expression of the drug influx transporter HOCT1 gene and/or high expression of the efflux transporter MDR1 gene), and abnormalities downstream of BCR-ABL, e.g., defects in the pro-apoptotic proteins BAD and Bim.

In GISTs, primary resistance is rare and only seen in <15% of cases. More often, resistance is acquired after more than 1 year of STI-571 therapy. Several groups have shown that such resistance is due to mutations in KIT.

Low Concentrations in the Central Nervous System (CNS)

STI-571 has limited activity against CML in the CNS because of poor penetration of the blood-brain barrier.

This results in an 88–100-fold lower concentration of STI-571 in cerebrospinal fluid, compared to that in plasma. Case reports have shown that CNS relapse of CML can occur in patients who have maintained major cytogenetic remission with STI treatment. Although this late CNS disease is still rare, it may become more common over time as more patients are treated successfully with long-term STI-571.

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Sticker Sarcoma

Synonyms

Canine transmissible tumor (CTVT)

Definition

A contagious veneral tumor found in the domestic dog and potentially in their social canids, such as the gray wolf and the cyote. The tumor cells themselves, rather than another agent such as a virus, constitute the contagious agent of the disease. CTVT is passed through the population by ▶ allografts, with the tumor cells from one animal directly seeding tumor formation in the next, usually during coitus. The tumor cells carry a particular marker, common to all tumors in the different host animals, which consists in a diagnostic long interspersed nuclear element (▶ LINE-1) near the ▶ *MYC* gene. The cells of any canine host do not have this diagnostic LINE-1 insertion. Thus, all tumors represent a single cell lineage that has been propagated over long time. The mechanism for tumor cells to evade the host immune system is not clear.

Stilbenes

Definition

Antioxidant compounds.

▶ Phytoestrogens

STK11

Definition

Serine/threonine protein kinase 11; the STK11 gene with a size of around 23 kbp is located on the short arm of chromosome 19 at band 13.3. It codes for a 433 amino acid long serine/threonine kinase inhibiting cellular proliferation, controlling cell polarity, and interacting with the ▶ TOR pathway. STK11 is the causative gene of the ▶ Peutz–Jeghers syndrome which is characterized by hamartomatous intestinal polyps, mucocutaneous pigmentation and an elevated risk for several cancers including ▶ breast cancer.

STK12

▶ Aurora Kinases

STK13

▶ Aurora Kinases

STK15

▶ Aurora Kinases

STK5

▶ Aurora Kinases

STK6

▶ Aurora Kinases

STK7

▶ Aurora Kinases

STMY1

▶ Stromelysin-1

Stomach Cancer

▶ Gastric Cancer

Stomatitis

Definition

An inflammation of the mucus lining of any of the structures in the mouth.

▶ Fluorouracil

Stop Codon

Definition

A stop codon signals the end of protein synthesis.

Stop codons are also referred to as "nonsense codon."

Three are known:

UAG (in RNA)/TAG (in DNA), "amber" UAA (in RNA)/TAA (in DNA), "ochre" UGA (in RNA)/TGA (in DNA), "opal", or "umber".

STR1

▶ Stromelysin-1

STRAP

▶ Serine-Threonine Kinase Receptor-Associated Protein

Stratification

Definition

In a broad sense, refers to the assignment of patients to subgroups according to specific characteristics. Patient stratification can allow to develop a trial design comprised of a genetically differentiated patient pool, using genomic ▶ biomarkers to assess the prognosis of patients carrying a particular biomarker or to evaluate the response of a group of individuals to a particular therapeutic regimen. In undifferentiated patient pools, the number of nonresponders could confuse a trial's endpoint, thereby possibly preventing advancement of a therapeutic to a genetically responsive subpopulation. For example, the ▶ amplification of the ▶ oncogene ▶ MYCN characterizes approximately 20% of the most frequent pediatric cancer ▶ neuroblastoma. With reference to all cases of neuroblastoma, this genomic change would merely be an interesting fact. However, patients carrying this biomarker all have particularly poor prognosis, independent of a classical assessment that before identification of this first cancer biomarker had indicated a good prognosis. As the consequence, neuroblastoma patients carrying amplified MYCN receive more vigorous therapeutic treatment than those lacking amplified MYCN. As an example for drug treatment, ▶ Herceptin may have initially been considered a failed drug due to its impact on only 25% of the patient

population during clinical trials. However, the 25% breast cancer for whom Herceptin was effective were all found to overexpress the ▶ HER-2/neu gene. Using testing for HER-2/neu overexpression as a means to stratify breast cancer patients, Herceptin is now widely used as a therapeutic drug for this subset of breast cancer patients.

Streptavidin

Definition

A tetrameric protein purified from *Streptomyces avidinii* that binds very tightly to the vitamin biotin.

Stress

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Definition

There are different ways to investigate the phenomenon of "stress." From the biologist's point of view, stress can be defined as the unspecific biological response of the body to any ▶ stressor. The biological ▶ stress response includes the activation of the sympathetic nervous system and the release of several hormones (most prominently adrenaline and cortisol) and is among other reactions associated with an increased heart rate, increased blood pressure, altered immune system response, and suppression of the digestive system.

From the psychological point of view, stress is a transactional process which depends on the meaning a person attributes to a stressor. In terms of the transactional stress model which was developed by Lazarus and Folkman, stress results from the subjects' appraisal that a certain stressor or situation is perceived as threatening (as opposed to challenging or harmless or irrelevant) and that the demands of the stressor exceed the individual's resources to deal successfully with the situation. In this model the same situation can lead to severe stress in one person while another person might not even recognize the situation as a potential stressor, depending on a variety of factors, including life experience, personality, coping resources, or social environment of the subject.

Generally, there are two main approaches in social research on stress. One approach to define stress is the concept of daily hassles. Following this approach, stress is a result of an accumulation of minor stressors in daily life (e.g., misplaced keys or overcrowded public transport). However, the majority of research related to stress and cancer occurrence defines stress as the experience of ▶ major life events (or synonymously ▶ stressful life events).

Major life events are defined as events which cause major changes in the life of the individual and are stressful to almost everybody regardless of individual coping capacity or social environment. In the social sciences, exposure to major life events is often measured by a checklist, the most prominent of these being a checklist which was constructed in the 1960s by Holmes and Rahe as a rank scale of life events. In a reanalysis of this scale in the 1990s, the five most stressful events in the list of 43 events were (in descending order) "death of a spouse," "divorce," "death of close family member," "marital separation," and "fired from work."

Characteristics

The assumption of an association between stress and cancer occurrence is common among cancer patients. Many patients believe that their cancer disease was caused or at least influenced by personal factors such as personality traits, coping strategies, depressive mood, or stress. Likewise, many clinicians believe based on their own clinical observations that stress or emotional trauma is associated with cancer risk. Since these beliefs persist among clinicians, researchers, patients, and the lay public, the topic has received intensive research attention in the past and many studies have addressed the question if stress is associated with cancer risk.

Possible Pathways of an Association of Stress and Cancer Risk

Different possible pathways for an association between stress and cancer risk have been postulated: The stress reaction of the body includes the release of several hormones and studies on the effects of stress on the immune response have shown that severe or chronic stress can adversely affect the immune system. It has been hypothesized that these changes can directly increase the risk for cancer. A competing theory of a relationship between stress and risk for cancer is based on the assumption that severe stress can influence behavioral factors and thus subsequently increase the risk for cancer. Studies on health behavior have shown that the experience of stress is associated with unhealthy behavior such as smoking, unhealthy diet, and lack of physical exercise. These behavioral factors themselves are risk factors for several cancers and an increase in cancer risk in the context of stressful living conditions might be ascribed to an increase of these unhealthy behaviors.

Methodological Problems

Research on the association between stress and cancer risk is methodologically challenging, because stress is difficult to conceptualize and cancer is a multifactorial disease. Any interpretation of research findings must take the methodological basis of the respective study into account, especially with regard to study design, different approaches in the measurement of exposure to stress, outcome measurement (development of a cancer disease), and the consideration of confounding variables.

Many studies on the association of stressful life events and cancer risk which have postulated a link between the two show methodological weaknesses. Some of them are based on small sample sizes and especially many early studies used a retrospective approach and were designed as case-control studies. In a ▶ case-control study, patients who are diagnosed with cancer are matched with healthy controls and both groups are asked about the experience of stressful events in the past (mostly referring to a period of 2, 5, or 10 years before the study). Naturally, a patient who was (maybe even recently) confronted with a cancer diagnosis might recall things differently (and might overreport the exposure to stress retrospectively) than a person who does not have to deal with a potentially life-threatening disease. This phenomenon is called ▶ recall bias and represents a fundamental problem in case-control studies which investigate past events.

The methodologically strongest solution to avoid the problem of recall bias is the ▶ prospective study design. In prospective studies on stress and cancer risk, only subjects who have not yet been diagnosed with cancer are included in the study. At the beginning of the study, all participants are investigated about their experience of stress and then followed up for a long period of time. The outcome of interest is cancer incidence and the analyses address the question if the subjects who develop cancer during follow-up had initially reported more stress (e.g., stressful life events) than the subjects who do not develop cancer.

In addition to study design, the assessment of exposure and outcome plays an important role with regard to the methodological quality of a study. Although it is likely that most people recall especially major events in their life correctly, there is no means to assure that their recollection represents the true exposure. One way to address this problem is the unbiased exposure assessment based on register data. Likewise, the strongest approach to assess the outcome (in this case: the development of a cancer disease) is the use of register data rather than self-report. Exposure in studies of stressful life events and cancer risk can be ascertained either from register data or from self-reports, which often comprises a checklist.

Another important factor in the examination of an association of stress and cancer risk is the adjustment for other risk factors for cancer. Since it is well established that behavioral factors such as smoking, alcohol consumption, diet, and exercise increase the risk for several cancers, these factors should be taken into account in order not to ascribe a potential finding to stress when it is in fact based on, for example, higher alcohol consumption in the exposed group.

Findings

Positive associations between severe stressors (i.e., stressful life events) and cancer risk have frequently been reported from case-control and ▶ retrospective studies. Since these studies suffer from the above-described methodological problems, this essay focuses on the results of studies which utilized a prospective approach.

Several prospective population-based studies which assessed both exposure and outcome with unbiased data sources such as administrative data from population registers and cancer registers investigated stressful life events such as death of a spouse, divorce, death of a child, or serious illness in a child. Most of these studies were conducted in Scandinavia where the availability of complete population registers and cancer registers enable ► cancer epidemiology researchers to link these data and investigate, for example, thousands of parents whose child died or whose child was diagnosed with a severe illness. Overall, these prospective population-based studies have shown no association between the investigated stressful life events and subsequent cancer risk.

Two studies found a slight increase in the risk for specific cancers though: One study on cancer risk in about 20,000 parents who had lost a child showed that bereaved mothers had a slightly increased risk for all cancers, which was confined to a slightly increased risk for smoking-related cancers. Likewise, a study of the cancer risk of about 20,000 parents of children in whom schizophrenia was diagnosed found that the mothers had an increased risk for ► lung cancer. However, elevated cancer rates were only found for smoking-related cancers and since these studies where based solely on administrative data there was no means to adjust for smoking behavior. Therefore these findings seem to support the hypothesis that stress does not directly influence cancer risk, but alters health-related behavior (such as smoking patterns) which in turn might increase cancer risk.

Two other studies utilized a more sophisticated approach by combining administrative data for the unbiased assessment of the outcome with personal information from the participants with regard to potential confounding variables in a prospective study design. Therefore, as opposed to many previous prospective studies, the analyses in these studies could be adjusted for a number of lifestyle factors known to be associated with cancer risk (e.g., smoking patterns and alcohol consumption).

A large prospective study of breast cancer in Finland took exposure to potential confounding factors into account and assessed the outcome on the basis of information in a population register, while experience of stressful life events was assessed from a checklist. It was found that experience of divorce or separation or death of a husband, a close relative, or a friend increased the risk for breast cancer. However, a Danish study which used a similar approach (assessment of stress by checklist, assessment of outcome by register data, adjustment for behavioral factors) and investigated all cancer sites did only show that the accumulated experience of stressful life events was associated with an unhealthy lifestyle but was not associated with an increase in cancer incidence.

Conclusion

Although the topic of the presumed association between stress and cancer risk received intensive research attention in the last decades, methodologically advanced studies on the subject are still rare. Due to methodological limitations, many previous studies contribute little to the question of the influence of stressful life events on the development of cancer. Concurrently lay theories on such an influence are widespread among cancer patients and the general public. Only prospective, population-based studies provide reliable information that may support a more evidence-based discussion of these issues.

Based on the repeated results of the prospective studies which have been conducted so far we can conclude that there is no convincing evidence that stress is an independent risk factor for cancer. This conclusion does not only imply that cancer patients have no reason to blame their psychological condition for the occurrence of the disease. The findings might also assist health-care professionals working with cancer patients or their relatives since they can reassure patients that there is no scientific proof that stress or the experience of stressful events such as bereavement or divorce causes cancer.

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Stress Response

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Synonyms

Damage response; Immediate early stress response

Definition

Stress response is a process that occurs in response to an altered balance of endogenous homeostasis following altered external and internal stimuli.

Characteristics

A cellular stress response consists of cellular changes required to accommodate internal or external insult. It can be induced following change in ▶ ROS which can take place in response to ▶ DNA damage, ▶ inflammatory cytokines, ▶ growth factors and irradiation, as well as osmotic or heat shock. Exposure to anticancer drugs or deprivation of survival factors also results in the activation of a stress response. A large set of cellular sensors, which include redox sensitive proteins, cell surface receptors, and proteins that recognize changes in ROS, ▶ ER stress, or DNA damage regulate the activity of stress kinases and their respective substrates. Depending on the type and degree of stress, the combined activation of stress kinases and their corresponding substrates dictate the cellular fate (survival or death) in response to the stress administered (▶ Apoptosis). The activation of one or more stress-activated kinase pathways allows the cell to determine whether DNA repair and growth arrest will prevail over the initiation of programmed cell death or cellular differentiation. The nature of the cellular stress response is therefore dependent on cell type, expression pattern of cell surface receptors, and stress kinases in concordance with respective phosphatases and kinase inhibitors.

Cellular Regulation

Recognition of cellular stress can be attributed to one of the following (as well as to any of their combinations):

- Altered organization of cell surface receptors, including changes in the oligomerization of EGFR, PDGFR, and IGFR as well as altered localization and conformation of membrane-anchored proteins including phosphoinositol 3 kinase (PI3K).
- Change in the redox potential within the cell, primarily due to an altered balance of reactive oxygen radicals (as a result of impaired activity of detoxification and homeostasis maintaining enzymes among which are thioredoxin and glutathione S-transferase π which were shown to inhibit, under non-stressed growth conditions, the activities of ASK1 and JNK, respectively).
- DNA damage which results in the activation of DNA-PK, JNK, and other kinases that sense changes in the presence of DNA lesions (Fig. 1). Each change, or the combination of these changes, has been implicated in the activation of a selective subset of stress kinases and in the subsequent activation of their respective substrates.
- ER stress, which results in activation of ▶ unfolded protein response (UPR) degradation of misfolded proteins (ERAD) with possible trigger for programmed cell death.
- Change in ubiquitin-mediated signaling that impacts cell cycle progression, activation of protein kinases, and DNA damage responses.
- IKK, a family of kinases implicated in the regulation of IκB phosphorylation and subsequent ▶ ubiquitination and degradation. Consequently, ▶ NFκB is free to enter the nuclei to mediate its transcriptional activities. NFκB has been associated with the cellular ability to cope with stress through its positive effect on proteins that antagonize the apoptotic cascade, including IAPs, TNFα, Fas, and Bcl2.
- MAPK, which is responsive to Ras and PI3K signaling, has been implicated in the cellular response to ROS and elicits the activation of p42/p44 ERKs.
- JNK and p38 which are activated by the upstream kinases MKK4/7 and MKK3/6, respectively. The latter are tightly regulated by ASK1 and MEKK1 signals. c-Jun/ATF2 are phosphorylated by JNK and p38, which represents divergent signaling cascades that result in transcriptional output that is often shared by the heterodimer ATF2/Jun. Other members of the p38 families are expected to play

a role in the phosphorylation of substrates that are not modified by JNK and vice versa, thereby conferring selectivity for the stress response.
- ATM and DNA-PK represents enzymes that recognizes DNA damage and contributes to cellular stress response via phosphorylation of key regulatory proteins including the tumor suppressor protein p53.

Activation of p53 is a good paradigm for stress response as this tumor suppressor stress inducible protein is phosphorylated by multiple stress kinases resulting in the dissociation of proteins that otherwise target its ubiquitination and degradation. Phosphorylation of p53 depends on the nature and degree of damage and stress and is carried out by ATM, DNA-PK, p38, and JNK. Phosphorylation on multiple residues (including 15, 20, 37, 46, 81, 389) depending on the type and dose of damage results in p53 that is able to elicit growth arrest or a signal triggering apoptosis.

Clinical Relevance

The interplay between stress-activated kinases, ligases, and other regulatory components appears to play a key role in the type of cellular response to stress. Changes in the expression, activation, or in the duration of stress kinase output are expected to result in an altered cellular response to stress. Tumors were found to harbor such changes, which conferred greater resistance to radiation or chemotherapy, with the example of TRAF2/GCK in human melanoma. Mutations in stress kinases are rarely found, although cases reported an MKK4 mutation in certain human tumors. Changes in the activation of stress kinases were also reported in heart and neurological disorders.

Our greater understanding of mechanisms underlying the regulation of stress kinases and their substrates is expected to allow the design and use of reagents that would specifically target the selective kinase to alter its activities, which will determine the ability of the cell to cope better (or worse) with the type of stress and DNA damage.

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Stress-activated Protein Kinase

► JNK Subfamily

Stressful Life Event

Definition

Critical event that causes severe stress and requires adjustment to the new situation (e.g., death of a loved one, divorce, job loss).

► Stress

Stressor

Definition

An agent, condition, situation, or other stimulus that causes stress to an organism.

► Stress

Striatonigral Medium Spiny Neurons

Definition

MSNs; projection neurons in the striatum, a major nucleus of the basal ganglia.

► Early B-Cell Factors

Stroma

Definition

Meaning mattress or support for parenchymal cells; Is the connective tissue of an organ or a gland. Historically, stroma was recognized as a supportive or scaffolding component. The stroma is believed to "sense" and "react" to paracrine and autocrine physiological or pathological disturbances, thus attempting to maintain the homeostatic balance. In the malignant process, refers to nonmalignant cells and connective tissue that surround and support a tumor.

▶ Desmoplasia
▶ Stromagenesis

Stromagenesis

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Synonyms

Stromal progression; Stromatogenesis; Tumor-associated stromal progression

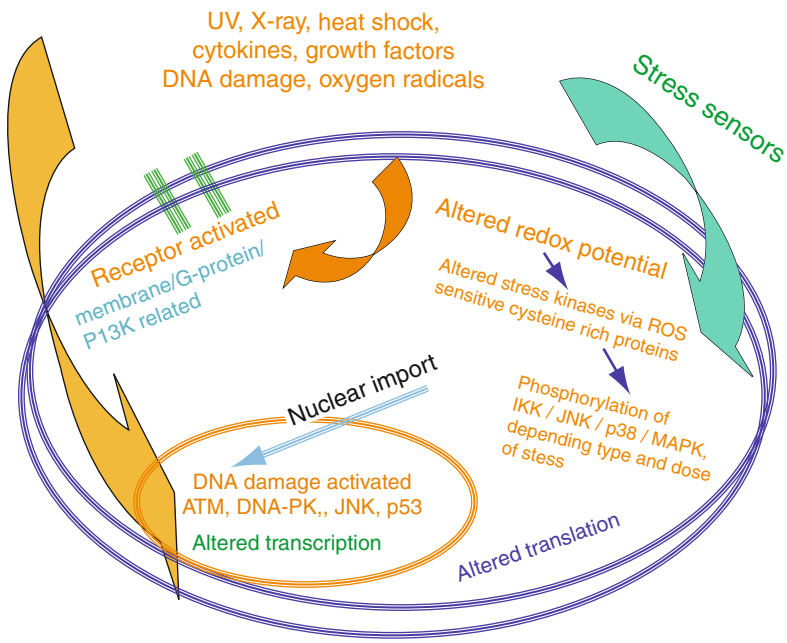
Definition

Stromagenesis, from the Greek term *stromatogenesis* (στρώμα ▶ stroma = mattress and γένεσις genesis = creation or birth), is used to describe the progressive changes that stroma undergoes during the process of ▶ epithelial tumorigenesis. Specifically, the term describes tumor-associated changes in fibroblasts and fibroblast-derived ▶ extracellular matrix, as opposed to the formation of new stromal endothelial blood vessels, identified as ▶ angiogenesis.

Characteristics

Although the term "*stromagenesis*" has only recently entered the scientific literature (2002) describing ▶ melanoma-fibroblast cross talk, the process of stromagenesis has been investigated for more than a century. Early publications from several pathologists in the late nineteenth century (e.g., Theodor Langhans in 1879) used the word "stroma" to refer to connective tissue and vessels associated with tumors. They thought of these areas as purely mechanical and nutritional supports with only mild insinuation of their active roles in ▶ tumorigenesis. The first meaningful hint that the stroma can influence tumor cell behavior was published in 1889, when Stephen Paget presented his "seed and soil" hypothesis. Paget reported that upon autopsy of 735 breast cancer patients, metastases were preferentially distributed to particular organ beds. He proposed that although randomly scattered throughout the vasculature, tumor cells, regarded as "seeds," could only generate viable metastases in specifically permissive territory, or "fertile soil." He further postulated that during cancer progression, tumor cells actively contribute to formation of a pro-metastatic microenvironment. Almost one century after Dr. Paget's theory, in 1975, Dr. Beatrice Mintz, from the Institute for Cancer Research at Fox Chase Cancer Center in Philadelphia, reported that normal host cells could restrict the neoplastic growth of ▶ teratocarcinoma cells microinjected into blastocysts. The injected blastocysts resulted in ▶ mosaic yet normal, healthy, and fertile mice. Dr. Mintz's work was the first to show that cancer cells are totipotent (e.g., stem cells) and that a "normal" microenvironment restricts tumorigenic processes, while a tumor-associated stroma promotes tumor development.

Today, it is believed that tumor cells can both influence and be influenced by their stromal microenvironment, and that some stromas are genetically and/or epigenetically more suitable than others to support tumorigenesis and metastases. Some radical theories, such as the "tissue organization field theory," propose that stroma is the sole tumorigenic component. This theory describes cancer as a problem of tissue organization comparable to organogenesis and proliferation as the default state of a cell. Even though the term "stroma" applies to both connective tissue's endothelial and fibroblastic cells, the term "stromagenesis" is often specifically used to describe the changes that tumor-associated fibroblasts (also known as cancer-associated fibroblasts) and their extracellular matrices undergo during tumorigenesis, while the term angiogenesis is used to describe the



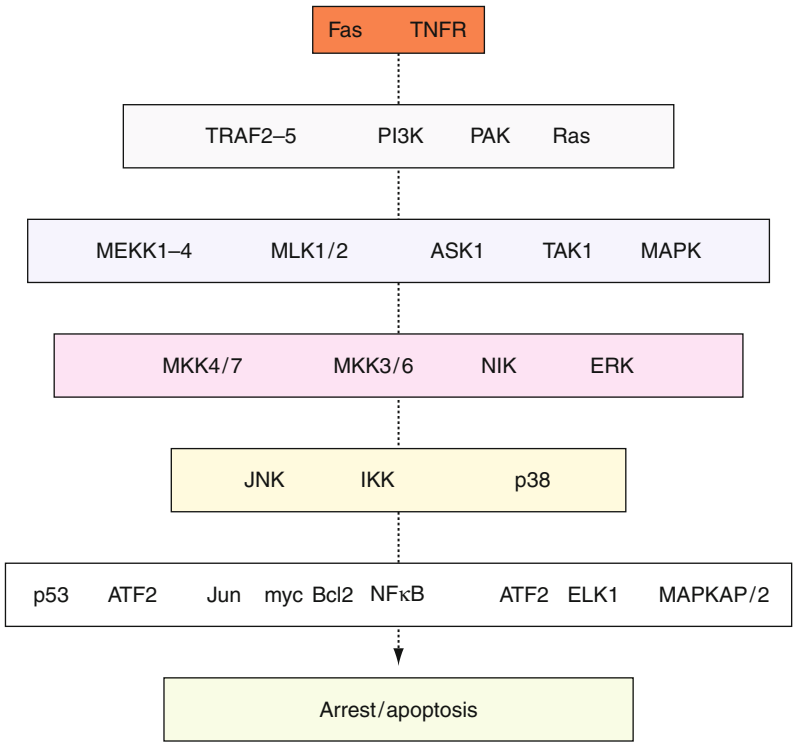
Stress Response.
Fig. 1 Outline of major stress sensors. Cell exposure to stress, as exemplified in the list of DNA damage and stress inducing treatments listed, affects one or more of the major cellular stress sensors. Those sensors include cell surface receptors, membrane-anchored proteins, and cysteine rich proteins which are sensitive to the formation of reactive oxygen radicals, and DNA lesions which are sensed by nuclear-residing protein kinases. The activation of one or multiple sensors depends on the type of stress, its dose, and duration. In turn, respective changes in the activities of stress kinases (Fig. 2) will determine cell fate

endothelial progression manifested as development of new vasculature in the vicinity of the tumor. Finally, the term "tumor-microenvironment" collectively includes stromagenesis, angiogenesis, and cells arising from the host inflammatory immune responses to the tumor, which recruits macrophages and lymphoid cells to the tumor milieu.

It is believed that stromagenesis involves a mechanical process whereby the stroma is stiffened due to changes in protein composition, which increases in intrinsic forces proximal to the tumor, inducing the tumor to progress. Stromagenic changes can be observed at the immediate vicinity of a developing tumor, as an integral part of the tumor, as well as at secondary tumor site (e.g., at the site of a metastasis). Investigators have attempted to describe stromagenesis by recounting its progression in at least three stages: (a) normal or repressive, (b) primed or inductive, and (c) tumor-associated or activated stroma. The best-described stromagenic reaction, known as ▶ desmoplasia, is regarded as fibrotic or scar-like by pathologists. Oncofetal stroma, less well described, presents a less organized stroma that is positive for the oncofetal fibronectin isomer and has been associated with, among others, aggressive oral ▶ squamous cell carcinomas, as well as some breast and colorectal cancers. Since the two described phenotypes share some markers, it is not yet clear whether they are entirely distinct or whether they are dynamically interchangeable, thus emerging as desmoplastic or oncofetal stroma at different times during tumorigenesis. Oncofetal stromal-reactions have been observed relatively far from the primary tumor site (e.g., in the skin of a variety of cancer patients), while desmoplasia has only been reported in direct association to neoplastic lesions. Nevertheless, a clear stroma reaction often represents more than 50% of the total tumor mass such as in the case of pancreatic cancers.

Activated fibroblasts are frequently characterized as myofibroblastic cells expressing ▶ alpha-SMA. Various tumor-stroma specific markers have been described, e.g., the fibroblast activation protein (▶ FAP). This protein has been shown to be expressed in the tumor-associated stroma but not in normal stroma or epithelial cells, or in cancerous tissues with the exception of melanoma. Antibodies against FAP were the first used to investigate the possibility of developing an effective chemotherapy by specifically targeting the tumor-associated stroma. Other stroma specific markers include extracellular matrix components such as tenascin-C, hyaluronic acid, and SPARC (also known as osteonectin).

It is now well recognized that both direct and indirect interactions between cancer cells and their stroma



Stress Response. Fig. 2 Stress kinases. Several stress-signaling cascades are based on their affinity to upstream and downstream components. Among the major stress kinases are ERKs (extracellular signal regulated kinases), IKK (inhibitory κ kinase), JNK (Jun kinase), and p38. Each of these signaling cascades is activated at different kinetics and with diverse affinities in response to stress. Thus, various types of stress can activate different subsets of stress-signaling cascades. Activation of the upstream stress components results in the phosphorylation of respective downstream targets. For example, TRAF2 (tumor receptor associated factor 2) can elicit the activation of NIK (nuclear factor κ B inducing kinase), MEKK1 (MAP/ERK kinase 1), ASK1 (apoptosis signal-regulating kinase 1), mitogen-activated protein kinase (MAPK), which in turn phosphorylates IKK, MKK4/7 (mitogen-activated kinase kinase 4/7), p38, or ERKs, respectively. Organization of these signaling cascades via "scaffold" proteins maintains their close contact and the high affinity for related family members. These kinases will in turn phosphorylate their transcription factors substrates. Depending on the combination of activated transcription factors and apoptosis regulatory proteins, the cell can undergo growth arrest, allows proper damage repair, or can undergo programmed cell death

are critical for promoting the growth and invasiveness of tumors. Nevertheless, the mechanisms by which tumor cells promote stromagenesis are not well defined, but some important triggers such as the cytokine ▶ TGF-beta have been identified. ▶ TGF-beta is produced and secreted as a latent molecule by normal epithelial cells, tumors, and stromal fibroblasts. It is activated by extracellular proteases (including metalloproteinases ▶ MMP-2, MMP-9, MT1-▶ MMP) and receptors (including integrins) on the surface of fibroblasts or early stage tumor cells. TGF-beta suppresses the growth of an emerging population of tumor cells. However, at later stages of tumorigenesis, TGF-beta is indirectly tumor supportive, because during tumor development TGF-beta receptors are often downregulated on the tumor cell surface causing a loss of TGF-beta responsive growth inhibition in tumor cells. However, because tumor cells continue to produce TGF-beta and stromal fibroblasts maintain high levels of its receptor(s), TGF-beta continues to drive fibroblastic responses (e.g., type I collagen fibrillogenesis), promoting the differentiation of stromal fibroblasts into myofibroblasts (myofibroblasts are also believed to be actively recruited to the tumor site from the circulation). Alpha-SMA also actively promotes stromagenesis, and mechanical force is generated by the myofibroblast through the isometric contraction of stress fibers containing alpha-SMA. This force is transmitted by integrin-dependent adhesion-structures, which connect stress fibers with the modified stromal extracellular matrix. It has been shown that TGF-beta induces desmoplastic

differentiation of normal fibroblasts into alpha-SMA expressing and collagen type I assembling myofibroblasts in vitro. Moreover, it is known that induction of connective tissue growth factor and a specific differential splice form of fibronectin known as ED-A enhance the profibrotic effects of TGF-beta.

The vital interplay between tumor and stroma means that, in principle, it is possible to target signaling pathways that regulate tumor-induced stromagenesis and, thereby, contain tumorigenesis.

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Stromal Cell Response

► Desmoplasia

Stromal Cells

Definition

Supportive cells, usually of a fibroblastic phenotype that provide both structural and nutritional support for the principal cells within a tissue or a tumor.

► Stroma

Stromal Progression

► Stromagenesis

Stromal-Derived Factor 1 Alpha

Definition

A ► chemokine that plays important roles in neovascularization; SDF1α binds CXCR4.

► Angiogenesis
► Antiangiogenesis

Stromatogenesis

► Stromagenesis

Stromelysin-1

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Synonyms

Matrix metalloproteinase 3; MMP-3; Proteoglycanase; SL-1; STMY1; STR1; Transin-1

Definition

Stromelysin-1 (EC 3. 4. 24. 17), a secreted proteolytic enzyme whose gene maps to 11q23, is a member of the ► matrix metalloproteinase (► MMP) family. Like the other MMPs, it is characterized by its ability to degrade the extracellular matrix (► ECM) components and its dependence upon Zn^{2+} binding for enzymatic activity. Stromelysin-1 is produced by fibroblastic cells and by normal and transformed epithelial cells in culture and in vivo. It plays a pivotal role in the degradation and remodeling of the ECM during a variety of normal and pathological processes. Besides digesting ECM components, stromelysin-1 modulates the activity of biologically active molecules by direct cleavage and release from bound stores, thus influencing many cellular functions.

Characteristics

Enzymatic Properties of Stromelysin-1

Human stromelysin-1 is secreted from cells as an inactive ▶ zymogen (M*r* latent 57,000 Da) and comprises four distinct structural domains: (1) an N-terminal propeptide that maintains the enzyme in latent form, until it is removed, owing to the interaction of a cysteine residue in this peptide with the zinc ion in the active site; (2) a catalytic domain containing the active site with the catalytic zinc moiety; (3) a linker or hinge region; and (4) a C-terminal hemopexin-like domain that is believed to play a role in the recognition of certain substrates and influence the binding of macromolecular inhibitors. Active enzyme (M*r* active 45,000 Da) is produced by cleavage of the propeptide that can be induced by proteinases like plasmin or in vitro by thiol-modifying agents, chaotropic agents, and heat, which destabilize and activate the molecule by dissociation of the zinc and the cysteine residue. Once activated, stromelysin-1 is susceptible to inhibition by tissue inhibitors of metalloproteinases (▶ TIMPs).

Stromelysin-1 has one of the broadest substrate spectra of the MMPs and can degrade most ECM components, but not fibrillar collagens. In addition, stromelysin-1 activates a number of proMMPs, and acts on non-matrix substrates including cell-surface and matrix-bound growth regulators, releasing them from stores (Table 1).

Regulation of Stromelysin-1

Stromelysin-1 is involved in ECM remodeling during physiological processes such as morphogenesis, growth, and wound repair. It follows that a tight and spatiotemporal regulation of its expression is critical for cell and tissue homeostasis. Stromelysin-1 expression is mainly controlled at the transcriptional level. A number of specific DNA elements in the human stromelysin-1 promoter have been shown to be important in the regulation of its transcription. They enable the gene to integrate through binding the

Stromelysin-1. Table 1 Stromelysin-1 substrates (Adapted from Ref. [1] and completed)

ECM substrates	Bioactive substrates	
Aggrecan	MCP-1 (monocyte chemoattractant protein-*1*)	Partial inactivation
Laminin	MCP-2	Agonist >> antagonist
Fibronectin	MCP-3	Agonist >> antagonist
Collagens III, IV, V, IX, X, XI	MCP-4	Agonist >> antagonist
Gelatin	SDF (stromal cell–derived factor)	Inactivation
Entactin	Pro-TNFα	Soluble active TNF-α
Perlecan	L-selectin	Shedding
Decorin	Pro-HB-EGF (heparin binding-EGF)	Active HB-EGF
Tenascin	Pro-IL-1β	Active IL-1β
Vitronectin	Perlecan	FGF2 release
Fibrin/fibrinogen	Decorin	TGF-β release
LP (link protein)	CTGF (connective tissue growth factor)	VEGF release
Elastin	SPARC (secreted protein acid rich in cysteine)	Angiogenic peptides
	Plasminogen	Angiostatin
	E-cadherin	Soluble ectodomain
	IGFBP-3 (IGF binding protein-3)	Active IGF release
	Pro-MMP-1	Active MMP-1
	Pro-MMP-3	Active MMP-3
	Pro-MMP-7	Active MMP-7
	Pro-MMP-8	Active MMP-8
	Pro-MMP-9	Active MMP-9
	Pro-MMP-13	Active MMP-13
	α1-proteinase inhibitor	Inactivation
	α1-antichymotrypsin	Inactivation
	α2-macroglobulin	Inactivation

transcription factors, which are a large number of stimuli from the different cellular signaling pathways. The specific DNA elements that have been well characterized are: (1) A proximal ▶ AP-1 site at −70 which binds the transcription factors of the ▶ Fos and ▶ Jun families. This site is necessary for the basal level of expression but is not sufficient on its own for activation by numerous inductors as the epithelial growth factor (EGF), the ▶ platelet-derived growth factor (PDGF), phorbol esters or the proinflammatory cytokine IL-1β. Nevertheless, this site must not be underestimated because it is important for combinatorial regulation by the different members of the Fos and Jun families. (2) Palindromic head to head ▶ Ets-binding sites (EBS) at −216/−201, which are not present in the promoter of the other MMPs. These sites bind the oncoproteins of the Ets family, which are transactivators such as Ets-1, Ets-2, or Pea-3 and repressors such as Tel. This EBS palindrome is important for the transcriptional regulation of the stromelysin-1 promoter in response to the oncoproteins ▶ Ras, Mos and ▶ Src, phorbol esters which are tumor inducers such as ▶ PMA and IL-1β. (3) An upstream regulatory sequence which is composed of an EBS and a NIP (Nuclear Inhibitory Protein) site at −94/−80. It cooperates with the EBS palindrome and the AP-1 site in response to IL-1β and PMA. (4) A stromelysin IL-1 responsive element (SIRE) at −1614/−1595, which is a distal negative response element to IL-1β responding also to the tumor necrosis factor α (TNFα), another proinflammatory cytokine. This element can present a single nucleotide polymorphism (▶ SNP) 5A/6A. The additional adenine in the 6A allele increases the binding of a NF-κB p50/p50 dimer leading to a lower level of stromelysin-1 transcription. This polymorphism has been associated with disease severity in diverse pathologies including ▶ cancer. (5) A stromelysin-1 PDGF response element (SPRE) at −1584/−1571 which binds the stromelysin-1 PDGF response element binding protein (SPBP). SPBP transactivates the stromelysin-1 promoter in response to PDGF through a κ/ι ▶ PKC-dependent pathway requiring cooperativity with the AP-1 site. (6) A nerve growth factor response element (NGFRE) at −241/−229, which binds the interferon response element binding factor-1 (IREBF-1). The transcription of stromelysin-1 was shown to be increased by IREBF-1 in response to the nerve growth factor (NGF).

Besides these well-defined responsive elements, variation in the levels of stromelysin-1 transcription by other compounds has been reported: (1) ▶ oncostatin M, which induces stromelysin-1 in human chondrocytes; (2) protein synthesis inhibitors such as cycloheximide and anisomycin, which induce an increase in levels of stromelysin-1 messenger RNA in human fibroblasts; (3) transforming growth factor β (TGF-β), which is able to repress the activation of the stromelysin-1 transcription by EGF, Ras, and Src in fibroblasts; and (4) hormones, which are also inducers or repressors of stromelysin-1 transcription. Glucocorticoids are able to inhibit the increase in stromelysin-1 messenger RNA induced by EGF, PMA, and IL-1β in fibroblasts. On the one hand, ▶ retinoic acid, like glucocorticoids in fibroblasts, is able to repress the stromelysin-1 expression and on the other hand to increase its induction by NGF in pheochromocytoma cells. Estradiol and progesterone inhibit stromelysin-1 expression during the menstrual cycle. Androgens have been reported to decrease the expression of stromelysin-1 induced by PMA in prostate carcinoma cells. Unfortunately, the mechanism whereby all these compounds exert their effects remains unclear. A post-transcriptional regulation of stromelysin-1 was also described. Indeed, activation of p38 alpha mitogen-activated protein kinase (▶ MAP Kinase) enhances stromelysin-1 expression by messenger RNA stabilization.

All the different responsive elements and effectors listed above point out the complexity and the sensitivity of stromelysin-1 regulation. It reflects more particularly the equilibrium found at every control level between positive and negative signals. This is a strategy required for all the MMPs to obtain a very subtle-tuned expression in response to various stimuli in different cellular types and biological events.

Stromelysin-1 and Cancer

It would be simplistic to consider stromelysin-1 just as a proteolytic enzyme hydrolyzing components of the ECM. Stromelysin-1 is able to generate and release numerous active and latent molecular signals or regulate pre-existing molecular signals. In this way, stromelysin-1, like other MMPs, can modulate the

cellular environment at different levels and is therefore able to influence the behavior and the future of the cells. It can be easily understood that misregulation of such a gene is associated with severe erosive and invasive pathologies such as tumor growth and invasiveness.

Stromelysin-1, like numerous other MMPs, is expressed in different tumors and its presence correlates with their aggressiveness. Its expression has been shown in vivo, notably in carcinomas: mammary carcinoma, colorectal carcinoma, lung carcinoma, prostate carcinoma, pancreatic carcinoma, esophageal carcinoma, or cutaneous carcinoma. It is noteworthy that stromelysin-1 has been often shown to co-express with the oncoprotein Ets-1, which is considered, among all the other Ets proteins, as an independent marker of poor prognosis in various cancer types. During the earliest stages of tumor development, stromelysin-1 is predominantly localized at the level of the fibroblasts of the ▶ tumor stroma.

Stromelysin-1 induces, by its proteolytic activity toward numerous compounds, the release of growth factors which act directly on tumor cells and other surrounding cells, including fibroblasts, inflammatory cells, and endothelial cells. Stromelysin-1 in degrading perlecan releases FGF2 (or basic ▶ fibroblast growth factor) which in the setting of cancer is known for its potential to induce ▶ angiogenesis and might be involved in desmoplasia in tumors, owing to its role in fibrosis. In degrading decorin, another proteoglycan, stromelysin-1 releases TGF-β, a growth factor implied in cell growth and proliferation. Stromelysin-1 is able to cleave extracellular proteins sequestering growth factors such as ▶ insulin-like growth factor binding protein 3 (IGFBP-3) which binds to insulin-like growth factor II (IGF II). Stromelysin-1 can also release active Heparin-binding EGF-like growth factor (HB-EGF) from cell surfaces by cleaving it at a site just outside the cell membrane. ▶ Chemokines are known targets of stromelysin-1 and other MMPs. For example, stromelysin-1 has been shown to transform, by partial proteolysis, the precursor of IL-1β in an active molecule, influencing tumor-infiltrating inflammatory cells. Stromelysin-1 is also involved in the loss of cellular adherence and the phenotypic modification of epithelial cells, which occur during the early stages of carcinomatoid tumor development. Thus, stromelysin-1 cleaves the extracellular domain of the ▶ adherens junction protein ▶ E-cadherin. The soluble fragment of E-cadherin which is released prompts cells to disaggregate and promotes tumor-cell ▶ motility in a paracrine manner, by interfering with the function of other full length E-cadherin molecules. Cleavage of E-cadherin also triggers the ▶ epithelial-to-mesenchymal transition (▶ EMT), promoting cancer cell invasiveness. Acquisition of an invasive phenotype by the tumor often goes with a gain of MMP expression by the tumor itself. Indeed, stromelysin-1 expression is associated with invasive carcinomas. Some ▶ squamous cell carcinoma (SCC) tumor cells can express stromelysin-1, providing further activity for a tumor-driven proteolytic cascade, in which MMP-13 can be activated by stromelysin-1 expressed by tumor cells. In addition, many compounds secreted by tumor-infiltrating inflammatory cells as well as by tumor or stromal cells are capable of modulating MMP expression. Tumor cells can also secrete factors, such as extracellular matrix metalloproteinase inducer (EMMPRIN), which enhances the expression of several MMPs, including stromelysin-1 by fibroblasts.

Stromelysin-1 has also a role in tumoral neoangiogenesis. Inhibition of the stromelysin-1 activity decreases neovascularization drastically in a murine model of ▶ colon cancer. In addition, the release of proangiogenic factors by stromelysin-1 is also in agreement with its known role in angiogenesis: (1) release of FGF2; (2) release of vascular endothelial growth factor (VEGF) isoform $VEGF_{165}$, as a consequence of degradation of its natural inhibitor, the connective tissue growth factor (CTGF); (3) release of active HB-EGF; and (4) production of an angiogenic polypeptide, owing to the cleavage of a matricellular protein, ▶ SPARC (secreted protein acid rich in cysteine). Nevertheless, stromelysin-1 can also generate ▶ anti-angiogenic factors. Indeed, stromelysin-1 generates angiostatin by cleaving plasminogen and might be involved in the generation of ▶ endostatin, a C-terminal fragment of the basement-membrane collagen type XVIII. It indicates that expression of stromelysin-1 in the tumor periphery might also serve to limit or regulate angiogenesis induced by the tumor. Another role of stromelysin-1 as a negative regulator of tumor expansion might exist. In fact, stromelysin-1 is able to cleave and inactivate ▶ CXCL12, a ligand for CXC chemokine receptor 4 (CXCR4) on leukocytes.

▶ Breast cancer cells express CXCR4 and it has been shown that inhibition of the binding of CXCL12 to CXCR4 by blocking antibodies reduces ▶ metastasis in vivo. Therefore, cleavage of CXCL12 by stromelysin-1 might inhibit metastasis.

Stromelysin-1 can act as a natural tumor promoter. It can induce premalignant lesions and favor tumor emergence on its own. These observations come from using transgenic mice overexpressing stromelysin-1 specifically in the mammary glands. Mammary tissue of these transgenic mice presents the characteristics of stroma reaction with collagen accumulation, neovascularization, tenascin-C expression, and upregulation of endogeneous stromelysin-1. These changes are hallmarks of cancer ▶ progression and may even predispose toward neoplastic epithelial transformation. Thus, overexpression of stromelysin-1 gives rise to changes that could potentially promote mammary ▶ carcinogenesis. This is confirmed in older animals from 6 to 24months of age, which develop with an important incidence, spontaneous premalignant lesions, and mammary cancers. A recurrent genomic instability has been shown by array comparative genomic hybridization (▶ ArrayCGH) in these different premalignant and malignant lesions. In addition, stromelysin-1 plays an active role in EMT. Thus, overexpressing this enzyme in normal mammary epithelial cells leads to an important morphological change with a loss of cell–cell adhesions and vimentin synthesis revealing EMT. This was confirmed in vivo using transgenic mice overexpressing stromelysin-1. A recent study indicates that stromelysin-1-mediated EMT is due to the expression of Rac1b, an isoform of Rac1 GTPase, which causes an increase in cellular ▶ reactive oxygen species (▶ ROS). This increase in the ROS stimulates the expression of the transcription factor ▶ Snail, promotes EMT, and causes oxidative damage to DNA and genomic instability. This may represent a key event in the stromelysin-1-induced phenotypic and genotypic malignant transformation in normally functioning cells.

These results are supported by studies in human cancers where the 5A allele of stromelysin-1 (see above, regulation of stromelysin-1) corresponding to higher levels of stromelysin-1 transcription may represent an unfavorable prognostic feature in breast cancer patients associated with more invasive disease. The 5A allele might also be associated with the increased susceptibility to non-small cell lung cancer among smokers and a risk of development and lymphatic metastasis in esophageal squamous cell carcinoma.

On the contrary, other studies using stromelysin-1 transgenic animals showed a reduction in the number of mice developing mammary tumors following treatment by a chemical carcinogen. An ▶ apoptosis induction of mammary epithelial cells in transgenic animals overexpressing stromelysin-1 (possibly by degrading laminin) has been reported. In addition, in stromelysin-1-deficient mice, topical applications of carcinogens resulted in skin tumors that grew at a faster initial rate than did carcinogen-elicited tumors in wild type mice. This enhanced tumor development was correlated with a reduction in the tumor inflammatory cell infiltrate. Thus, the presence of stromelysin-1 appears to be a protection by an increased influx of inflammatory cells to the area of carcinogenesis.

All these data point out that the proteolytic activity of stromelysin-1 and other MMPs toward an always growing number of matrix and non-matrix substrates renders it difficult to predict the effect of cleavage in a complex biological context. This is all the more real in cancer progression which involves a continuous interplay between tumor cells, stroma cells, and inflammatory cells. It becomes clear that the biological consequences of this activity can lead to either stimulation or inhibition of tumoral development. Validation of true substrates in vivo and a better understanding of the biological properties of the cleavage products are required to find an efficient therapeutic use for the inhibitors of stromelysin-1 and other MMPs.

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Structural Biology

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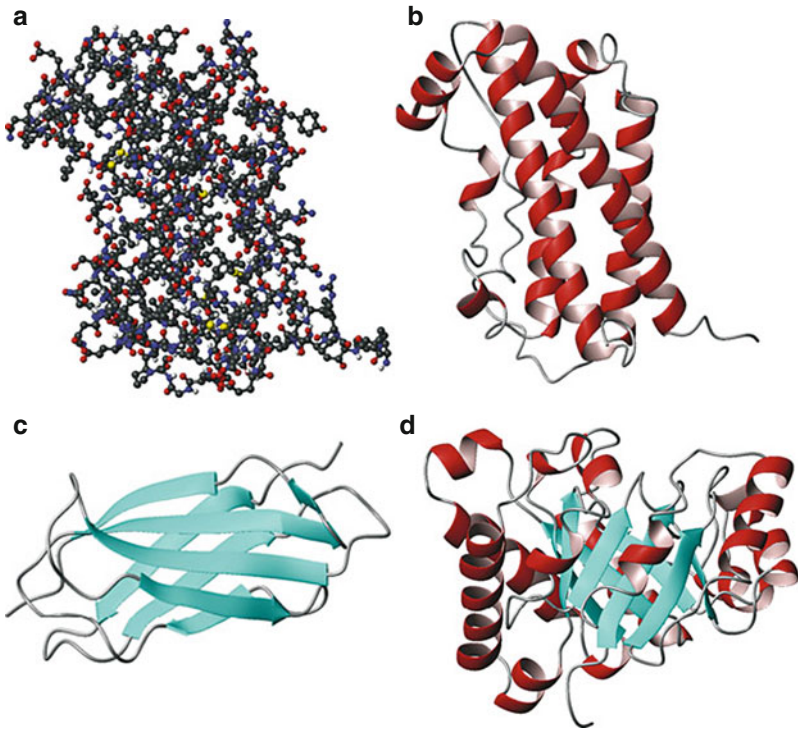
Definition

Structural biology involves biophysical methods that can determine the three-dimensional structure of macromolecules. These structures can lead to an understanding of the molecular basis of cancer and identify the atomic details necessary for drug design, optimization, and development.

Characteristics

The two methods that can elucidate atomic-level structures of biological macromolecules – for example, proteins, DNA, RNA, and complexes between/among these molecules – are ▶ X-ray crystallography and ▶ nuclear magnetic resonance spectroscopy (▶ NMR). For X-ray crystallography, crystals are necessary for determining structures. In contrast, NMR is used to determine structures of molecules in solution. In crystallography, X-rays are diffracted by the crystal creating the data necessary to determine the structure. NMR experiments are based on the property of nuclear spin, inherent to specific nuclear isotopes such as ^{1}H, ^{13}C, and ^{15}N. A majority of NMR-derived structural restraints are derived from magnetic couplings between nearby nuclei. Structures of the same macromolecule determined by each method are essentially identical. However, each technique presents its own advantages and disadvantages and, together, the two techniques are highly complementary. In practical terms, NMR spectroscopy is limited in the size of the protein investigated, with structures <30 kDa being relatively routine and structures up to around 100 kDa anticipated. On the other hand, X-ray crystallography has been successfully utilized to determine the structures of very large macromolecular complexes such as a virus or the large ribosomal subunit. A final important difference involves static versus dynamic views of protein structure. X-ray crystallography excels at providing high-resolution detail of the equilibrium, or most stable, state of a macromolecule. NMR structures are normally more dynamic and indicate regions of the structure that have multiple conformations. While this is also possible in crystallography, the end result of an X-ray structure looks more like a static macromolecule. Together, these two views are highly complementary in that knowledge of both the preferred molecular conformation and the possible dynamic excursions around this low energy state are important to understanding molecular function.

The final output of a structure determination project consists of a set of Cartesian coordinates for every atom in the macromolecule, that is, a listing of their relative positions in the commonly named X, Y, and Z geometric axes. Armed with an understanding of the covalent connections between protein atoms, these spatial coordinates allow construction of three-dimensional visualizations of the overall molecular structure. Whereas the "ball-and-stick" representation (Fig. 1a), localizing each atom (ball) and covalent bond (stick), provides the greatest wealth of detail about the molecular structure, it fails to convey important patterns about the overall structural topology. The simpler "ribbon diagram" (Fig. 1b–d) illustrates only the backbone secondary structural elements, which together assemble into an overall topological pattern. The basic elements of protein ▶ secondary structure include alpha-helix, beta-sheet, loop and coil regions. Initially produced as a linear chain of amino acid residues, the secondary structure folds to form the ▶ tertiary structure of the protein. Two or more linear chains or subunits are sometimes necessary to form the final ▶ quaternary structure. Despite the evolution of tens of thousands of different proteins in the human genome, it appears that far fewer structural topologies are required to carry out all the necessary functions of human biology. Examples of these fundamental three-dimensional topologies are the four alpha-helical bundle (Fig. 1b), the beta-sandwich (Fig. 1c), and the alpha–beta barrel (Fig. 1d). Additional examples of experimentally determined protein structures can be found in the ▶ Protein Data Bank (PDB, http://www.pdb.org), a centralized, public database of nearly all published macromolecular structures. The PDB is a powerful resource for researchers, students, and teachers alike, providing graphical tools for



Structural Biology.
Fig. 1 Graphical depictions of protein structures. (**a**) "Ball-and-stick" representation of human growth hormone (PDB code 3HHR) compared to (**b**) a backbone "ribbon diagram" of the same protein, which more clearly demonstrates its four helical bundle structural topology. Also illustrated are two additional examples of protein structural topologies: (**c**) the beta-sandwich from human fibronectin (PDB code 1FNF) and (**d**) the alpha–beta barrel, represented by human triosephospate isomerase (PDB code 1HTI)

visualization of protein structures along with a variety of accompanying genetic, structural, and functional data on each submitted molecule.

Macromolecules serve as the cell's machinery and are responsible for a vast majority of the biological activities necessary for life. The atomic-level description of macromolecules derived from structural biology provides the framework for defining the fundamental mechanisms by which these molecular machines function. Oftentimes, a newly determined structure of a long-studied macromolecule provides critically needed explanation of previous experimental results. Other times, structural analyses of newly discovered proteins provide important insights into their potential biological functions and serve as a powerful guide for future investigations. The catalytic mechanisms of protein enzymes or nucleic acid ribozymes can be aided by observation of the chemical groups located in the active site of the macromolecule, generally in a series of structures with separately bound reactants, products, and intermediates of the reaction. Similarly, the molecular basis of allostery, such as that which occurs for the binding of multiple ligands to hemoglobin, can be elucidated by comparison of multiple, differently ligated structures. Analysis of transmembrane-spanning and membrane-associated receptor proteins reveals the structural basis of cell–-cell communication.

One of the most illustrative case studies of the important role played by structural biology in cancer research involves the tumor suppressor ▶ p53 Protein. Inactivating mutations in p53 are seen in over 50% of all tumors, highlighting its central function as a cell cycle regulator and tumor suppressor. The p53 polypeptide contains four distinct domains, which vary in their structural stability and their degree of conformational mobility. The most stable region is located in the central portion of the chain and folds into a DNA-binding domain (DBD). A vast majority of inactivating mutations are located in this domain and a combination of X-ray crystallographic structural models of the DBD complexed to nucleic acid along with complementary NMR studies have revealed the precise molecular interactions involved in transcriptional activation. Inactivating mutations directly interfere with DNA-binding, structurally destabilize the DBD, or disrupt critical protein–protein interactions required for cooperative binding of p53 oligomers to DNA.

The first 100 residues in p53 are natively unfolded, as demonstrated by NMR and other complementary biophysical techniques. However, portions of this unstructured region are believed to undergo disorder-to-order transitions upon binding various regulators of p53 function. For example, co-crystallization of the p53-binding domain of MDM2, a critical regulator of p53, with its recognition sequence located in the p53 N-terminal domain reveal a helical structure for these residues. Lastly, a large body of structural studies on the "tetramerization domain," located in the C-terminal to the DBD in p53, have elegantly demonstrated the importance of p53 oligomerization in its function as a transcriptional activator. The tetramerization domain forms a four helical bundle with pairs of beta strands connecting adjacent helices, in what is often referred to as a "dimer of dimers" arrangement. In solution, p53 exists as mixture of dimeric and tetrameric forms in equilibrium, with the tetrameric form preferred for recognition of its DNA consensus sequence. Hence, knowledge of the structural properties of p53 has contributed to a better understanding of its function and has aided the rational design of potential anticancer agents, which function by enhancing the tumor suppressing activities of p53.

In the remaining portion of this entry, we will review how the three-dimensional structure of a target involved in cancer, whether a protein or nucleic acid, can be used in a variety of ways to contribute to the development of an anticancer drug. We will consider three different methods: (1) the screening of a target by libraries of small molecules using computational techniques, also called *in silico* design, (2) the use of "molecular fragments" or small molecules in crystallography or NMR, respectively, to design novel compounds, and (3) optimization of a lead compound or the study of a known therapeutic to meet unmet clinical needs.

If no small molecule inhibitors are initially available, the functional site involved in inducing cancer can directly reveal important properties – such as the volume of the functional site, the electrostatic potential of the macromolecule, atoms that can act as hydrogen bond donors or acceptors, and the overall hydrophobicity of the site – that are important for identifying a drug. Sophisticated software has been developed for ▶ small molecule screening of millions of compounds into this site and score the best fit of each molecule with respect to all molecules in the library. The molecules predicted to bind most tightly can then be experimentally screened against the target protein. If any of these predicted compounds have the desired chemical or biological effect, they can be used to develop a molecule that goes into clinical trials against a specific tumor or cancer.

An alternative experimental approach is to use either organic solvent molecules (in crystallography) or multiple molecules that bind to adjoining sites (in NMR) to design specific, novel molecules that bind to the active site. The crystallographic approach is known as multiple solvent crystal structures (MSCS) and attempts to find functional groups (such as amides, various alcohols, demethylformamide, acetone, etc.) to create a chemical map of where these functional groups can bind in the active site. The information gained from this approach is used by chemists to design small molecules that incorporate the functional groups in the exact position to create a new therapeutic agent. An analogous approach in solution is known as SAR by NMR. In this case, larger molecules can be used that bind to adjoining regions and can be chemically linked to create a specific compound for the molecular target.

When the structure of an approved drug is studied in complex with its molecular target, it reveals how it binds and what would be necessary to improve potency, if it becomes necessary. A perfect example of this is the ▶ Small molecule drug Gleevec, which is used to specifically target a specific protein that causes chronic myelogenous leukemias. In some patients, mutations occur in the protein that leads to resistance to Gleevec. To overcome these problems, the three-dimensional structure of Gleevec bound to its target, ▶ BCR-ABL, and mutants of this protein have been determined, which has led to both an understanding of how the mutants avoid binding Gleevec and the development of new compounds that are effective against the mutant protein.

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Structural Maintenance of Chromosomes Protein

Definition

SMC; protein complexes consisting of structural maintenance of chromosomes (SMC) are critical for the accurate segregation of chromosomes during cell proliferation cohesion and condensin are two members of this family. Cohesion is required to hold sister chromatids together during mitosis while condensin is required for proper organization of mitotic chromosomes to allow segregation.

► S-Phase Damage-Sensing Checkpoints

Structural Vascular Stabilization

► Vascular Stabilization

STS

► Steroid Sulfatase

Stuart-Prower Factor

Definition

► Factor X.

Subacute Toxicity Studies

Definition

► Repeat Dose Toxicity Studies

Subarachnoid Space

Definition

Is the space between the brain surface and arachnoid tissue layer filled with cerebral spinal fluid.

► Convection Enhanced Delivery (CED)

Subarachnoidal Spread

► Leptomeningeal Dissemination

Subcellular Compartments

Definition

Parts of the intracellular space divided by various organelles, most of which are enclosed by limiting membranes.

► Modular Transporters

Suberoylanilide Hydroxamic Acid

Definition

SAHA; is an inhibitor of ► histone deacetylases, undergoing clinical trial.

► Vorinostat

Substrate Channeling

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Synonyms

Enzymic mouth to mouth feeding

Definition

The preferred local utilization within a protein complex of a locally synthesized substrate by shielding the nascent substrate from a molar excess of the same moiety in the surrounding bulk solution. The converse also occurs where that moiety is preferentially drained by an intracomplex "substrate steal" mechanism depriving the complex of essential substrate. The direction of the flux also depends on the accessibility of the relevant precursor pools. Membrane-linked multienzymes in the glycolysis pathway attached to red cell transporter proteins and the fatty acid synthase multienzyme assembly are key examples. Reference [1] and its recent update from the same group (J Biol Chem in press) provide a good example.

The relationship to cancer follows a newly discovered example that utilizes a protein–protein shield that includes one isoform of the first metastatic suppressor ever to be discovered, nucleoside diphosphate kinase (NDPK, nm23, awd) in a complex that can also include ► G proteins. This is described in reference [3]. Briefly, depending on the subcellular site of the complex, the isoform of NDPK and cell type, interacting components (effectors) may include an ion channel in the membrane (e.g., potassium channels). Mitochondrial examples are also known that may maintain *trans*-matrix flux across the inner/outer membranes to/from the cytosol during high demand. Substrate channeling makes it possible to repeatedly create local shielded products and arranges their flux toward or away from a process or organelle without local substrate buildup or deficiency.

Characteristics

Molecular collisions in a liquid such as a cytosol or in a semiliquid lipid membrane typically occur at over one billion per second, which generates a problem for the fidelity of precursor–product relationships in protein–protein-based reactions. These reactions require repeatedly identical products from a pair of substrate reactants say, for example, small ligands at millimolar levels collide in the surrounding water phase that itself exists at a concentration of 55,000 times excess over solute. These solute concentrations are assumed to be even throughout the cell cytoplasm. Substrate channeling challenges that notion.

The Key Features

1. A multiprotein complex contains one or more effector proteins controlling function(s) essential for cell viability (critical nodes of interacting proteins – viability interactomes) that bind one or more interacting regulatory partners involved in sensing some substrate flux. The idea posits a subspace environment (microcytosol) in the interstices of the interface between the protein components forming a shield against the bulk phase 55 M water. See latest work from Sariban-Sohraby's group (in press 2007).
2. A relay signal may or may not link to feed the complex of proteins that reflect the prevailing bulk cytosol status. However, small molecules dissolved in water outside the complex do not have unfettered access to the interstices of the multiprotein complex (restricted gate).

The Prototypical Paradigm

Individually, one such protein component (NDPK) of one such complex has been repeatedly linked to the spread of different cancers either by mutation of specific residues that are either (1) known to be permissive for binding/phosphorylation interactions (protein kinase CK2 and NDPK) or (2) by virtue of altered absolute levels of each component (disordered concentrations of either NDPK or CK2 are linked to cancer). The exact partner protein stoichiometries are unknown but examples exist in cytosol and membrane with different effectors constituting the complex such as ion channels. Thus, the proposed sensor complex has the potential to utilize more than one effector protein (ion channel, G protein, etc.).

Each effector (output enzyme) shares the property of slotting into some unknown surface of interaction between complexed protein kinases. One kinase is constitutively active when isolated from cells – a necessary prerequisite for a sensor! This cancer-related kinase is CK2 (formerly casein kinase 2); the second is the above dual serine–histidine kinase NDPK (NDPK-A or nm23-H1). In vitro data suggest that AMP activated kinase is also present with NDPK but the function is unknown. AMPK is occasionally misnamed by some authors as AMP-dependent kinase but AMPK was deliberately given the name AMP activated kinase by DG Hardie because of its substantial AMP-independent activity. These three kinases are always active to some degree in a resting cell replete with ATP.

Details

Epithelial Membrane. CK2 also controls the lipid transporter ABCA2 and the efflux pump function of another ABC protein (the MDR protein). Heterotrimeric G proteins receive histidine phosphate on their beta subunits directly from the high energy phosphohistidine on one isoform of NDPK. This idea is similar to control of potassium channel function by direct transfer of phosphohistidine to the target potassium channel, for example, and its removal by a local phosphohistidine phosphatase.

Mechanisms

NDPK not only transphosphorylates nucleoside diphosphates using a high energy phosphohistidine intermediate (38 kJ/mol, compared with 8 kJ/mol for phosphoserine or phosphotyrosine) but can make this energy available for transfer to a G protein or a K channel. The key authors in the field may be found at http://www.dundee.ac.uk/mchs/ndpk.

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Subtractive Hybridization

Definition

A technique based on differential hybridization, linker-determined ▶ PCR and sequencing for determining differences in gene expression under two different conditions or between two tissues.

▶ Class II Tumor Suppressor Genes

Subtype AML-M7

▶ Acute Megakaryoblastic Leukemia

Subunit Vaccine

Definition

A vaccine that uses merely one component of an infectious agent, rather than the whole, to stimulate an immune response.

Succinate Dehydrogenase

Definition

An iron-containing flavoprotein enzyme that catalyzes reversibly the dehydrogenation of succinic acid to fumaric acid in the presence of a hydrogen acceptor and that is widely distributed especially in animal tissues, bacteria, and yeast – called also succinic dehydrogenase.

Sugarbaker

Definition

Peter Sugarbaker, a surgeon from the Washington Cancer Institute, Washington DC, USA, who pioneered intensive loco-regional multi-modality therapy in the management of peritoneal surface malignancies. This is also referred to as ▶ cytoreductive surgery and hyperthermic intraperitoneal chemotherapy.

Sugar-Remodeling System

Definition

One of the experimental systems for analyzing the biological functions of oligosaccharides. Cells or animals are

manipulated by gene transfection or ▶ knock-down, leading to changes in their oligosaccharide structures.

▶ Fucosylation

SUI1 Domain

Definition

In budding yeast, SUI1 is a translation initiation factor that along with eIF-2 and the initiator tRNA-Met directs the ribosome to the proper translation start site.

▶ MCT-1 Oncogene

Suicide Gene

Definition

Genes used in ▶ cancer gene therapy or ▶ oncolytic virotherapy encoding proteins that convert nontoxic prodrugs in toxic derivates. A gene when expressed in cells induces cell death. For example, *herpes simplex virus-thymidine kinase* gene. Cells expressing the *thymidine kinase* gene phosphorylate a prodrug ganciclovir, and phosphorylated ganciclovir kills the cells.

▶ Oncolytic Adenovirus
▶ Oncolytic Virotherapy
▶ Suicide Gene Therapy

Suicide Gene Therapy

Definition

Refers to the two-step process of delivering a gene to tumor cells that is able to transform a nontoxic prodrug into a toxic metabolite and thus killing the cells. Transfer of a gene which, when expressed in cells, activates a normally nontoxic prodrug to a toxic form.

▶ HSV-TK/Ganciclovir Mediated Toxicity
▶ Pannexins

Sulcus

Definition

Plural: sulci; is a normal anatomical feature of the brain and represents the groove or fissure of the brain matter seen on gross inspection.

▶ Convection Enhanced Delivery (CED)

Sulfasalazine

Definition

Sulfasalazine is used to treat bowel ▶ inflammation, diarrhea (stool frequency), rectal bleeding, and abdominal pain in patients with ulcerative colitis, a condition in which the bowel is inflamed. Sulfasalazine is in a class of medications called anti-inflammatory drugs. It works by reducing inflammation inside the body.

Sulfatases

Definition

Enzymes hydrolyzing sulfuric acid esters. They contain the unusual amino acid formylglycine in their active centre. Formylglycine is post-translationally formed from cysteine (primarily in eukaryots) or serine (mainly in bacteria). Together with sulfotransferases, which form sulfuric acid esters, they regulate local and systemic levels of various hormones and the sulfation/*N*-sulfonation state of glycan structures.

▶ Sulfotransferases

Sulfate Group

Definition

Is a polar, very water soluble ester of sulfuric acid group that is chemically attached to a drug in a ▶ phase II metabolism reaction.

▶ ADMET Screen

Sulfatide

Definition

Galactosylceramide with a sulfate ester on C-3 of the sugar, a strongly acidic sphingolipid.

▶ Sphingolipid Metabolism

Sulfation

Definition

The process of adding sulfate groups as esters to proteins or other biomolecules. Sulfation is one of the main types of post-translational modifications made during the protein synthesis process in eukaryotes in addition to ▶ glycosylation and ▶ phosphorylation.

▶ Osteopontin

6-Sulfatoxymelatonin

Definition

The major metabolite of melatonin resulting from the hydroxylation of melatonin followed by its sulfation in the liver; it is excreted in the urine.

▶ Melatonin

Sulfokinases

▶ Sulfotransferases

Sulforaphane

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Synonyms

4-Methylsulfinylbutyl isothiocyanate; (−)-1-Isothiocyanato-4(R)-(methylsulfinyl) butane

Definition

Sulforaphane belongs to the isothiocyanate family. It is a hydrolysis product of glucoraphanin, a glucosinolate found in broccoli. Its molecular formula is $C_6H_{11}NOS_2$, and its molecular weight is 177.29 Da. Glucoraphanin is also known as 4-methylsufinylbutyl glucosinolate. Sulforaphane is the aglycone breakdown product of glucoraphanin, also known as sulforaphane glucosinolate (SGS). Glucosinolates are beta-thioglucoside-N-hydroxysulfates and are primarily found in cruciferous vegetables (cabbage, broccoli, broccoli sprouts, brussels sprouts, cauliflower, cauliflower sprouts, bok choy, kale, collards, arugula, kohlrabi, mustard, turnip, red radish and watercress). Young broccoli sprouts and young cauliflower sprouts are especially rich in glucoraphanin. The enzyme ▶ myrosinase present in cruciferous vegetables converts glucoraphanin to sulforaphane upon damage to the plant (such as by chewing).

Characteristics

Actions and Pharmacology

Sulforaphane has anticarcinogenic activity in laboratory animals that are exposed to chemical carcinogens. It inhibits mammary gland carcinogenesis in rats induced by ▶ dimethyl benzoanthracene, colon tumorigenesis in rats induced by ▶ azoxymethane, and lung carcinogenesis in mice exposed to tobacco carcinogens, ▶ benzo(a)pyrene and 5′AMP-activated protein kinase (▶ NNK) (▶ Carcinogenesis, ▶ carcinogen metabolism, ▶ chemical carcinogenesis). It appears

to be effective during both initiation and post-initiation (progression) stages. In addition to other cell lines, for example, human colon cells, studies have also shown that in prostate cancer cells, sulforaphane can induce ▶ cell cycle arrest and ▶ apoptosis, suggesting its potential in preventing prostate cancer. However, so far no tumor bioassays in animals have been reported to test its chemopreventive activity for prostate cancer. It also has anti-bacterial activity as consumption of broccoli sprouts is effective at inhibiting *Helicobacter pylori* growth with sulforaphane being at least one of the active agents.

Mechanisms of Action

Several possible mechanisms have been studied for the chemopreventive activity of sulforaphane, notably inducing phase II detoxification enzymes, such as glutathione S-transferase and quinone reductase [NAD(P)H: (quinone-acceptor) oxidoreductase] (▶ Detoxification). These enzymes may confer protection against certain carcinogens and other toxic electrophiles, including reactive oxygen species. In addition to phase II enzyme induction, the induction of apoptosis and cell cycle arrest and enhancement of the transcription of tumor suppressor proteins are also likely mechanisms. Numerous studies have shown that SFN activates multiple signaling pathways in various cell lines involving protein kinases. Chief ones among them are the mitogen-activated protein kinase (MAPK) family of serine/threonine kinases including ERK1/2, JNK1/2, and p38 that play an important role in cell proliferation and apoptosis in response to stimuli and ERK activation mediates PEITC or SFN-induced apoptosis in PC-3 cells. SFN has been shown to induce apoptosis via a p53-dependent or p53-independent pathway. Together, these studies show that ITCs can modulate the kinase pathways and transcription factors, often in a cell-specific manner. As an electrophile, sulforaphane can covalently modify thiols in proteins. The binding to cystein residues in critical proteins has been suggested to underlie its functions as a phase II enzyme inducer and may be one mechanism by which it induces apoptosis. However, detailed molecular mechanisms for the apoptosis induced by sulforaphane remain to be investigated.

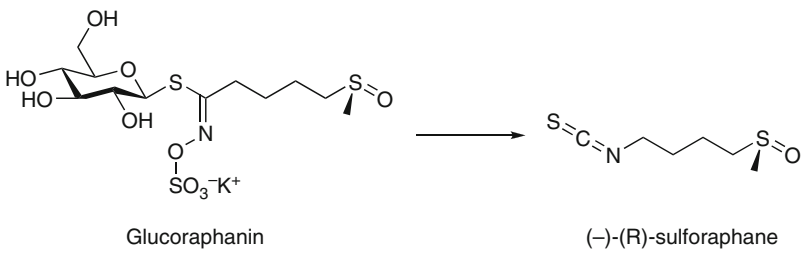
Metabolism and Pharmacokinetics

Few studies have been conducted in humans on sulforaphane or its glucosinolate, glucoraphanin. However, broccoli is a rich source of sulforaphane, containing 44–56% of glucoraphanin. It has been used as a substitute of sulforaphane in several human studies to understand how it is metabolized. Like other ITCs, a major route of metabolism of sulforaphane is via the mercapturic acid pathway, involving first conjugation with glutathione (GSH) followed by enzymatic degradation to subsequent cysteinylglycine, cystein, and finally N-acetylcysteine. The N-acetylcysteine conjugate is then excreted in urine as a major metabolite of sulforaphane. The bioavailability of sulforaphane from fresh broccoli is approximately three times greater than that from cooked broccoli, in which myrosinase is inactivated. Considering the cancer-chemopreventive potential of ITCs, cooking broccoli may markedly reduce its beneficial effects.

Summary

Results from cell culture and animal studies indicate that sulforaphane can inhibit chemical-induced carcinogenesis (chemical carcinogenesis); however, little direct evidence is available to support its role in protecting against cancers in humans. Epidemiological studies have shown an association of broccoli intake with a reduced risk of colon cancer. Although the active ingredients responsible for the protective effect were not identified, based on results of the animal and cell culture studies, sulforaphane may be involved (Fig. 1).

Sulforaphane.
Fig. 1 Hydrolytical conversion of glucoraphanin to sulforaphane mediated by myrosinase



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Sulfotransferases

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Synonyms

Sulfokinases

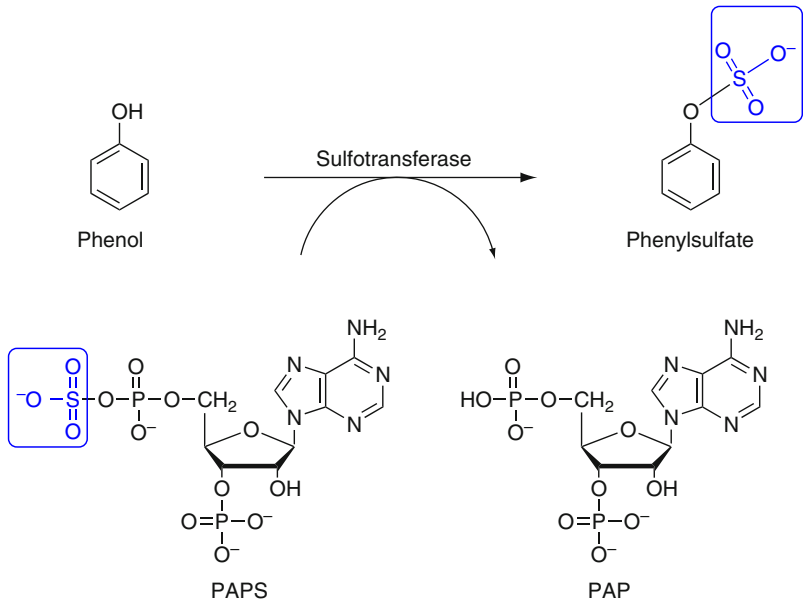
Definition

Sulfotransferases transfer the sulfo (SO_3) group from a donor substrate to a nucleophilic site of an acceptor substrate (Fig. 1). Whereas 3´-phosphoadenosine-5´-phosphosulfate (PAPS) serves as the sulfo donor for all eukaryotic sulfotransferases studied, the acceptor substrates are highly variable (small endogenous and ▶ xenobiotic molecules as well as various macromolecules) depending on the individual sulfotransferase enzyme. Sulfotransferases must not be mistaken for ▶ sulfatases. The latter enzymes hydrolyze sulfo conjugates. Together with sulfotransferases they regulate local and systemic levels of various hormones and the sulfation state of glycan structures.

Characteristics

Sulfotransferases can be classified on the basis of the sulfo acceptor as: (1) glycan structures of proteoglycans; (2) carbohydrates of glycolipids; (3) tyrosine residues of proteins; and (4) small molecules. The amino acid sequences and the gene structures are very different between these four classes.

Proteoglycan carbohydrate sulfotransferases modify sugar and aminosugar residues of glycoproteins. Nearly 30 forms are present in the human. Most forms are localized in membranes of the Golgi apparatus. Some classes of products, such as the glycosaminoglycans (heparan, chondroitin, dermatan, and keratan sulfates), are modified in many different positions. This complex and variable modification



Sulfotransferases.
Fig. 1 Sulfotransferase reaction illustrated for phenol as the acceptor substrate. In this case the sulfo group is transferred to an oxygen atom, generating a sulfooxy (sulfate) group. Other possible acceptor sites are amino and – rarely – thiol groups, leading to the formation of sulfoamino and sulfothio groups, respectively

requires several different sulfotransferases (including combined *N*-deacetylases/*N*-sulfotransferases). *O*-Sulfonated (sulfated) and/or *N*-sulfonated glycoproteins are important components of the cell surface and the extracellular matrix. The negative charges introduced by sulfo transfer affect interactions with other molecules, such as receptors and proteases, and can modulate their function (e.g., as co-receptors or protease inhibitors).

Protein tyrosine sulfotransferases (two forms in the human) modify tyrosine residues of proteins. The tyrosine sulfation motif is identical to the tyrosine phosphorylation motif, and both modifications involve the same increase in mass (80 Da). Nevertheless, there is no competition between these post-translational modifications, as they occur in different cellular compartments. The protein tyrosine sulfotransferases are integral membrane proteins of the Trans Golgi Network. Most tyrosine-sulfated proteins are secretory, others are incorporated into lysosomal and plasma membranes. Protein tyrosine sulfation is irreversible.

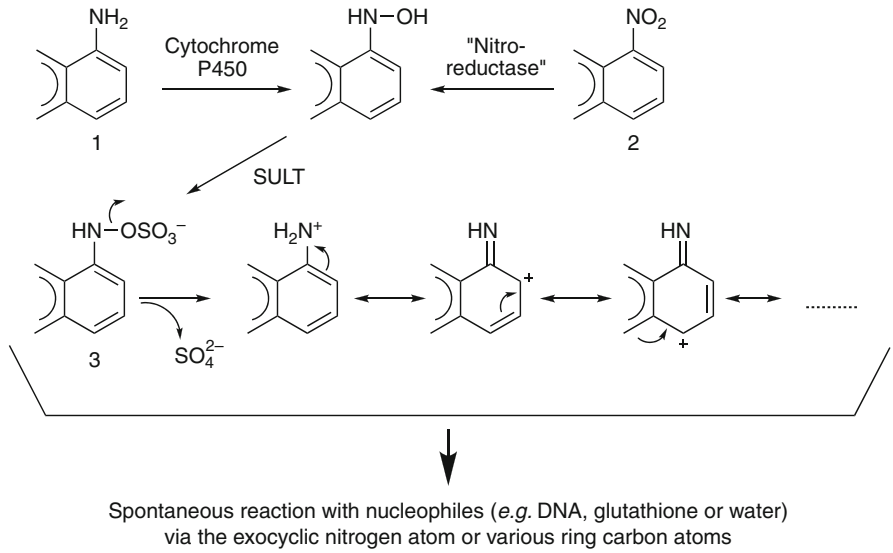
A glycolipid sulfotransferase localized in the Golgi apparatus catalyzes the formation of various sulfated glycolipids, such as cerebroside sulfate (a major component of myelin) and seminolipid (found in testis).

The fourth class of sulfotransferases modifies small molecules. These enzymes are soluble proteins and are usually localized in the cytoplasm. However, some forms are enriched in cell nuclei in some tissues. Most are present as homodimers with subunit masses of 30.5–41.3 kDa (human forms). Monomeric and heterodimeric proteins may also occur. These sulfotransferases are members of a single superfamily, termed ▶ SULT. Using the degree of amino acid sequence identity as a guide, the SULT superfamily is further classified into families (SULT1, 2, 4 and 6 in humans), subfamilies, and individual forms of subunits (13 in humans). Some SULTs play an important role in the regulation of local and systemic levels of endogenous compounds: SULT1A3/1A4 (identical protein encoded by two different genes) for catecholamines, ▶ SULT1E1 for estrogens, and SULT2 enzymes for various alcoholic steroids, bile acids, and cholesterol (hydroxysteroid and alcohol sulfotransferases are trivial names for the SULT2 enzymes). However, all these forms also metabolize certain xenobiotics with high efficiency. ▶ SULT1A1, the most abundant SULT form in humans, is characterized by very broad substrate tolerance toward xenobiotics and a moderate catalytic efficiency for various endogens substrates (estrogens, iodothyronines, and catecholamines), for which it may serve as a backup enzyme in the absence of more specific SULT forms. The primary functions of the remaining human SULT forms are less clear, although a number of endogenous and xenobiotic substrates have been identified, except for SULT4A1, a form specifically expressed in brain. Not only that there is no substrate found for this form, it also lacks the ability to bind PAPS, the common co-substrate of sulfotransferases. The amino acid sequence of SULT4A1 is conserved to an unusually high extent between different mammalian and other vertebrate species, suggesting an important, hitherto unknown function. Other forms may also have undetected functions in addition to their sulfo transfer activities.

With the striking exception of SULT4A1, the SULT superfamily shows high evolutionary dynamics. While a single SULT1A gene has been detected in all non-primate species investigated, four genes occur in the human. The non-primate enzymes (SULT1A1) metabolize both xenobiotics and catecholamines; they are supported in these functions by SULT1D1. On the contrary, humans have SULT1A enzymes specialized for catecholamine metabolism (encoded by the *SULT1A3* and SULT1A4 genes), and other forms with enhanced xenobiotic-metabolizing activity but strongly decreased affinity for catecholamines (SULT1A1 and SULT1A2); moreover, the SULT1D1 gene is degenerated to a pseudogene in humans. An opposite situation is found for the SULT2A subfamily, which comprises a single form in humans, but at least three different forms in mice and rats. Sexual dimorphism of hepatic expression is another species-dependent characteristic of many SULTs. This dimorphism is dramatic in young adult rats, with Sult1c1 and Sult1e1 exclusively expressed in males and the various Sult2a enzymes exclusively or predominantly expressed in females. Such sexual dimorphisms are absent or minute in humans, much below the interindividual variations within the sexes.

Sulfotransferases and Cancer

The expression of many sulfotransferases strongly depends on the type and the differentiation state of cells. Changes in levels and patterns of proteoglycan carbohydrate sulfotransferases, glycolipid sulfotransferases, and SULTs have often been reported between normal, preneoplastic, and neoplastic cells,



Sulfotransferases. Fig. 2 Metabolic activation of aromatic amines (1) and nitroarenes (2) to reactive sulfuric acid esters (3). In general, these esters are very short-lived in water ($t_{1/2} < 1$ s). Note that the heterolytic cleavage is not a reversal of the SULT reaction, as the leaving sulfate group contains an additional oxygen atom. "Nitroreductase" is not a specific enzyme in mammalian cells, but a side activity of various enzymes primarily catalyzing other reactions (such as xanthine oxidase, cytochrome P450 reductase, and quinone reductase)

but without elucidation of the causal relationships. In principle, sulfation-dependent alterations in cell–cell, cell–matrix, and protein–protein interactions on the plasma membranes could trigger the process of carcinogenesis. More specific connections with carcinogenesis can be made for SULTs, as they metabolize hormones, carcinogens, and anticancer drugs.

Estrogen-dependent tissues are major sites of the expression of SULT1E1, which probably is used for tuning the estrogen responsiveness. In the endometrium, SULT1E1 expression strongly varies during the menstrual cycle, roughly in an anti-parallel manner to the expression of the ▶ estrogen receptor, and is upregulated in pregnancy. SULT1E1 is present in normal mammary cells, but not in tumor cells. This difference may lead to an increased local level of active estrogen hormones in tumor cells.

Numerous chemical carcinogens require metabolic activation to electrophilically reactive intermediates that can covalently bind to DNA and induce mutations. 2-Acetylaminofluorene was the first carcinogen for which an activation pathway was elucidated. It involved *N*-hydroxylation followed by sulfo conjugation. The in vitro findings were corroborated in vivo: inhibition of SULT or PAPS deficiency abolished, or drastically decreased, the hepatocarcinogenicity of 2-acetylaminofluorene and *N*-hydroxy-2-acetylaminofluorene in mice and rats. Meanwhile, it is evident that SULTs are involved in the formation of reactive, mutagenic, and potentially carcinogenic metabolites from many compounds. The reason is in the fact that sulfate is a good leaving group in certain chemical linkages, for example, when the resulting cation is resonance-stabilized (Fig. 2). Thus, SULT- or *N*-acetyltransferase-mediated esterification usually represents the final activation of various homo- and heterocyclic nitro-, amino-, and amidoarenes (Fig. 2). The relative importance of the various SULTs and *N*-acetyltransferases in this activation strongly depends on congener, species, and tissue. Other rodent carcinogens that can be activated by SULTs to mutagens include some alkylated polycyclic aromatic hydrocarbons (e.g., 1-methylpyrene), alkenylbenzenes (e.g., safrole), nitroalkanes (e.g., 2-nitropropane), steroidal drugs (e.g., cyproterone acetate), schistosomacidal drugs (e.g., hycanthone), and anti-estrogens (e.g., ▶ tamoxifen).

SULT-mediated activation is not detected in standard in vitro mutagenicity tests, as these systems are SULT-deficient. Addition of an external SULT system is not a reliable remedy for this shortcoming, as sulfo conjugates are charged and may not penetrate into the

target cells. cDNA-mediated expression of SULTs within the target cells is a more appropriate alternative. In general, a given promutagen is only activated by a small number of SULT forms, which vary for different promutagens.

In the rat, SULT expression is strongly concentrated to the liver. This is true in particular for those forms that are capable of activating many different promutagens. Indeed, SULT-dependent carcinogens usually induce liver tumors in the rat. The kidney is a potential target for those reactive sulfo conjugates that are sufficiently stable to be transferred via the circulation to this site. Various human SULTs have a much wider tissue distribution than rat SULTs. Thus, it is important to consider other candidate target tissues in humans beside the liver and kidney.

Human SULT1A1 is able to activate a particularly wide range of diverse promutagens. However, it also forms stable sulfo conjugates from a much larger number of other xenobiotics and thus may contribute to their detoxification. SULT1A1 shows a common polymorphism, involving a ^{213}Arg/His exchange, which affects enzyme expression and activity. Furthermore, this polymorphism is genetically linked with another functional polymorphism in the neighboring SULT1A2 gene. The incidence of various tumors significantly differed between SULT1A1 genotypes in epidemiological studies. The high-activity genotype was associated with a reduced risk in some cases and an elevated risk in other cases. Possibly, these findings reflect the dual role of SULT1A1 in detoxification and toxification of xenobiotics.

► Aminoflavone, an aromatic amine, is a new cytostatic drug entering clinical trials. It requires bioactivation by ► Cytochromes P450 and SULTs (pathway 1–3 in Fig. 2). The growth-inhibiting activity of aminoflavone in 60 human tumor cell lines was primarily determined by the level of expression of SULT1A1.

The antiestrogenic activity of tamoxifen, a drug used in the treatment of mammary tumors, is mainly due to its metabolite 4-hydroxytamoxifen, which has much higher affinity for estrogen receptors than the parent drug. 4-Hydroxytamoxifen is a good substrate of SULT1A1. Surprisingly, the high-activity genotype of SULT1A1 was associated with an increased survival time in tamoxifen-treated women with breast cancer; among patients who did not receive tamoxifen, there was no association between survival and SULT1A1 genotype. Thus, it appears that sulfation of 4-hydroxytamoxifen provides a benefit whose mechanism remains to be elucidated.

In conclusion, SULTs are involved in the metabolic activation and inactivation of carcinogens, the regulation of hormones interacting with tumors, and the activation and inactivation of some anticancer drugs. Sulfotransferases that modify macromolecules might also play a role in carcinogenesis, as sulfated and *N*-sulfonated macromolecules are major constituents of the plasma membrane and the extracellular matrix, which are important for cell–cell interactions.

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SULT

Definition

Acronym for the gene superfamily encoding soluble sulfotransferases.

► Sulfotransferases

SULT1A1

Definition

Most abundant soluble sulfotransferase in the human organism. It facilitates the excretion of numerous drugs and other ► xenobiotics. It also catalyses

the final activation step of various carcinogens and the anti-cancer drug ▶ aminoflavone.

▶ Sulfotransferases

SULT1E1

Definition

Sulfotransferase form with high affinity for estrogens, primarily expressed in estrogen-dependent tissues (for tuning estrogen responsiveness) and in human small intestine (for inactivating dietary estradiol/estrone) and liver. A small number of promutagens are activated by SULT1E1 with much higher efficiency than by other sulfotransferase forms.

▶ Sulfotransferases

Sumoylation

Definition

Is the reversible, covalent attachment of SUMO, a ubiquitin-like protein (Ubl). Many proteins, such as transcription factors, are regulated by sumoylation. Often sumoylation acts antagonistic against ▶ ubiquitination, preventing proteins from being degraded. Sumoylation is important for the regulation of DNA damage repair and for the maintenance of genome integrity. There is evidence that sumoylation is involved in cancer metastasis.

Sun Light Induced Cancer

▶ Photocarcinogenesis

Sunbeds

Definition

The device used for tanning with an artificial source of ▶ UV radiation may be referred to as a sunbed, a sunlamp, artificial UV, artificial light, a tanning bed, among other terms. Also, a number of terms are used to define a place where indoor tanning may occur: solarium, tanning salon, tanning parlor, tanning booth, indoor tanning salon, and indoor tanning facility. In addition, indoor tanning may take place in private, non-commercial premises.

Sunitinib

Definition

Is a small molecule that blocks the ▶ vascular endothelial growth factor receptor as well as other receptors. It has antiangiogenic and anticancer properties.

▶ Antiangiogenesis

Superantigens

Definition

A class of antigens, including certain bacterial toxins, that unleash a massive and damaging immune response.

Supercooled Phase

Definition

Phase without ice formation, which occurs firstly when tissue is subjected to a constant lowering temperature.

▶ Cryosurgery in Bone Tumors

Supercooling

Definition

In cryosurgery, pure water with a subzero temperature.

▶ Cryosurgery in Bone Tumors

Superoxide Dismutase

Definition

SOD; is an important intracellular antioxidant defense mechanism that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. The three mammalian types of enzyme differ with respect to their intracellular localization and the metal atom within their active center (Cu, Zn, or Mn). Overexpression of SOD in cancer cell lines induces an increase in H_2O_2 production and reduces tumor growth.

► Oxidative Stress
► Photodynamic Therapy

Superoxide Radical

Definition

Chemically, an oxygen molecule to which one extra electron has been added, giving it a negative charge. O_2^-

► Nickel Carcinogenesis

Supervised (Machine) Learning

► Supervised Classification

Supervised Classification

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Synonyms

Supervised (machine) learning

Definition

Supervised classification is the process in which an artificial system (usually a computer program) is used to generate a predictive model (► Classifier) based on numerical feature descriptions of real-world observations (samples) that are grouped in at least two different categories (classes). The objective of this process is to establish a classifier that predicts with a minimal error the class of new samples that have not been used for construction of the classifier.

Characteristics

Clinical management and basic research of heterogeneous diseases, such as cancer, increasingly covers sophisticated technical systems and laboratory assays that may generate large volumes of high-dimensional data. A prominent example from the field of cancer research is genome-wide expression analysis using ► microarray technology, an approach that may yield tens of thousands of data points (gene-expression levels) for a single biological sample. These comprehensive analyses may reveal important information on the underlying biological phenotype of the sample and are frequently applied to identify novel ► biomarkers for disease outcome or response to therapy. However, processing, analysis, and interpretation of this high-dimensional data are not trivial. Deriving proper conclusions from such complex measurements therefore requires sophisticated computational methods in order to reliably classify the phenotype of each sample. The process in which a computational method is applied to generate or apply a diagnostic or prognostic tool for phenotype classification of a biological sample is called supervised classification.

The techniques applied in supervised classification have their origin in machine learning research and have been developed for the task of learning by examples: Certain real-world observations of interest (samples) can be grouped in different categories (classes). The attributes (features) of the samples can be described, for example, by numerical quantification through a measurement. The central question addressed by supervised classification approaches is: "Given the features and class labels of an exemplary set of samples (training set), can a pattern of feature values be derived that is indicative of a certain class

and is therefore able to accurately predict the class of new samples that were not used for learning (test set) based on their feature description?" Different techniques have been contrived for the task of learning by examples, however, the general framework for the process of supervised classification typically follows a certain standard: A learning machine (a computer program) is confronted with a data set of training samples for which the class affiliation is known and derives a predictive model. This subprocess is known as ▶ model selection. The predictive value of the derived model is then determined by its ability to generalize from the given set of training samples, i.e., to predict with minimal error the class of new samples that have not been used for classifier generation. This subprocess is also referred to as ▶ model testing.

Clinical Relevance

In cancer research, techniques for supervised classification have been used particularly for data from molecular and cell biology endeavoring to identify novel biomarkers from ▶ DNA microarray experiments for gene-expression profiling or comparative genomic hybridization (▶ ArrayCGH), ▶ microRNA expression analyses, mass spectrometry or ▶ proteinchip profiling. The endpoints predicted in such studies usually either cover the classification of disease subtypes, the prediction of patients' outcome or the prediction of response to a certain therapy. With growing experience in dealing with high-dimensional data from these techniques it has become evident that a universally best method for classifier creation does not exist. Therefore, an abundance of strategies and algorithms have been described that may be successfully utilized for supervised classification. Some of the more frequently used learning algorithms are: Linear Discriminant Analysis, Decision Trees, Support Vector Machine (SVM) Learning, Artificial Neural Networks (ANN), and the Nearest Shrunken Centroid Analysis (also known as "Prediction Analysis of Microarrays," PAM).

Caveats and Recommendations

Before tools derived from supervised classification approaches can be used in a clinical setting, it is mandatory that they are both highly accurate and reproducibly applicable. Studies that propose novel predictors by supervised classification should therefore mind the following recommendations:

1. A classifier is not sufficiently characterized by a list of its features. Therefore, a detailed description of the algorithm used and the parameters of the derived predictive model is mandatory. Only a thorough description of the application of supervised classification techniques guarantees reproducible results.
2. Proper evaluation of generated predictive models is mandatory before these models can be introduced into a clinical setting. With high-dimensional data, the number of measured features exceeds that of available samples for classifier construction by far (the feature to sample ratio is high). As a consequence, there is a big threat for "overfitting" classifiers to the unique set of samples from which they have been derived. In this context, overfitting means that although the classifier performs good results on the training set of samples (and therefore seems to "fit" this set quite well) its predictive power might turn out to be poor in an independent data set. To avoid the problem of overfitting, stringent evaluation of a generated classifier should be performed by proper (cross) validation. This implies the use of large sets of samples that have not been used for classifier generation (independent samples).
3. In principle, it is advisable to follow the recommendations of Simon and Altman, who suggest classifying studies that promote new diagnostic or prognostic tools into one of three phases. Studies showing an association of a new prognostic factor with real outcome or an improved prognostic performance in comparison to established markers are categorized as phase I or II. Studies investigating the hypothesis that a newly created prognostic tool emanating from phase I and II studies can be independently validated on a large cohort of new patients are of phase III. Only classifiers that successfully pass all three study phases should be considered for utilization in the clinical setting.
4. High classification accuracy of a predictor does not imply biological relevance of its features. A big caveat needs to be put to this fact as even predictive models with extraordinary accuracy will comprise neither all nor only features that are functionally related to the phenotype of the disease. Deducing biological importance from high discriminative power of features will be misleading in the vast majority of cases. Furthermore, utilizing different

algorithms will lead to distinct predictive models, all of which may classify a given set of samples with similar accuracy although they are composed of varying sets of features.

Abiding by these recommendations may save from many of the pitfalls of supervised classification. When applied and evaluated properly, supervised classification approaches will undoubtedly play an important role in the development of improved cancer diagnostics, optimized risk stratification and more individualized, patient-tailored therapy.

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Supportive Care

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Synonyms

Palliative care

Definition

Supportive care refers to those services, both specialist and generalist, that might be required by people affected by cancer (patients and family members) to meet the many needs associated with cancer and cancer treatment. Thus supportive care is provided across the continuum of the cancer journey, from the point when the possibility of cancer is first raised, through diagnosis and treatment and into palliative care or survival.

Characteristics

Supportive care has been referred to as an "umbrella" term to describe the many services involved in assisting people affected by cancer and their families to live well and manage the many needs that arise from having cancer and receiving cancer treatment. There is growing evidence that the needs of people with cancer are numerous and that despite increased awareness by health professionals about these needs they frequently remain unmet. Needs relate to many domains but are often classified as informational, physical, psychological, and existential. There is also growing recognition that these unmet needs affect both short- and long-term outcomes for people with cancer and their families and require more formal approaches to ensure support outcomes are improved. The increasingly organized approach to supportive care in cancer is notable in the formation of the Multinational Association of Supportive Care in Cancer (MASCC) in 1990. MASCC recognizes supportive care to encompass all aspects of care beyond those involved in direct cancer disease management and as involving a range of disciplines and specialists including doctors, nurses, social workers, psychologists, dentists, researchers, educators, and others.

Supportive care encompasses many common elements of care provision such as symptom control, rehabilitation, psychological support, self-help, information provision, patient education, complementary therapies, spiritual care, and palliative care. High-quality supportive care assists patients to continue potentially curative cancer treatment, to live well with advanced disease, to manage the return to "normal" as a cancer survivor, and to focus on quality living at the end of life. Supportive interventions can range from pharmacological interventions such as antiemetics through to psychological interventions such as cognitive behavioral therapy. All health professionals involved in cancer care delivery will be involved in the delivery of supportive care to people affected by cancer and their families, and thus such care requires a team approach.

Perhaps the most important related term is palliative care as in many ways the term supportive care seeks to

widen the commonly understood elements of palliative care to aspects of cancer care not traditionally understood as requiring palliative care involvement. Palliative care, as distinct from ▶ palliative therapy aims to improve the quality of life of people facing life-limiting and terminal illness through provision of pain and symptom relief and attention to the broader psychosocial and existential needs. While many aspects of palliative care are applicable earlier in the course of cancer, many people affected by cancer, their families, and health providers find the close association between palliative care and end-of-life care a barrier to earlier engagement of these services in meeting non-end-of-life support needs. Palliative care is also not recognized as extending beyond cancer treatment into survivorship. Examples of support needs where palliative care services might usefully be included in earlier parts of the illness trajectory include the management of symptoms associated with cancer treatment such as pain from chemotherapy-induced mucositis and the psychosocial support of patients and families who face a crisis of meaning following the diagnosis of cancer. However, it is more difficult to define other supportive needs as being palliative care, such as pre-chemotherapy education, learning to talk after a laryngectomy, or managing issues such as return to work following successful cancer treatment. The rise of modern palliative care has been the catalyst for increased attention to the wider supportive care needs of people with cancer and their families but the term supportive care captures this broader focus of meeting these other support needs.

Supportive care services rely on several key elements for success: assessment of supportive care needs, discussion with patients and families about their priorities for support, referral to appropriate specialist services including the possibility of self-referral and access to appropriate health and support information to enable the patient to understand what is happening to them, to engage in self-care, and to participate fully in health-related decision making.

Four key principles appear to drive the development of supportive care services internationally in that they are commonly featured in the literature on this topic:

- Supportive care needs require an individualized approach.
- Patients and carers are critical members of the supportive care team.
- A multidisciplinary and coordinated approach is essential.
- The development of an evidence base for interventions to improve supportive care outcomes.

Supportive Care Needs Requiring an Individualized Approach

While supportive care needs are able to be identified, classified, and measured among groups of patients with similar cancers or receiving similar cancer treatments, to be effective approaches to supporting people with these needs must take account of the person's unique situation. Providing information and support that is tailored to a person's expressed needs improves outcomes. Tailoring of supportive care services to the individual's situation means taking account of their life orientation, values, response to illness, existing support system, and goals for care. What is a major problem and cause of distress for one patient or family member might be of only minor concern to another. Thus, central to the provision of supportive care services is an ongoing dialogue with the person affected by cancer and their family to ensure appropriate responses to the needs they experience.

Patients and Carers: Key Members of the Supportive Care Team

Importantly, supportive care is not the sole domain of qualified health professionals, as is the case in disease-related treatment. Indeed patients and their families are considered to be an important component of supportive care delivery. The premise is that supportive care services should allow care to be delivered in home and community settings and that people affected by cancer should be involved in service design and in the development of novel interventions for testing in robust clinical trials.

For many people affected by cancer being at home is made possible through personal and family/friend involvement in supportive care delivery. Supportive care provided by patients and their family/friends can range from technical care such as the management of medications and central venous access devices, through instrumental and daily living care such as assistance with hygiene, mobility, and household chores through psychosocial support to cope with the emotional demands of a cancer diagnosis, its treatment, and ongoing adverse side effects. Cancer treatment is now largely delivered in the ambulatory setting

and thus supportive care interventions increasingly focus on improving the capacity of patients and their significant others to undertake self-management of the many demands of cancer treatment.

A Multidisciplinary and Coordinated Approach

The supportive care needs expressed by people affected by cancer are varied and often complex, this complexity often requiring the input of multiple health professionals. For example, the management of the oral mucosa during chemotherapy might involve a dentist to undertake a pretreatment check and correction of caries, a dental hygienist to remove plaque build-up, a nurse to assist with daily oral hygiene reinforcement and oral assessment to minimize oral complications, enable early detection, and manage oral analgesics, a palliative care physician to manage severe pain, and a dietician to assist with maintenance of the patient's nutritional status. No one discipline will successfully manage these support needs alone.

Supportive care also often requires the involvement of several settings of care such as acute, primary, and home care. Coordination of care becomes a key feature of supportive care delivery with a high need for attention to information flow and the avoidance of gaps and/or duplication of care.

In reality, many of the disciplines required to be involved in supportive care, such as dieticians, physiotherapists, and clinical psychologists, are not well established as specialist disciplines in cancer care in many parts of the world and are also poorly captured in the systems that fund cancer services. This means that the burden of paying for supportive care services, such as those involved in return to work programs or long-term enteral feeding, are part of the large out-of-pocket expenses faced by many people following cancer treatment.

The Development of an Evidence Base to Interventions to Improve Supportive Care Outcomes

As the multiple supportive care needs of people affected by cancer have become better recognized so has the need for an evidence base to guide clinicians in how to best address these needs. The increasing focus on supportive care highlights the paucity of evidence to guide interventions to prevent or reduce supportive care needs. In the United Kingdom, national guidance has been developed for supportive and palliative care but is largely based on levels of evidence from consensus or descriptive studies with few randomized controlled trials. As a result in many western countries there is an emerging emphasis being placed on the development of research capacity in supportive care. Governments, such as in Canada, the United Kingdom, and Australia, recognize that appropriately used *Supportive Care Interventions* can reduce time in hospital, improve quality of life, and increase patient satisfaction with health services.

One of the most critical areas of supportive care where there is a growing evidence base is in meeting the psychosocial needs of people affected by cancer, with a specific emphasis on evidence-based communication. Guidelines in this area establish a strong mandate for enhancing the training of health professionals in good communication skills, particularly around the delivery of health information and in eliciting and responding to the patient's concerns. However, even in this area much of the research has focused on women with breast cancer and may miss some of the subtleties of providing for the psychosocial needs of men and individuals with other cancers.

The increasing attention on research is evidenced by the development of specialist journals on supportive care such as Supportive Care in Cancer and The Journal of Supportive and Palliative Care and the creation of a WHO Collaborating Center for Supportive Cancer Care at the MD Anderson Cancer Center in Houston, Texas. However, there remain many gaps in the knowledge on how best to prevent, minimize, manage, and recover from the many adverse effects of having, receiving treatment for, living with, dying from, or surviving cancer.

Conclusion

Supportive care is becoming recognized as a key component of cancer management, standing alongside medical, surgical, and radiation treatments to ensure best physical, psychological, and social outcomes for people affected by cancer and their families. However, there are many barriers to best practice in supportive care including funding, the lack of a strong evidence base in many areas, and shortages of specialist services in many disciplines. It is imperative that cancer control programs to capture the supportive care needs of people affected by cancer and foster research into the best interventions to prevent and minimize these needs are established.

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Suppressive T Cells

► Regulatory T Cells

Suppressor of Fused

Definition

Abbreviated Su(Fu) in mouse; SU(FU) in human; a negative regulator of hedgehog signaling that regulates the nuclear translocation of the Gli transcription factors.

► Hedgehog Signaling

Suppressor of Invasion, Metastasis, and Angiogenesis

► RECK Glycoprotein

Suppressor T Cells

Definition

► Regulatory T Cells.
► T Regulatory Cells
► Treg

Suppressors of Cytokine Signaling

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Synonyms

SOCS

Definition

Suppressors of cytokine signaling (► SOCS) and the cytokine-inducible SH2 protein CIS regulate the responses of various cells to ► Interleukins and other cytokines. They are rapidly induced after treatment with a cytokine. SOCS prevent prolonged activation of the signaling pathway of Janus kinase (► JAK)/ ► signal transducer and activator of transcription (► STAT).

Characteristics

The SOCS/CIS family has eight members: SOCS-1, -2, -3, -4, -5, -6, -7, and CIS. They are composed of an amino-terminal domain of variable length, central ► SH2 domain, and a carboxy-terminal 40-amino-acid module known as SOCS box. Expression and function of SOCS-1, -2, -3 and CIS were characterized in most studies.

SOCS-1 and SOCS-3 downregulate JAK tyrosine kinase activity due to the presence of an inhibitory region in their amino-terminal domain. SOCS-1 could bind directly to JAK kinase through its SH2 domain, whereas SOCS-3 SH2 domain binds the signal-transduction subunit gp130 of a cytokine receptor. The SOCS box is important for interaction with the ubiquitin-transferase system. It suppresses oncogenic activity of the TEL gene fused to JAK2 and STAT phosphorylation in hematopoietic cells. This part of the molecule is involved in the degradation of the nucleotide exchange factor Vav and the papilloma virus oncoprotein E7. SOCS-1 and -3 are major negative regulators of signaling of ► interleukins (IL), in particular ► IL-6, through inhibition of prolonged

activation of the JAK/STAT pathway. Mice deficient of SOCS-1 die due to a myeloproliferative disorder caused by uncontrolled ▶ interferon-gamma and tumor necrosis factor-alpha signaling. Deletion of SOCS-3 is associated with polycythemia, a premalignant form of erythroid ▶ leukemia.

SOCS proteins also interact with either ▶ insulin-like growth factor or ▶ insulin receptors thus diminishing the effect of these antiapoptotic molecules. Both regulators of cytokine signaling degrade insulin receptor-substrate-1 and -2 thus inhibiting action of the ▶ insulin-like growth factors in target tissues.

CIS and SOCS-2 bind to phosphorylated tyrosine residues on activated cytokine receptors. Cytokines which induce STAT5, such as ▶ prolactin, ▶ erythropoietin, IL-2, -3, and growth hormone signal via upregulation of CIS. Similarly, SOCS-2 acts as a ▶ negative feedback regulator of growth hormone signaling. SOCS-2-deficient mice present with liver hypertrophy, increase in serum levels of ▶ insulin-like growth factor-I, and weight.

Role of SOCS-1 in Cancer

SOCS proteins are considered tumor suppressors in most human malignant neoplasms on the basis of expression and functional studies. The expression of SOCS-1 and SOCS-3 is reduced in several tumors in which IL-6 acts as a pathogenetic factor. Mice with haploinsufficiency of the *SOCS-1 gene* develop severe liver fibrosis. In these animals, the development of ▶ hepatocellular carcinoma was accelerated. Expression of SOCS-1 is decreased in hepatocellular carcinoma because of frequent hypermethylation of CpG islands. It was found in 65% of analyzed liver carcinoma samples. Transfection of hepatocellular carcinoma cells with SOCS-1 cDNA resulted in a reduced growth rate and anchorage-independent growth.

▶ Epigenetic changes in the *SOCS-1* gene promoter were also observed in tumors derived from gastrointestinal tract, ▶ lung cancer, ovary, pancreas, as well as myeloma and chronic lymphoid leukemia. SOCS-1-deficient animals increasingly develop ▶ Colorectal tumors. Decreased SOCS expression because of changes in ▶ epigenetic mechanisms or increased degradation leads to continuous activation of JAK2 and STAT thus promoting tumor growth.

JAK activation in cancer could be caused not only by IL-6 and related cytokines but also by ▶ Src kinase. SOCS could inhibit JAK/STAT activity induced by the cytokines of the IL-6 family but not that induced by ▶ Src. SOCS-1 is a negative growth regulator of cells transformed by the Kit receptor tyrosine kinase or the v-Abl oncogene. Tumorigenicity and metastatic activity of Tel-JAK2 and Bcl–Abl cells were diminished by expression of SOCS-1. Tumors that overexpress v-Abl may bypass the inhibitory effect of SOCS-1 through phosphorylation, disruption of its interaction with ▶ elongin, or inhibition of proteasomal (▶ Proteasome) targeting of JAK.

SOCS-1 expression increased in human ▶ melanoma in comparison to melanocytes in normal skin and melanocytic nevi. In contrast to several solid neoplasms, SOCS-1 expression correlates with tumor invasion and could be considered a progression marker in melanoma. SOCS-1 mutations were frequently detected in primary mediastinal B-cell and ▶ Hodgkin lymphoma and are associated with increased nuclear phospho-STAT5 accumulation. Thus inadequate action of SOCS-1 in cancer leads to a hyperactivation of signaling through the STAT pathway.

SOCS-3 in Human Cancer

The role of SOCS-3 in human cancer is similar to that of SOCS-1. Its expression is decreased in lung, head and neck, and liver cancer. SOCS-3 promoter hypermethylation was frequently observed in these tumors. Deregulation of the STAT3 pathway was reported in cutaneous lymphoma cells in which constitutively active STAT3 and SOCS3 are simultaneously expressed. SOCS-3 levels in lymphoma were reduced by transfection of the dominant-negative STAT3. Under these experimental conditions, the cells became more sensitive to an effect of ▶ interferon-alpha.

SOCS-1, -2, and -3 and CIS transcripts and immunoreactive proteins are elevated in in situ ductal and invasive ▶ breast cancer. It seems that SOCS exhibit their action in regulation of STAT3 in tumors in cooperation with other proteins such as ▶ caveolin. These interactions may be critical for the outcome of STAT3 phosphorylation in breast and ▶ Prostate Cancer. Estrogenic induction of SOCS3 in breast cancer cells was blocked by the pure estrogen receptor antagonist ICI 182,780.

IL-6 causes a variety of biological effects in prostate tumors. They may include inhibition of proliferation but also promotion of survival of tumor cells and

stimulation of ▶ angiogenesis. In prostate cancer, the expression of SOCS-3 was investigated in a number of cell lines. SOCS-3 mRNA and protein were found in cells in which there is no expression or phosphorylation of STAT3. In contrast, SOCS-3 was not detectable in LNCaP cells in which treatment with IL-6 induces STAT3 phosphorylation, growth inhibition, and terminal neuroendocrine differentiation. Loss of SOCS-3 in that cell line is a consequence of gene promoter hypermethylation. SOCS-3 expression is higher in samples of prostate cancer compared with those obtained from benign tissue. Upregulation of SOCS-3 in prostate and pituitary cancer cell lines was observed after treatment with an AMP derivative. Agents that elevate intracellular ▶ cAMP cause an inhibition of proliferation and stimulation of ▶ apoptosis in prostate cancer cells. siRNA approach revealed that SOCS-3 acts as a negative feedback regulator of action of hormones that induce increased cAMP levels.

SOCS-2 in Malignant Diseases

Compared with SOCS-1 and -3, there is a more limited evidence supporting global role for SOCS-2 as a tumor suppressor or promoter. SOCS-2 levels are elevated in BCR–ABL tyrosine kinase-positive in comparison to BCR–ABL-negative chronic myeloid leukemia cell lines. They also increased in patients with chronic myeloid leukemia in blast crisis. SOCS-2 is a component of a BCR–ABL-▶ negative feedback mechanism. It could inhibit some of its effects in leukemia cells. Leukemia cells, however, develop a resistance to inhibitory effects of SOCS-2. SOCS-2 is induced by estrogen and growth hormone in hepatoma and breast cancer cells.

In short, SOCS proteins are involved in regulation of cellular events in cancer tissue. In most tumors, their expression decreases because of epigenetic changes in respective gene promoters. SOCS reexpression in some of the cancer cell lines leads to retardation of tumor growth. In breast and prostate cancer, SOCS elevation may prevent oncogenic signal transduction through the JAK/STAT signaling pathway.

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Suprachiasmatic Nucleus

Synonyms

SCN

Definition

A region in the hypothalamus of the brainstem that consists of small, bilaterally paired clusters of nerve cells that comprise the central circadian pacemaker or biological clock of the brain.

▶ Melatonin

Supraparamagnetic Nanoparticle

Definition

A type of nanoparticle composed of iron oxide that is used as a contrast agent for magnetic resonance imaging.

▶ Nanotechnology

Suramin

Definition

Is a polysulfonated naphthyl urea, inhibiting ▶ heparanase with an IC50 of 48 μM. Suramin inhibited B16 melanoma cell invasion ($IC_{50} = 10$ μM)

through reconstituted basement membrane but had no effects on melanoma cell growth. Suramin has not been widely used because it has significant toxic effects in humans, including neurotoxicity, renal toxicity, adrenal insufficiency, and anticoagulant-mediated blood dyscrasias. In efforts to avoid these side effects, analogs of suramin have been synthesized and are undergoing evaluation. Compounds NF 227, NF 145, and NF 171 are three such analogs, all of which possess heparanase inhibitory activities more potent than that of suramin (the IC_{50} values were 20–30 μM). These compounds effectively inhibited heparanase-mediated ▶ angiogenesis in an animal model.

▶ Heparanase Inhibitors

Surface Glycoproteins

Definition

Proteins imbedded in the outer membrane of a cell that have polysaccharides attached, particularly on the outer side.

▶ CD Antigens

Surface Molecules

▶ CD Antigens

Surface Plasmon Resonance

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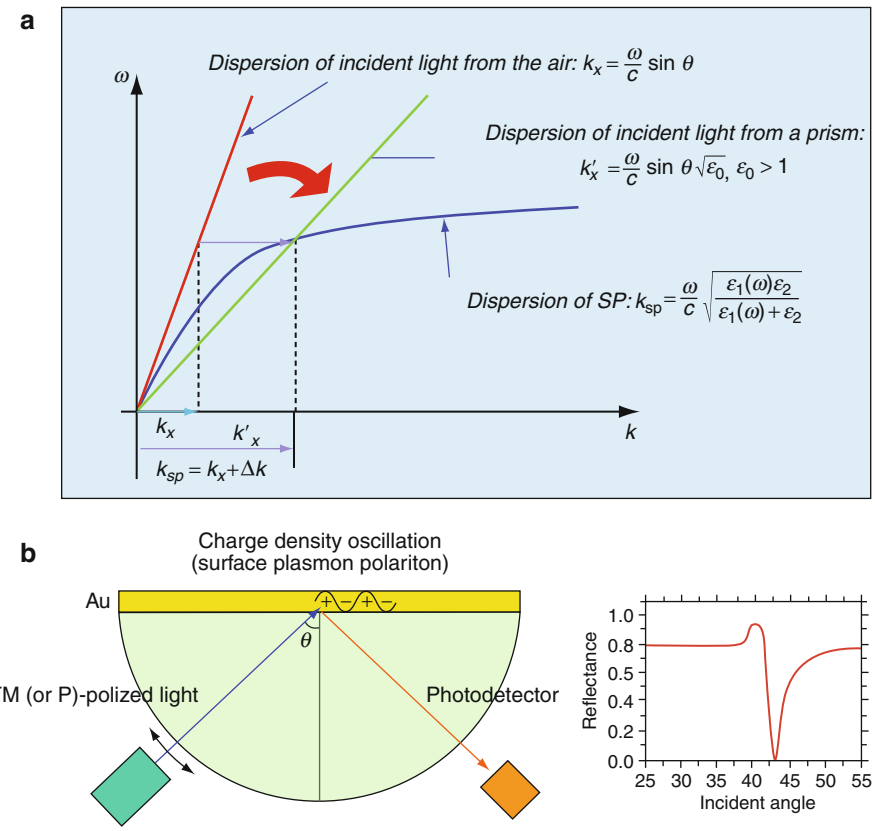
Definition

Surface plasmon resonance (SPR) is one of several optical phenomena known to occur on two-dimensional metal surfaces (typically gold or silver films) when a total internal reflection of incident light occurs at the interface of two different substances, one with a high refraction index and the other with a low refraction index. The SPR ▶ biosensor, which exploits the SPR phenomenon, is a label-free and surface-sensitive spectroscopic system, which utilizes measured changes in the local refraction index upon adsorption. This sensor may be applicable to disease diagnostics and ▶ high-throughput screening (HTS) in drug discovery, as well as to studies of biomolecular interaction.

Characteristics

Detection Principle of SPR Biosensor

Surface plasmon polaritons (SPPs), which are also referred to more simply as surface plasmons (SPs), are longitudinal and collective oscillations of electrons occurring on a metal–dielectric interface. The frequency of these longitudinal oscillations is linked to the wave vector (k_{sp}) by a dispersion relation. The incidence of light can induce the excitation of the SPs when the momentum of the SPs matches that of the incident light. In such cases, the well-characterized surface plasmon resonance phenomenon occurs. As SP waves are inherently longitudinal, the waves can be coupled only with the transverse magnetic (TM) mode of electromagnetic waves, the polarized direction of which is parallel to the incident plane. It has been determined that this wave-matching condition can be quite readily disrupted by even miniscule changes in the interface conditions. Hence, in cases in which the light excitation condition is fixed, the SPR technique not only allows for the precise measurement of changes in the refractive index or the thickness of the medium adjacent to the metal film, but also enables the detection of changes in the adsorption layer on the metal surface. As is shown in Fig. 1a, the surface plasmons have a larger wave vector than do light waves of the same energy $\leq \omega$. In order to excite the SPs with photons, the wave vector of the photons must be increased. Thus far, two basic apparatus can be utilized to achieve this: (1) a prism coupler and (2) a grating coupler. The Kretschmann geometry of the ATR method, using a prism, is currently the approach most often employed in SPR sensors. This is a special case of ATR, in which a thin metal film exists at the ATR interface. As can be observed in Fig. 1b, the light wave is reflected completely at the



Surface Plasmon Resonance. Fig. 1 (**a**) The dispersion relation of non-radiative SPs and (**b**) the configuration of the ATR method. See text for details

interface, and excites the SP via the evanescent field. Detection is thus accomplished by recording the changes in the resonance angle or the wavelength.

Properties of the SPR Biosensor

As has been thoroughly documented, the SPR biosensor is a versatile optical spectroscopic system, and represents a promising technology for the real-time, ▶ label-free analysis of affinity-based measurements in the fields of analytical biochemistry, experimental biology, and medicine. Following the introduction of the SPR biosensor by Jonsson et al. in 1991, the applications of SPR technology have expanded significantly, coming to encompass a wide-ranging field of topics. However, the applications of SPR technology in biomedical science are particularly salient; as many as 5,000 review and research articles concerning SPR have been published over the last 15 years. SPR-based biosensor technologies remain a subject of intensive research, and technical advances in the approach are continually opening new opportunities for the application of the general method. Numerous SPR apparatus are being developed and exploited on the basis of theoretical developments. One such promising approach is the coupling of SPR to mass spectrometry (SPR-MS), an approach that may prove to be extraordinarily useful in the field of functional proteomics. This hybrid SPR-MS system has shown itself to be a rapid and effective method for the identification of interaction partners in complex biological mixtures. The other principal SPR-associated technology involves the application of the technique to imaging systems. With regard to SPR imaging (SPRI), this system detects the change in the reflectivity of incident light, due to the binding of biomolecules to chip surfaces at a fixed angle of incidence, in contrast to SPR systems involving the detection of shifts in the SPR angle or wavelength. SPR imaging using optical array detectors appears to constitute a promising new direction in parallel or multi-channel biosensing, and thus may allow for high-throughput drug screening. Additionally, SPRI technology may also be applicable to the diagnosis of different types of disease including human cancers in the near future. Currently, commercially obtainable SPR instruments are large and expensive, and are therefore inappropriate for applications

requiring portability and affordability, such as point-of-care technology (POCT). For this reason, miniaturization efforts that render the development of a portable system feasible have recently been undertaken in parallel with other components of research into more advanced SPR instrumentation.

Biomedical Applications

The most extensively employed application of SPR sensor technology is the monitoring of affinity scale in the study of biomolecular interactions. The many SPR-based affinity analysis applications currently available have become extremely significant in biomedicine and other fields. Biomedical applications of SPR can be categorized into three general fields: (1) biomolecular interaction analysis, (2) high-throughput screening, and (3) proteomics research.

Biomolecular Interaction Analysis (BIA)

The most common application of SPR biosensors is biomolecular interaction analysis (BIA), a critical component of protein function research. SPR technology has been applied to the monitoring of a variety of biological events, including kinetic analyses of ligand–receptor interactions, kinetic analyses of DNA binding to proteins with captured DNA, interaction analyses of enzymes with their substrates, dynamic analyses of antigen–antibody binding, epitope mapping, DNA hybridization, and real-time monitoring of DNA manipulation. In addition, the spectral SPR profile has been shown to be influenced by changes in the optical thickness of the sensor metal film, as well as by changes in the refraction index occurring near the metal surface (within ~200 nm). Because these optical indicators can be affected by structural transitions in proteins, SPR has also been utilized in the characterization of the conformational alterations of immobilized proteins upon binding to small molecules or in a variety of environments.

High-Throughput Screening (HTS)

SPR-based biosensors can be employed not only for the real-time monitoring of the kinetics of ligands with their receptors, but also in the development of pharmaceuticals, as SPR can be employed in drug-screening procedures for single molecules in drug discovery studies. The application of SPR technology to high-throughput screening (HTS) is another recent trend in drug screening. SPR systems have been configured into a variety of formats, including array format, multi-channel unit format, and SPR imaging (SPRI) format, which allow for simultaneous real-time measurement in the range of hundreds to thousands of binding reactions on the surface of a chip. Despite the profound versatility of SPR technology, SPR biosensors are also known to have a significant drawback that makes them inappropriate for high-throughput screening, as this system does not allow for the analysis of many samples in parallel. By way of contrast, SPR imaging technology using optical array detectors not only allows for high-throughput multiplex analysis, but also provides a sensitivity almost identical to that of classical SPR. Therefore, SPR imaging systems are more appropriate for high-throughput label-free detection than any other optical technique. SPR imaging methods allow for the quantitative characterization of biomolecular interactions, including DNA–DNA duplexes and DNA–drug interactions, in an HTS manner. Furthermore, the targeting of single-base mismatches in the alteration of DNA–DNA hybridization properties has been achieved in DNA arrays, using SPRI biosensors. Another technology that uses the SPR imaging system has been applied to the monitoring of real-time interactions of proteins to a DNA-patterned chip surface in a high-throughput manner. For example, this approach has been used for the high-throughput analysis of interactions occurring between the ▶ p53 Protein and multiple DNA sequences. Recently, a novel SPR imaging-based HTS system for anti-cancer drug discovery was developed by Ro et al. in 2006. In the research, in order to determine whether the SPR imaging system was capable of screening for small molecules that inhibit protein–protein interactions, the interaction between the retinoblastoma tumor suppressor ▶ RB1/pTP53 and the ▶ human papillomavirus (HPV) E7 protein was selected for use as a model system. The RB–E7 interaction was challenged by the spotting of the RB protein in the presence of the RB binding peptide (PepC). The SPR imaging results showed that PepC inhibited the RB–E7 interaction in a concentration-dependent manner, thereby indicating that SPR imaging–based HTS technology could potentially provide a versatile tool for the selection of small molecule inhibitors, via the targeting of protein–protein interactions.

Proteomics Research

One powerful method by which the biological function of most proteins can be anticipated is the identification of the interaction partners of "bait" proteins, which results in the discovery of protein biomarkers for disease diagnosis and drug screening. These functional proteomics studies, including "ligand fishing" from complex biological mixtures, can be performed efficiently using SPR biosensors coupled with mass spectrometry (MS). This combined SPR–MS system, when utilized as a tool for ligand fishing, allows for the identification of interaction partners with the desired drug candidate characteristics, biomarkers in a variety of therapeutic areas, epitopes or antibody-binding sites on protein antigens, and enzyme inhibitors in extracts constructed from diverse organisms. Conventional SPR–MS approaches can be used to characterize unknown proteins that have been captured on the sensor surface, both via SPR technology and by exact direct mass measurement with ▶ matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS. As SPR detection is non-destructive and non-labeling, the combination of these two systems is quite relevant to possible approaches to the identification of binding partners directly after interaction analysis, followed by mass spectrometric assays. Recently, a new analytical protocol, in which SPR is coupled to ▶ electrospray ionization (ESI) MS, has created a new opportunity for the identification and secondary characterization of interaction partners. This system is, potentially, an extremely effective method for the identification of novel binding partners. Therefore, the combined SPR–MS system is expected to become a powerful tool in the area of quantitative analysis of functional proteomics, a field which includes large-scale "ligand fishing" assays.

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Surface-Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry

Definition

SELDI-TOF MS is an approach that combines two powerful technologies, retentate chromatography and MS. The core of the SELDI-TOF MS platform is the proteinchip arrays, which have varying chromatographic properties, such as anion exchange, cation exchange, metal affinity, and reverse phase. The SELDI-TOF MS provides on-chip separation as well as the capability to perform enzymatic reactions directly on the chip. Various complicated biological materials can be uniformly captured, concentrated, and purified on the small chemical surface of the chip. To be able to identify the proteins, the complexity of the sample was reduced by fractionation approaches. A complex mixture of proteins from cells or body fluids can be reduced to sets of proteins with common properties by binding the sample to chips with differing surface chemistries in parallel and in series.

▶ Proteinchip

Surgery

Definition

Removal of the tumor by a surgeon.

Surgical Biopsy

Synonyms

Open biopsy

Definition

Use of surgery to sample or remove tissue.

▶ Fine Needle Aspiration

Surgical Debulking

Definition

Debulking surgery (synonym), also ▶ cytoreductive surgery, is used to remove just a portion of a cancerous tumor. It is recommended in situations when removing an entire tumor might damage an organ or other parts of the body. It is common for other cancer treatments, such as chemotherapy or radiation, to be used after a debulking procedure.

Surgical Menopause

Definition

Menopause that occurs when a premenopausal woman has both of her ovaries removed.

▶ Menopausal Symptoms After Breast Cancer Therapy

Surgical Pathology

▶ Pathology

Surgical Trauma and Cancer Recurrence

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Definition

Surgical trauma occurs after every surgical procedure by using traumatic tools, gauzes, and by rubbing the tissue. This causes an inflammatory response during which loads of cytokines and growth factors are produced. These factors will create an outstanding environment where tumor cells can adhere and flourish into a tumor lesion. Understanding the specific mechanism of tumor cell adhesion can lead to develop specific tools to prevent local and distant recurrence in the future.

Characteristics

The process of cancer metastasis could be compared to an exhausting obstacle race, during which the tumor cell has to pass a series of sequential interrelated steps to become a clinically relevant lesion. In distant metastasis, the tumor cell must succeed in invasion, embolization, survival in the circulation, adhere to a distant capillary endothelium, followed by extravasation and multiplication in another organ. Locoregional tumor recurrence after intra-abdominal seeding or spill of tumor cells seems to be less complicated; the tumor cell has to adhere, after which it can flourish and grow out as a lesion.

Although the process of locoregional tumor recurrence gives the impression to be highly comprehensible, peritoneal and local recurrence is a persistent hurdle after curative resection of colon or pancreatic tumors. The resection site is preferable and recurrence to locoregional sites is common. Several theories on local tumor recurrence have been advocated. The most feasible theory is the development of local recurrence after resection of locally advanced disease, which already penetrates the peritoneal surface or adjacent organs. Another source for local recurrence is the presence of cancer cells in the abdominal cavity prior or during surgery. Peritoneal washings, before manipulation of the tumor, are positive in 20–30% of the patients with colorectal cancer. Furthermore, extensive manipulation of the tumor during surgery will cause leakage of tumor cells out of the dissected lesion or out of the transected lymphatic channels or veins. The free floating tumor cells or tumor emboli will precipitate on raw tissue, followed by an inflammatory response during which an outstanding environment for the tumor cells will be created.

Approximately 40% of the patients with colorectal cancer, who underwent surgery for local or locally advanced disease, develop recurrent disease. The intra-abdominal recurrence rate after curative resection for pancreatic cancer is even more deplorable. Methods of prevention and treatment of locoregional metastasis, like neoadjuvant radiotherapy, brachytherapy, adjuvant chemotherapy, photodynamic therapy, (hyperthermic)

intraperitoneal chemotherapy, and peritonectomy, are developed and implemented in daily surgical practice. The benefit of adjuvant chemotherapy on overall survival for patients with node-positive, locally advanced colon cancer is well established. The adjuvant therapy regimen in pancreatic cancer has not been elucidated yet. The ESPAC-1 randomized controlled trial showed no survival benefit for adjuvant chemoradiotherapy after R0 resection, however, it revealed a potential benefit for adjuvant chemotherapy. However, a study from Smeenk et al. showed that adjuvant chemoradiotherapy, after irradical resections of pancreatic cancer, gives a significant improvement of local control, nevertheless treatment with chemoradiotherapy does not improve survival.

These (neo)adjuvant treatment strategies are mainly based on diminishing advanced disease. An adequate understanding of the pathophysiology of the tumor cell adherence is needed to clarify the initial step of implantation.

Trauma and Inflammation

Since Virchow's studies in the mid-nineteenth century, the role of inflammation and wounding as an initiator and promoter of tumor development has been implied. Virchow indicated that cancers tended to occur at sites of chronic inflammation.

The investigation aiming to clarify the relationship between inflammation and cancers first led to the determination, whether the reactive oxygen species (ROS) and nitrogen species generated by inflammatory cells, such as leucocytes recruited to the inflammatory foci to kill infectious agents, may cause mutagenic assaults and result in tumor initiation. Nowadays, it has been realized that the development of cancers from inflammation might be a process driven by inflammatory cells as well as a variety of mediators, including cytokines, chemokines, and enzymes, which altogether establish an inflammatory microenvironment.

Inflammation is a process in response to tissue damage caused by microbial pathogen infection, chemical or mechanical induced wounding. At the very early stage of inflammation, neutrophils are the first cells to migrate to the inflammatory sites under the regulation of molecules produced by rapidly responding macrophages and mast cells present in tissues. As the inflammation progresses, various types of leucocytes, lymphocytes, and other inflammatory cells are activated and attracted to the inflamed site by a signaling network involving a great number of growth factors, cytokines, and chemokines.

Surgical trauma induces an acute phase response, during which tissue damage will be controlled, infective organisms will be killed, and the repair process will be induced. The acute phase response is initiated by macrophages and monocytes entering the surgical traumatized site, which release proinflammatory cytokines, tumor necrosis factor alpha (TNF-α), and interleukin-1 beta (IL-1β). TNF-α and IL-1β stimulate the production and release of other cytokines, like interleukin 6 (IL-6). These cytokines are potential directors of the expression of cell adhesion molecules (CAMs) and regulating in that way the adhesiveness between leucocytes and the endothelium. Parallel to the endothelium, the inflammatory cascade following surgical trauma in the peritoneal cavity creates an outstanding environment for residual or spilled tumor cells to adhere to mesothelial cells.

Laboratory Investigations

The process of tumor recurrence can be separated into tumor cell adhesion and tumor growth. Initially the tumor cells have to adhere before they nurture and develop into a metastatic lesion. To study the specific pathways of tumor cell adhesion we developed an in vitro model, in which we investigated the interactions of different human colon and pancreatic tumor cell lines on mesothelial monolayers and the endothelium. Our study focuses on the influence of surgical derived inflammatory factors on tumor cell adhesion. IL-1β and TNF-α are crucial enhancing factors of tumor cell adhesion to the mesothelial monolayers and the microvascular endothelium. In addition, IL-1β and TNF-α significantly upregulated the expression of adhesion molecules ICAM-1, VCAM-1, and CD44 on mesothelial cells and endothelial cells.

These cytokines are produced during the acute inflammatory response, which is induced by peritoneal trauma and has to initiate the wound-healing process. IL-1β, TNF-α, and IL-6, produced by activated leukocytes, are the major mediators of inflammation and tumorigenesis. Together they generate the production of adhesion molecules, growth factors, nitric oxide, and the activation of the NF-κB pathway. In that way the proinflammatory cytokines will stimulate tumor adhesion, growth, and invasion. Furthermore, mesothelial cells have additional active participation

in the inflammatory response by producing proinflammatory cytokines and in that way stimulating the expression of adhesion molecules. TNF-α has a dominant function in the abdominal cavity and might modulate the production of ILs from mesothelial cells. Production of IL-1 and IL-8 by mesothelial cells is enhanced after stimulating the cells with TNF-α. In malignant disease, high-dose local TNF-α selectively destroys tumor blood vessels and thereby induces apoptosis, but when chronically produced this cytokine may act as an endogenous tumor promoter. It contributes to the modulation of the cell, that is, CAMs, necessary for tumor spread and growth. The influence of IL-1β on tumor cell metastasis is inevitable as well. In mouse metastasis models, treatment with an IL-1 receptor antagonist significantly decreased tumor development. Additionally, IL-1β-deficient mice are resistant for developing metastases.

During the inflammatory response, PMN are attracted to the site of injury, which is mediated by chemotactic factors and proinflammatory cytokines. PMN are known to aggravate an overwhelming burst of ROS to destroy invading organisms and inducing additional tissue destruction. Furthermore this oxidative burst can be induced by TNF-α, which causes an upregulation of the FMLP receptors (N-formyl-methionyl-leucyl-phenylalanine) on PMN. In our studies, FMLP-stimulated PMN induce a significant enhancement of tumor cell adhesion. Moreover, the ROS producing system (X/XO) exhibits an even superior enhancement of adhesion of tumor cells and this enhancement was inhibited by antioxidant scavengers.

Clinical Applications

Notwithstanding that surgery remains the treatment of choice in colorectal and pancreatic cancer, local recurrence after curative surgical resection is an incessant drawback. Different pathways of local tumor recurrence have been unraveled, nonetheless in what way can these investigational results be implemented in clinical practice?

Peroperative diminishing surgical trauma, by using less traumatic tools, gauzes, and techniques, seems to be an adequate option. The study done by Bouvy et al. showed in a rat model that laparoscopic surgery is associated with less tumor growth stimulation compared with conventional surgery, due to reduced surgical trauma. Additionally laparoscopic surgery is correlated with less immunological alterations and this may imply less local tumor recurrence as well. Lacy et al. showed that laparoscopic-assisted colectomy was associated with a significantly lower probability of tumor recurrence and a higher probability of overall and cancer-related survival in stage III cancer. Although the recent publication of Law et al. is not a randomized controlled trial, this study put forward a significant survival benefit for patients, who underwent a laparoscopic resection in stage I–III colon cancer. The two randomized controlled trials, in which laparoscopic-assisted colectomy was compared with open surgery, could not reveal this benefit concerning tumor recurrence in the laparoscopic group.

Since the expression pattern of adhesion molecules on tumor cells is tremendously diverse, it is not feasible to use single monoclonal antibodies to confront spilled tumor cells. The development of cell adhesion peptides (i.e., RGD peptide) provides promising results by blocking the adherence of tumor cells to the components of the extracellular matrix. The use of RGD peptides has been expanded with preliminary results by using the RGD peptides for delivering drugs to tumor cells that express certain integrin types after which an internalization process takes place of the integrin adhesion complex.

Interfering with the inflammatory process during and after surgery is a reliable option to prevent tumor recurrence; however, wound healing is depending on this process. This interference should be very selective, otherwise the healing process will be disturbed. Interference with the inflammatory response might be accomplished by inhibiting the influx of PMN into the peritoneal cavity with antineutrophil serum (ANS) after curative resections of gastrointestinal tumors. An in vivo study showed a significant decrease of peritoneal tumor recurrence after intraperitoneal injection of ANS.

Another pathway of interest is the influence of ROS on tumor cell adhesion. Accumulation inhibitors of ROS are superoxide dismutase, catalases, glutathione peroxidases, and vitamins C and E; these antioxidant enzymes and nonenzymatic systems are engineered during stress. Since the release of ROS is enormous following surgical trauma, additional exogenous administration of antioxidants might be a therapeutical option.

An antioxidant with potential is melatonin. Melatonin, produced mainly in the pineal gland,

possesses a wide spectrum of biologic activities, including its function as a naturally occurring oncostatic neurohormone by inhibiting cell proliferation, inducing apoptosis, and reducing metastatic spread. Regarding the scavenger function of melatonin, a synthetic form might be of interest in prevention of tumor recurrence.

Since the discovery of NF-κB in the mid-1980s, this transcription factor has been a subject of intense investigation. The NF-κB transcription factor complex is a pleiotropic activator that participates in the induction of a wide variety of cellular and viral genes. Binding sites for NF-κB are present in the promoter region of many CAMs, cytokines, and growth factors. Antisense inhibition of NF-κB activity causes a block of cellular adhesion to the extracellular matrix, inhibition of in vivo growth of adherent cells, and inhibition of in vivo tumorigenicity in nude mouse models. Recent investigations also showed that ROS is engaged in a unique reciprocal cross-talk with NF-κB. The exact mechanism has not been unraveled yet; however, remarkable is that the induction of NF-κB is abrogated by overexpression of ROS scavenging enzymes.

A promising NF-κB inhibitor is pentoxifylline (trental). The inhibition of the transcription of NF-κB might cause a suppression of CAMs and in that way a potential decrease of lung metastasis. In addition, pentoxifylline may reduce the TNF-α-induced oxidative burst by reduced binding of FMLP to PMN surface receptors.

Interference with the invasion of tumor cells through the extracellular matrix is another therapeutic option. Matrix metalloproteinases (MMPs), a group of zinc-dependent endopeptidases, play an important role in the growth and invasion of colorectal and pancreatic cancers by degradation of the extracellular matrix. The levels of certain MMPs can be used to estimate the metastatic capacity and recurrence of disease as well as prognosis of patients. However, for effective therapy using MMP inhibitors, highly selective administration may be required. Since MMP-7 is the most critical MMP for colorectal cancer progression, developing selective inhibitors against this protease and their administration in the early stage of disease may be worth trying.

The inflammatory sequelae enhance tumor cell adhesion to the mesothelium in vitro. The pathways involved are orchestrated in a meticulous way by proinflammatory factors produced preoperatively in response to surgical trauma. Interference with the inflammatory sequelae (i.e., cytokines, PMN, ROS, adhesion molecules, NF-κB) produced preoperatively must be well balanced, without disturbing the wound-healing process and the systemic immune response. Interference with these pathways may lead to specific tools to conquer the adhesion and growth of spilled tumor cells in vivo.

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Surrogate Endpoint

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Synonyms

Intermediate endpoint; Surrogate endpoint biomarker; Surrogate outcome

Definition

A surrogate endpoint is an outcome observed prior to a health outcome of interest (called the true endpoint) that is used to make conclusions about the effect of intervention on the true endpoint.

Characteristics

Role of Surrogate Endpoints in Cancer Research

The role of surrogate endpoints in cancer research depends on the purposes of the analysis. The main roles are to

- Shorten the length of time needed to evaluate a new treatment for clinical use.
- Quickly and cheaply evaluate interventions at an early phase of development as a prelude to more rigorous evaluation.

Ideally, these roles require the surrogate endpoint to be validated. As with so many terms used in the medical literature, the meaning of the term "validation" can vary among medical scientists. We define validation of a surrogate endpoint as a formal process of data analysis for determining how well the surrogate endpoint can be used to make conclusions about effect of treatment on true endpoint. Some authors use the term "surrogate endpoint" to mean the early outcome has been validated, based on the literal definition of a surrogate as a substitute; here, we use the term surrogate endpoint to refer to an outcome that is considered as a possible substitute for the true endpoint but that still requires validation. If a surrogate endpoint is validated it can, with reasonable confidence, be used to evaluate a new intervention, but subject to very important *caveats*.

Surrogate endpoints can take a variety of forms related to characteristics of genes, proteins, cells, tissues, or individual health status.

- Surrogate endpoints have been proposed to shorten the time needed to evaluate a new cancer treatment or preventive intervention. For example, 3-year disease-free survival has been evaluated as a surrogate endpoint for 5-year overall survival in patients in ▶ colorectal cancer adjuvant ▶ clinical trials, and ▶ prostate-specific antigen (PSA) has been proposed as a surrogate endpoint for ▶ prostate cancer recurrence but its validation has been questioned. Although adenoma occurrence has been used as a surrogate endpoint for colorectal cancer occurrence in cancer prevention trials, it has not been validated.
- Surrogate endpoints are also frequently used to evaluate possible treatments to identify those agents that are the best candidates for more rigorous evaluation using a true endpoint. Examples of these surrogate endpoints are markers of cell ▶ proliferation and ▶ apoptosis. The use of these surrogate endpoints involves much smaller sample sizes than with a true endpoint of cancer incidence which is a major reason (besides shortening the time for evaluation) they are so attractive to researchers. The smaller sample size for the surrogate endpoints arises because the surrogate endpoint is more common than the true endpoint. The more common the endpoint, the smaller the sample size needed to detect a given percentage decrease in probability of endpoint. However, the fact that the sample size is much smaller with the surrogate endpoint than the true endpoint is an indicator that these surrogate endpoints can almost never be validated as currently used in drug development. In this situation, the underlying problem with validation is that a common outcome is not a good substitute for a rare outcome because most subjects with the common outcome do not develop the rare outcome, leading to a large amount of noise. Basically, the only way to adjust for the extra noise is with a sufficiently large sample size to reliably predict the effect of treatment on true endpoint. Nevertheless, because selection of candidate ▶ chemoprotectants (often termed "chemopreventive agents") is needed, these un-validated surrogate endpoints are used in cancer prevention research. Recently developed schema for assigning levels of evidence may be useful for ranking surrogate endpoints for preliminary evaluation of chemoprotectants.
- A third use of surrogate endpoints is for clinical decision-making for individual patients. In this setting, the clinician measures a biomarker on a patient after the start of treatment and uses this marker to predict outcome and inform possible changes in therapy. Validation of surrogate endpoints for this use is not well developed. It is important to realize that most of the literature on validating surrogate endpoints applies to use of surrogate endpoints for evaluating the effect of treatment on true endpoint among a population. This type of validation does not necessarily imply validation for clinical decision-making.

Methods of Validation

This discussion of validation focuses on the role of surrogate endpoints to shorten the duration of cancer trials used to make a clinical recommendation. At the

onset of this discussion, it is important to dispel a common misconception that a high correlation between a surrogate endpoint and a true endpoint implies the surrogate endpoint has been validated. It has been shown mathematically that even perfect correlation between a surrogate and a true endpoint does not guarantee a validated surrogate endpoint. The reason is that the relationship between the surrogate and the true endpoint can differ in different arms of the trial while still being perfectly correlated within each arm. This difference in relationships between arms could lead to incorrect conclusions about the effect of the treatment on the true endpoint when using only information about the effect of treatment on the surrogate endpoint.

When validating a surrogate endpoint, it is preferable to use data from randomized trials rather than from observational studies. When data are collected from a randomized trial, each of the two key components, the effect of treatment on surrogate endpoint and the effect of treatment on true endpoint, are not biased from unmeasured baseline covariates. This is not the case when data are collected from an observational study.

There are two major approaches to validation of surrogate endpoints. One approach involves only a single randomized trial with data on a surrogate and a true endpoint. The other approach involves data from multiple randomized trials with surrogate and true endpoints. Once a surrogate is validated it would be applied to a new trial with only a surrogate endpoint.

Many early methods for surrogate endpoint validation were based on data from a single trial and relied on the Prentice criteria. The Prentice criteria, named after the criteria formulated by Ross Prentice in an influential 1989 article on surrogate endpoint validation, were developed to ensure that rejection of the null hypothesis under the surrogate endpoint implies rejection of the null hypothesis under the true endpoint. The main criterion, sometimes called the Prentice criterion, is that the distribution of the true endpoint conditional on the surrogate endpoint does not depend on the intervention. In other words, the Prentice criterion says that, for all treatments under consideration, there is a single pathway from treatment to true endpoint that goes through the surrogate endpoint, so once the surrogate endpoint is known, no other information is needed to determine the distribution of the true endpoint. With data on a surrogate and true endpoint in a single trial, one can statistically test if the Prentice Criterion holds. If one statistically rejects the Prentice Criterion, the surrogate endpoint is poor. If one cannot statistically reject the Prentice Criterion, further investigation is needed because lack of rejection of a null hypothesis does not automatically imply that the null hypothesis can be accepted. One approach for validation when the Prentice Criterion is not rejected is to compute the proportion of treatment effect explained by the surrogate endpoint. This computation involves fitting two regression models: (1) a model for the effect of treatment on true endpoint and (2) a model for the effect of both treatment and surrogate endpoint on true endpoint. The proportion of treatment effect explained equals one minus the ratio of the coefficient for treatment effect in model (2) to the coefficient for treatment effect in model (1). For a perfect surrogate endpoint, the proportion of treatment effect explained equals one, because the surrogate endpoint captures all the information about the true endpoint. Despite the popularity of the proportion of treatment effect explained, it has many drawbacks including wide confidence intervals. However, the major drawback is the difficulty in reaching any consensus as to the proportion needed to validate a surrogate endpoint. Opinions vary, and the "acceptable" threshold may differ from early phases of drug development to definitive testing for drug approval. The acceptable level may also vary among diseases.

Another approach for single-trial validation with a binary surrogate endpoint involves constructing a model for two levels of the surrogate endpoint: (1) the level observed based on the actual assignment of the randomization group and (2) the level inferred if, contrary to fact, the person were assigned to the other randomization group. While this validation approach has attractive mathematical and conceptual underpinnings, it has not been applied in practice. Also the assumptions required for estimation are very stringent, and may not hold in many situations.

Validation methods based on data from single trials are inherently limited because they do not account for variability over trials in the mechanism by which treatment affects the outcome. This was a major criticism of a single-trial validation of ▶ prostate-specific antigen as a surrogate endpoint for survival among patients with ▶ prostate cancer.

Validation methods based on data from multiple trials of surrogate and true endpoints are becoming

more popular. These surrogate endpoint validation methods are sometimes called meta-analytic because, like standard ▶ meta-analysis of true endpoints in randomized trials, they combine data over multiple trials although the techniques and purpose differ. A variety of meta-analytic methods for validating surrogate endpoints have been proposed, and there is no consensus as to best approach. In fact, some investigators advocate multiple approaches to meta-analytic validation.

Many meta-analytic validation methods are based primarily on two statistics for each trial, the effect of treatment on surrogate endpoint and the effect of treatment on true endpoint, which are often plotted as a set of points. A regression line is then fit to these points to construct a model relating the effect of treatment on the surrogate endpoint to the effect of treatment on the true endpoint. The simplest model is a linear regression based on least squares. More complicated regression models account for additional variability by specifying that the coefficients in the regression vary over trials according to a specified distribution. Different versions of these complex regression models are needed for different types of data (binary, survival, continuous) along with special software. Sometimes there are numerical problems when fitting these models. To apply the results to a new trial, the effect of treatment on surrogate endpoint in the new trial is plugged into the regression model to yield the predicted effect of treatment on true endpoint in the new trial. Alternatively, an investigator can compute a surrogate threshold which is the minimum effect of treatment on surrogate endpoint that corresponds to a statistically significant effect of treatment on true endpoint.

Another meta-analytic approach has been developed for use with binary surrogate endpoints. This approach has been applied to survival data where the binary endpoint is cancer recurrence at an early time and the true endpoint is probability of overall survival to a later time. The basic idea is that each arm of each trial in the meta-analysis has information relating the surrogate and true endpoints. The same relationships can be applied to the surrogate endpoint in the new trial to obtain an estimate of the predicted effect of treatment on true endpoint in the new trial based on data from each previous trial. These estimates are averaged over predictions from all previous trials to obtain an average estimate of the surrogate-based predicted effect of intervention on true endpoint in a new trial.

Meta-analytic methods are most straightforward when each trial involves two randomized groups, with subjects in one group receiving a control treatment and subjects in another group receiving an experimental treatment that is never used as a control.

Special considerations arise when a trial has more than two randomization groups, when it is not clear which treatment is the control, or when a treatment applies to the control group in one trial and the experimental group in another trial.

There are various approaches for using regression type meta-analytic models to summarize the quality of the surrogate endpoint. One summary measure is an individual-level association, which is the squared correlation between surrogate and true endpoints after adjusting for trial and treatment effects. (Unlike the other validation measures discussed, this measure could be useful for clinical decision-making for individual patients.) Another summary measure is a trial-level association, which measures the association between the effect of treatment on surrogate endpoint versus the effect of treatment on true endpoint. Related summary measures based on information theory have also been developed. Sufficiently high values of the summary measure indicate a surrogate endpoint is validated, but more guidance is needed to determine the threshold level.

Another summary measure for the quality of the surrogate endpoint in a meta-analysis is the average prediction error. To compute this measure, one trial is removed from the meta-analysis and treated as a new trial. The other trials are used to predict the effect of treatment on the true endpoint in the "new" trial. The error is the absolute value of the difference between predicted and true effects of intervention on true endpoint in the "new" trial. This procedure is repeated over all trials with a different trial selected as the "new" trial on each iteration. The error is averaged over all trials to obtain the average prediction error. The average prediction error is compared with the average clinically meaningful difference that each trial was designed to detect (which can be inferred based on the sample size of the trial and variability of the true endpoint). As a rule of thumb, a ratio of average prediction error to clinically meaningful difference of 1:10 indicates a good surrogate endpoint (because errors due to the use of the surrogate are relatively small compared to the difference

one hopes to detect), while a ratio of 1 or greater indicates a poor surrogate endpoint. Again this may vary according to how much tolerance one has for making a mistake. This in turn may vary with phase of drug development, lethality or stage of the disease, and toxicity of the intervention.

Caveats in the Use of Validated Surrogate Endpoints

Regardless of the method of validation, there is no guarantee that in a new study the validated surrogate endpoint will in fact yield the correct conclusion about the effect of treatment on a true endpoint in a new trial.

One reason for caution with validated surrogate endpoints is that the treatment under study in a new trial may have different mechanisms for affecting true endpoints than the treatments in previous trials. For example, a new treatment to reduce adenomas may have a different impact on the fraction of adenomas that develop into ▶ colorectal cancer than would previous treatments. A different mechanism for the effect of treatment on true endpoint could mean that the extrapolation from previous trials, even accounting for extra variability, may be incorrect. To avoid this problem, the intervention in the new trial is often restricted to being in the same "class" as the intervention in the trials used for validation, although this is still no guarantee that the surrogate endpoint will yield the correct conclusions in a new trial. In fact, even the same drug could have a different spectrum of mechanistic actions at different doses. For example, beta-carotene is likely an antioxidant at low concentrations but a pro-oxidant at high concentrations and acetaminophen is a safe antipyretic drug at low doses, but is a potent liver toxin at higher doses.

A validated surrogate endpoint should also be viewed with caution when the presence of the surrogate endpoint necessitates additional treatments that could affect the true endpoint. For example, a surrogate endpoint of cancer recurrence will often lead to secondary treatments designed to alter the true endpoint. If a new type of secondary treatment is adopted in a new trial, results from previous trials using the older secondary treatment will no longer be applicable.

Another caution in using a validated surrogate endpoint is that it applies only to one particular endpoint, usually the primary health benefit. In many trials, harmful side effects are a serious consideration. It is possible that in the interval between the surrogate and true endpoints, the intervention can cause serious adverse effects unrelated to the true endpoint. In such circumstances, reliance on the biomarker for decision-making can miss a net harm. In essence, this is what happened with ▶ celecoxib for the prevention of colon cancer (▶ COX-2 in Colorectal Cancer). In a clinical trial, celecoxib decreased the risk of incident adenomatous polyps in patients with prior polyps; but an excess of cardiac deaths occurred prior to the average time interval between polyp formation and cancer development. In summary, if a trial is terminated at the time the surrogate endpoint is observed, it may not have continued sufficiently long enough to provide information about harmful side effects.

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Surrogate Endpoint Biomarker

▶ Surrogate Endpoint

Surrogate Marker

Definition

A measurement of a drug's biologic activity that substitutes for a clinical endpoint such as death or pain relief. It is a laboratory measurement of the biological

activity of the drug within the body that indirectly provides information about the effect of treatment on disease stage. Surrogate marker not necessarily must have a relationship with the real treatment endpoint. Surrogate markers can take a variety of forms related to characteristics of genes, proteins, cells, or cellular processes. Surrogate markers have been proposed to shorten the time needed to evaluate a new cancer treatment or preventive intervention; ► surrogate endpoint.

Surrogate Outcome

Surrogate Outcome

Survival

Is the measure of elapsed time from some well-defined point in time, such as the enrolment into a clinical trial, until an event of interest, such as death, occurs.

► Kaplan–Meier Survival Analysis

Survivin

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Synonyms

API4; Apoptosis inhibitor 4; Baculoviral IAP-repeat containing protein 5; BIRC5

Definition

As a structurally unique member of the ► inhibitor of apoptosis protein (IAP) family, survivin is highly expressed in fetal tissues, but not in most adult tissues. Most human cancers return to the fetal pattern of survivin overexpression, thus suggesting a pivotal role of survivin for tumor cell survival.

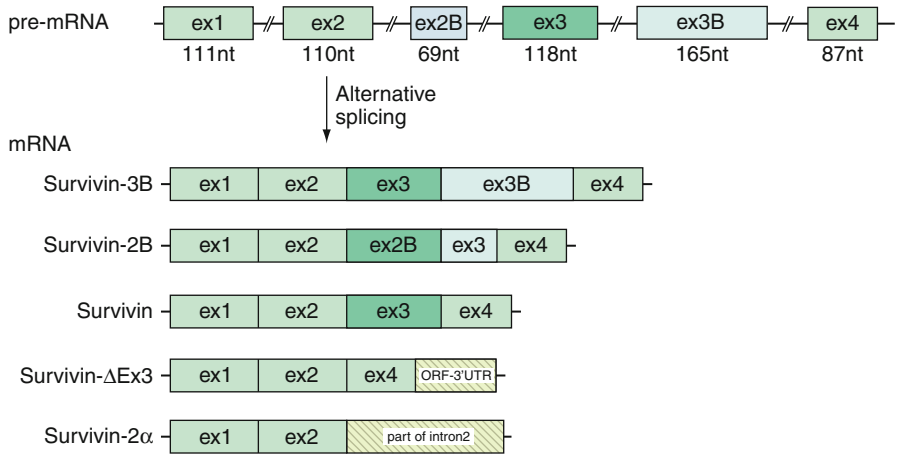
Characteristics

The human survivin gene is located on chromosome 17 (band q25) and encompasses 14.796 base pairs that comprise six exons. The coding strand is preceded by a TATA-less promoter and a GC-rich region corresponding to a ► CpG island. The coding sequence of survivin is largely complementary to the coding strand of the effector cell protease receptor-1 (EPR-1) gene. This suggests that survivin and EPR-1 transcripts originate from duplicated genes that were arranged in opposite orientations. The human survivin gene encodes five different splice variants, which may contribute to the fine-tuning of survivin actions (Fig. 1):

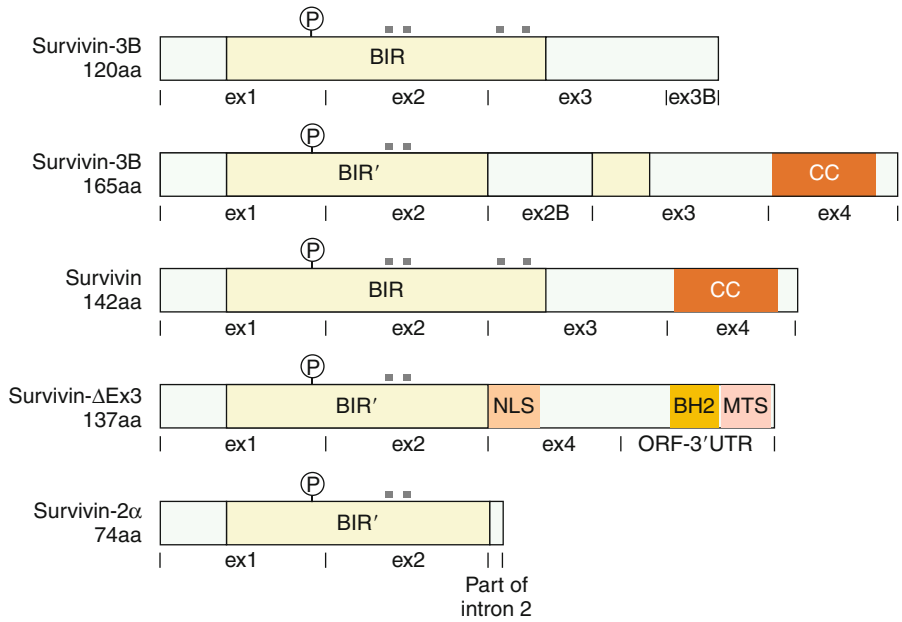
- Survivin is the first of five transcripts identified and consists of exon 1 (111 bp), exon 2 (110 bp), exon 3 (118 bp), and exon 4 (87 bp).
- Survivin-2B is characterized by the insertion of an additional exon 2B (69 bp) between exon 2 and 3.
- Survivin-ΔE 3 shows a loss of exon 3 as well as a frame shift with extension of the open reading frame into the 3′ untranslated region.
- Survivin-3B contains an additional exon 3B derived from a 165 bp long part of intron 3.
- Survivin-2α is characterized by an addition of 197 nt of intron 2, of which 195 nt are noncoding.

The structure of the corresponding survivin proteins is unique as compared to other IAPs. Thus, most mammalian IAPs contain a carboxy-terminal RING finger domain, a caspase recruitment domain (CARD) and, most importantly, two or three copies of a baculovirus IAP repeat (BIR), a zinc finger domain essential for the inhibition of apoptosis by IAPs. In contrast, survivin proteins exhibit only a single BIR domain, an extended carboxy-terminal α-helical coil that forms a bowtie-shaped homodimer, and lack both the RING finger and the CARD region. The splice variants survivin-2B, survivin-ΔE 3, and survivin-2α exhibit pronounced structural alterations that also affect their single BIR domain (Fig. 2). The BIR domain in survivin-3B is not impaired by alternative splicing. Survivin is a 16.5 kDa protein (single chain) and can act as a homodimer, while heterodimerization with its splice variants are also described. To date, research has been mainly focused on the functional properties of the survivin protein, whereas little is known about the four different survivin variants.

Transcription of survivin shows a marked cell cycle–dependent pattern with a pronounced upregulation in the G2/M phase (► G2M-transition).



Survivin. Fig. 1 Structural organization of the alternatively spliced transcripts of the survivin gene. Abbreviations: *ex* exon, *ORF* open reading frame, *UTR* untranslated region, *nt* nucleotides



Survivin. Fig. 2 Protein domains of functional divergent survivin splice variants. Abbreviations: *P* phosphorylation site at Thr34, *z* zinc finger ligand, *BIR* baculoviral IAP repeat, *BIR′* truncated BIR domain, *NLS* nuclear localization signal, *BH2* bcl-2 homology domain 2, *CC* coiled-coil motif, *MTS* mitochondrial targeting sequence, *aa* amino acids, *ex* exon

This cell cycle periodicity has been related to the presence of two Sp1 sites in the proximal promoter region. They might interact with zinc finger transcription factors of the Sp family that are also implicated in the control of other cell cycle–related genes. Suppression of survivin transcription in the G1 phase may be further regulated by a cell cycle homology region (CHR) and by three cell cycle–dependent elements (CDE) of the promoter that are also known from other G2/M-expressed genes.

Functional and Cellular Characteristics

In accordance with other mammalian IAPs, survivin antagonizes a broad range of apoptotic stimuli by inhibiting caspase-3, -7, and -9. Survivin-dependent inhibition of caspase-9 is restricted on phosphorylation at Thr34. Other mechanisms of action in a cell cycle–dependent manner are the association to the ▶ p53 network, as well as the binding to CDK4, and moreover, the activation of survivin by CDK1-dependent phosphorylation at Thr34 has been demonstrated. Survivin has been reported to interact with several other apoptotic factors, such as XIAP and smac/DIABLO. Moreover, many other proteins are known to also interact with survivin: HBXIP, INCENP, ▶ Aurora B kinase, tubulin, ▶ HSP90, etc..

Survivin is abundantly expressed in fetal tissues as revealed by immunohistochemistry in lung alveolar

epithelium, proximal tubule epithelium of the kidney, pancreatic islets, endometrial glands, intestinal crypt epithelium, thymic medulla, and neurons of the spinal cord. Survivin is predominantly localized in the cytoplasm, whereas survivin-ΔE 3 is found exclusively in the nucleus.

In contrast to other mammalian IAPs, however, survivin expression is not detectable by Northern blot, in situ hybridization and immunohistochemistry in normal adult tissues, with the exception of thymus and placenta. The predominant restriction of survivin expression to fetal tissues suggests a key role of this IAP protein for the regulation of developmental apoptosis. Of note, several highly proliferating adult cells and tissues could express increased levels of survivin possibly to escape from cell death (e.g., T lymphocytes).

Survivin actions have been located to the microtubules of the mitotic spindle apparatus. Survivin binding to the polymerized microtubules is mediated by a carboxy-terminal coiled-coil domain. The increased levels of survivin expression during the G2/M phase of the cell cycle might protect the mitotic apparatus from degradation. Therefore, survivin has been suggested to be an active component of the G2/M checkpoint control that preserves chromosomal ploidy and genetic stability by induction of apoptosis in aberrant cells. The downregulation of survivin expression in adult tissues may lower the threshold for apoptosis in replicating cells harboring genetic defects.

Clinical Relevance

Many types of human cancer, such as carcinomas of the lung, stomach, colon, breast, prostate, skin, as well as non-Hodgkin lymphomas, neuroblastomas, and melanomas, return to the fetal pattern of survivin expression. Survivin reexpression may be an early step of malignant transformation, as evident from its presence in precancerous lesions such as colorectal adenomas and Bowen's disease of the skin. The exact molecular mechanisms involved in the reactivation of the survivin gene in human cancers are currently unknown. Nevertheless, the overexpression of survivin in many histogenetically distinct tumor types indicates a strong selection advantage from survivin-related resistance to apoptosis. This selection advantage may result from the loss of an effective G2/M checkpoint control that permits ▶ progression of genetically unstable tumor cells through mitosis. Moreover, tumor cells may profit from the increased resistance to many different pro-apoptotic stimuli, including – inter alia – hypoxia and death signals from immunocompetent cells.

The clinical implications of survivin-related resistance to apoptosis are profound. First retrospective studies on gastric, colorectal, and bladder carcinomas as well as neuroblastomas suggest that survivin may be a prognostic factor, helping to identify patients with an increased risk of rapidly progressive disease.

The presence of survivin in urine may also act as a biological marker for bladder cancer. Different expression patterns of the alternative splice variants of survivin, such as survivin-2B and survivin-ΔE 3, are defined as ▶ biomarkers on mRNA level for tumor staging and progression in certain tumor entities.

Because survivin also confers increased resistance to certain anticancer drugs, e.g., ▶ paclitaxel and methotrexate, the level of survivin overexpression may be used as a predictive parameter for anticancer-drug sensitivity. Finally, the disruption of survivin-related antiapoptosis may become an attractive therapeutic target, selectively increasing the susceptibility of cancer cells to apoptosis-based treatment strategies without affecting the viability of non-neoplastic tissues that do not express survivin. Survivin is proposed to play a central role in the progression and resistance to therapy of diverse tumor types. The clinical utility of survivin and its variants as a diagnostic tumor marker can be profoundly improved if the marker is also a therapeutic target. Many studies suggest that an inactivation of survivin prevents tumor progression.

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Susceptibility Loci

▶ Modifier Loci

Sustained Release

Definition

Drugs released slowly from carriers for a sustained and continuous supply of drugs.

▶ Drug Delivery Systems for Cancer Treatment

Sutent

Definition

▶ Sunitinib malate.

SV40

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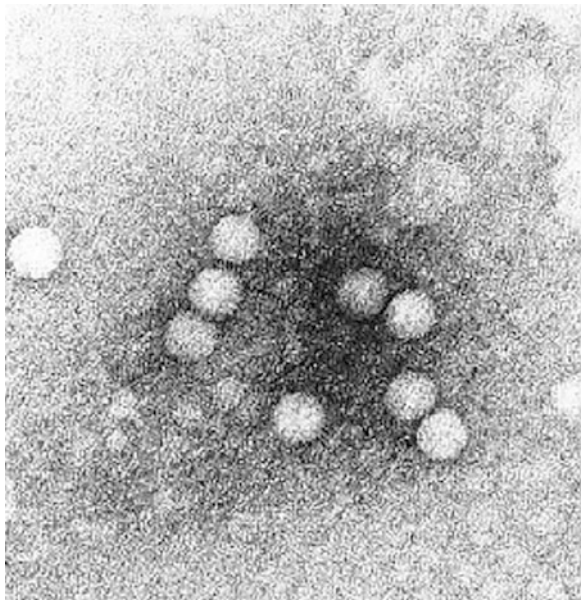
Synonyms

Simian virus 40

Definition

A DNA tumor virus (genus *Polyomavirus*) found to be a contaminant of Salk and Sabin polio vaccines (1955–1961) that propagates naturally in kidney cell lines of Asian macaque species, specifically the rhesus and African green monkey. SV40 in these species, and related primates, produces no cytopathic effects upon the animals, but the virus injected into hamsters and other rodents causes ependymomas, lymphomas, osteosarcomas, sarcomas, and ▶ mesotheliomas. Subsequent research has shown a possible correlation between SV40 (Fig. 1) and human mesotheliomas.

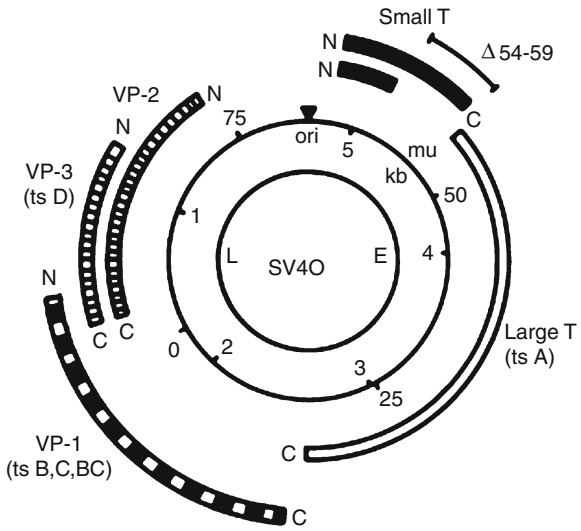


SV40. Fig. 1 Electron micrograph (250,000×) of SV40 virions (kindly provided by Dr. R. Fresco)

Characteristics

SV40 particles lack a lipid envelope and have a diameter of ~40–50 nm with spherical icosahedral symmetry. The molecular mass of the SV40 virion has been estimated to be 270 kDa. The icosahedral capsid contains three viral proteins (VP1, VP2, VP3), with VP1 being the major protein and VP2/VP3 being minor proteins. Along with the viral proteins, the SV40 virion contains cellular histones (H2A, H2B, H3, H4) that aid in condensing the viral DNA. The SV40 genome comprises of a closed circular dsDNA (5,243 bp) that associates with the various histones to achieve condensation, similar to that of cellular DNA in the form of chromatin. Nucleosomes that number between 24 and 26 on the viral DNA are made from the assembly of the histone-DNA complexes.

The genome of SV40 is numbered in a clockwise direction beginning at the origin (Ori) and continuing around until the site of Ori is reached again, marking the end of the genome. SV40 genome is responsible for



SV40. Fig. 2 Genetic map of SV40. *Outer circular segments* represent specific proteins (Tag/tag, VP1, VP2, and VP3). *Inner circle* represent the direction of transcription and region of origin

coding six genes, synthesizing VP1, VP2, VP3, LP1, and the large (T-ag) and small (t-ag) t-antigens. SV40 large T-ag is comprised of 708 amino acids, while the small t-ag contains 174 residues. The initiation of viral DNA synthesis is mediated by the essential replication protein large T-ag, which in turn is regulated by phosphorylation. The transformation of cells and induction of tumor formation by SV40 is another function of the large T-ag, which causes the inactivation of products made from several tumor suppressor genes, including ► p53, ► pRb, p107, p130/Rb2, p300, and p400. One of the crucial consequences of binding large T-ag to p53 is the inactivation of an essential checkpoint that halts the process of mitosis if DNA damage is present, causing the cell to continue cycling. The small t-ag protein is not an essential mediator in SV40 replication, but it does play a significant role by causing an increase in the production of large T-ag and aiding in inactivating p53.

Different regions of the SV40 genome, designated "early" and "late," are expressed at different times during the stages of infection. The early region codes for the large T-ag, the small t-ag, which are the SV40 oncogenes, and for a 17 kDa protein of uncertain biological significance. The late region codes for the capsid proteins VP1, VP2, VP3, and for LP1, a protein involved in the process of SV40 particle assembly late in infection (Fig. 2).

Replication

The early and late regions of the SV40 genome distinctly separate the replication process into two events: Similar to other viruses, SV40 virions come into contact with the outer membrane surface of the host cell and attach to receptors located throughout the outer cell surface. These receptors are thought to represent the major histocompatibility complex class I molecules (MHC class I). Upon being transported into the cell, SV40 virions are moved to the cell nucleus. Once inside the nucleus, the virion capsid disassembles and the viral DNA is released.

At this point in replication, the early region of the SV40 genome is transcribed first, synthesizing the small t-ag/large T-ag proteins. This causes the cells to enter S phase. The SV40 72-bp enhancer elements help positively regulate transcription in the early region of the genome.

It has been identified that there are SV40 strains with either one 72-bp enhancer (archetypal) or two 72-bp enhancers (nonarchetypal). Nonarchetypal SV40 replicates more rapidly compared to archetypal SV40. Three G+C-rich domains, also referred to as 21-bp repeat regions, are binding sites for cellular factors. Upon the initiation of S phase, the viral DNA replication and transcription can now begin from the late region, causing the production of necessary structural proteins (VP1, VP2, VP3, LP1). After the necessary proteins and DNA replication is complete, the various viral particles assemble together forming the next generation of SV40 virions. When a high number of virions accumulate in the cells, the cell is lysed and infectious SV40 is released. Infected mesothelial cells, however, can release infectious SV40 without undergoing lysis.

Clinical Relevance

Poliomyelitis was a devastating disease that swept throughout the western world until 1955 when the Salk vaccine began to be used against this crippling disease. Polio vaccines made from monkey kidney cell cultures between 1955 and 1961 and sold until 1963 were found to have been contaminated with numerous supposedly harmless viruses. Knowledge of this contamination was well known, but there was no evidence to suggest any tumorigenic properties of any viruses present in the vaccines. In 1960, Sweet and Hilleman established that an unknown percentage of polio vaccines produced from monkey kidney cell lines was

contaminated with SV40. In 1962, Eddy and colleagues produced investigations showing that newborn hamsters injected with rhesus monkey kidney cell cultures developed sarcomas. The resultant sarcomas were attributed to the DNA tumor virus SV40.

It was shown that SV40 was able to successfully replicate, produce infection, and spread throughout humans by oral and respiratory routes. In 1964, when SV40-transformed human cells were injected subcutaneously into volunteer terminally ill patients, those cells were found capable of growth. Vaccines that were produced after 1961 were required by federal law to be tested for SV40, but by that time it has been estimated that ~98 million people, both adults and children, had already been exposed to SV40 through a contaminated polio vaccine. SV40 was able to transform both human and rodent cells in tissue culture. However, epidemiological studies suggested that SV40 was not oncogenic in humans, because the overall incidence of cancer in cohorts injected with contaminated polio vaccines was similar to that of cohorts who had not been exposed to SV40 contaminated polio vaccines.

Subsequent investigations in 1993 showed that when hamsters were injected with SV40 into the pleural space, all of the animals developed mesotheliomas within 3–6 months. Mesotheliomas are tumors that have increased from almost zero to 3,000 cases per year in the USA during the past 50 years. In the USA mesotheliomas are mostly caused by ▶ asbestos, the finding that SV40 caused mesothelioma in hamsters prompted investigations into the possibility that some mesotheliomas in humans could be attributed to SV40 infection directly or with SV40 acting as a cocarcinogen with asbestos. Mesothelioma samples studied in 1994 showed that 60% of the samples contained SV40 DNA and expressed the SV40 large T (tumor) antigen. The results were confirmed by numerous laboratories using a variety of techniques such as PCR, in situ hybridization, Western blot, immunohistochemistry, Laser dissection/PCR, etc., but the percentage of positive samples varied from 6% to 83%, and a few studies were completely negative. Technical and geographical differences may count for these variances. Significant geographical differences in exposure to SV40 were confirmed by a recent study showing that the polio vaccines used in the former USSR and in the countries under its influence contained infectious SV40 until at least 1978. These findings supported a previous conclusion of the Institute of Medicine of the National Academy of Sciences that the epidemiological data were flawed and therefore it was not possible to accept or reject a causal association between SV40-containing polio vaccines and cancer. In fact, it was not possible to clearly distinguish exposed from nonexposed cohorts. Although the epidemiological data are not available, mechanistic experiments in human mesothelial cells, and animal experiments strongly support a pathogenic role of SV40 in mesothelioma. More recently, SV40 has been shown to be a cocarcinogen in causing mesothelioma in animals and malignant transformation of mesothelial cells in tissue culture. In addition, the data showed that in the presence of SV40 lower amounts of asbestos were sufficient to cause mesothelioma. Co-carcinogenesis was mediated through the activation of the ▶ extracellular signal-regulated kinases (ERKs) and ▶ activator protein-1 (AP-1) activity that led to cell proliferation and stromal invasion.

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SVA Repeat

Definition

A hominid-specific composite repetitive DNA element named after its main components, *S*INE-R (Short Interspersed Nuclear Element derived from the

Human endogenous retrovirus K10), VNTR (► Variable Number Tandem Repeat), and ► *Alu*, elements.

► LINE-1 Elements

SWI/SNF

Definition

Highly conserved multiproteic structures involved in the ATP-dependent chromatin remodeling. Among the core subunits of SWI/SNF, hSNF5/INI1 is responsible for the oncogenesis of rhabdoid tumors.

► hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily b, member1

► hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

SWISS-PROT

Definition

A highly annotated biological database of protein sequences developed by the Swiss Institute of Bioinformatics and the European Bioinformatics Institute.

► Intrinsically Unstructured Proteins

SWOG

Definition

Southwest Oncology Group is one of the largest cancer clinical trials cooperative groups in the United States. Funded by research grants from the National Cancer Institute, part of the National Institutes of Health, the Group conducts clinical trials to prevent and treat cancer in adults, and to improve the quality of life for cancer survivors. Southwest Oncology Group studies many adult cancer types, including breast, gastrointestinal, genitourinary, gynecologic, and lung cancers, as well as melanoma, myeloma, leukemia, and lymphoma. Approximately 120 clinical trials are underway at any given time. http://www.swog.org/

Syk

► Syk Tyrosine Kinase

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Synonyms

Spleen tyrosine kinase; Syk

Definition

Syk ► tyrosine kinase activity was originally recognized in spleen, thymus, and lung and was later cloned from spleen. A 72 kDa protein, Syk contains 635 amino acids and the human gene maps to chromosome 9q22. It contains two N-terminal, ► SH2 domains in tandem and a C-terminal tyrosine kinase domain. Interdomain A separates the tandem SH2 domains and interdomain B links the kinase domain to the tandem SH2 domains. An alternatively spliced site in interdomain B deleting 23 amino acids gives rise to a short form of Syk that can be differently expressed according to the tissue. Syk together with the related Zap70 (zeta-activated protein of 70 kDa) constitute a separate tyrosine kinase family.

Characteristics

Distribution and Function

Syk is critical to immune cell signaling where it promotes ▶ proliferation, survival, ▶ phagocytosis, and ▶ differentiation and is required for lymphocyte development, innate immune recognition, osteoclast maturation, and platelet activation. Syk also influences ▶ angiogenesis and ▶ lymphangiogenesis. Animals lacking Syk die around birth from a failure of blood and lymphatic vessel separation. Although Syk is now studied as a target for therapeutic control of B-lineage leukemias and lymphomas, and inflammatory and autoimmune diseases due its function in immune cells, there are several indications that Syk is also a potential target for cancer therapy. Syk seems to be involved either positively or negatively in tumor formation and ▶ progression, depending on the cell type. In hematopoietic malignancies, Syk can be constitutively activated or overexpressed. The TEL-Syk gene fusion, resulting from a ▶ chromosomal translocation identified in a myelodysplastic syndrome patient, was found to result in constitutively autophosphorylated Syk that promoted growth factor independent growth in a hematopoietic cell line. The fusion kinase ITK-SYK drives oncogenesis in conditional mouse models of peripheral T cell lymphoma. Pharmacological inhibition of Syk has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. Syk overexpression has been reported in anaplastic lymphoma kinase-positive tumors, and in splenic marginal zone and mantle cell lymphomas; however, its direct responsibility in lymphoma development is not known. In contrast, loss of Syk has been reported in ▶ Reed–Sternberg cells of classical Hodgkin disease.

It is now appreciated that Syk, unlike Zap70, is found not only in hematopoietic cells, but also in many other cell types including mammary, gastric, and lung epithelia, and in hepatocytes, melanocytes, neuronal, muscle and endothelial cells, and in some fibroblasts. And, Syk appears to behave as a ▶ tumor suppressor in a growing list of tumors, including ▶ breast cancer, ▶ gastric cancer, ▶ hepatocellular carcinoma, pancreatic adenocarcinoma, oral squamous cell carcinoma, and ▶ melanoma in which its expression is lost or significantly decreased compared with normal cells. In breast, Syk is progressively lost from normal to hyperplastic to ▶ ductal carcinoma in situ to invasive cancers.

Experimental Evidence for a Tumor/Metastasis Suppressor Role of Syk

Animal studies using breast carcinoma, pancreatic adenocarcinoma, or melanoma cells have shown that reexpression by gene transfer of Syk in tumor cells that are Syk negative, blocks primary tumor growth when tumor cells are injected into the mammary fat pad or in the skin of mice, respectively. Conversely, inhibition of Syk function in breast cancer cells that express Syk is accomplished by gene transfer of a kinase-defective, dominant-negative Syk. This results in more efficient tumor initiation and increased tumor growth when cells are injected into mice. An ▶ experimental metastasis model reveals that reintroduction of Syk potently blocks lung metastasis of breast and melanoma tumors. In keeping with its role in blocking metastasis, in vitro studies correlate Syk loss in breast cancer cells with invasive growth of cells in a three-dimensional ▶ extracellular matrix culture system. Reexpression of Syk in Syk-negative cells blocks this invasive growth, as well as inhibiting directional cell ▶ migration due to ▶ chemotaxis by tumor cells, and ▶ anchorage-independent proliferation, processes that are associated with the ability of tumor cells to metastasize. Thus, Syk can behave not only as a tumor suppressor, but also as a metastasis suppressor in non-hematopoietic tumors. Mice that lost one SYK ▶ allele exhibit an increased proliferation in the mammary gland, invasion of epithelial cells through the mammary fat pad and develop mammary carcinomas by 1 year, emphasizing the critical role of Syk as a tumor suppressor for breast cancer.

Mechanism of Syk Activation and Signaling

Activating receptors on hematopoietic cells contain one or more cytoplasmic ▶ immunoreceptor tyrosine-based activation motifs (ITAMs) or hemi-ITAMs that, following activation of the receptor and phosphorylation on tandem tyrosine-containing motifs by Src family kinases, form binding sites for the Syk tyrosine kinase. This occurs via tandem SH2 domains contained in the N-terminus of this molecule. Syk then phosphorylates itself on multiple tyrosine residues, thereby creating binding sites for its substrates and downstream effectors that are gradually being uncovered by proteomic studies. Among these, one can distinguish intermediates of the major intracellular signaling pathways (e.g., ▶ MAP kinase and ▶ PI3 kinase) and final effectors (e.g., alpha-tubulin and

► cortactin). These signaling cascades finally affect a complex series of cellular responses such as cell proliferation, differentiation, ► adhesion and migration, ► apoptosis, and phagocytosis. Signaling in hematopoietic cells requires immune cell receptors or C-type lectin receptors that are lacking in epithelium and other Syk positive cell types raising the question of the mechanism of Syk activation in epithelial cells. However, Syk can also be activated by transmembrane ► integrin receptors in hematopoietic cells. There is some evidence for β1 integrin-mediated activation of Syk in epithelial cells. ► Integrin signaling is involved in cancer and most particularly in the ► invasion and metastasis processes. In epithelial cells, Syk can also be activated when membrane-bound ITAM motifs are introduced into cells either experimentally, or, associated with ► Epstein–Barr virus (EBV) infection. Relevant to EBV infection in ► nasopharyngeal cancer, Syk activation is required for increased cell migration. Oppositely, in breast cancer and melanoma cells, Syk suppresses chemotaxis when reexpressed following gene transfer experiments. Downstream, following Syk activation, a number of pathways can be activated or inhibited depending upon the cell type and microenvironment. Thus, across the spectrum of cancers, Syk function likely depends on the cellular and molecular context, which varies widely between hematopoietic cells and other cell types.

Syk might also influence cell behavior via its differential subcellular localizations in breast epithelial cells. Syk possesses a nuclear targeting sequence that is absent in the alternatively spliced short form, and nuclear localization is associated with suppression of tumor cell invasion and alteration in expression of transcription factors via interaction with SP1 transcription factor in breast cancer cells. Syk also negatively regulates SP1 activation during hypoxic stimulation as occurs during tumor cell growth in areas of low oxygen abundance. An early observation was that reintroduction of Syk into Syk-negative breast cancer cells results in abnormal cell division and cytokinesis. Correspondingly, Syk was found to be present also at the centrosome, the major microtubule organizing center of the cell. This localization is dependent upon its tyrosine kinase activity, and its presence there is tightly regulated during cell cycle progression suggesting a potential to regulate cell proliferation. Syk has also been observed in adherens junctions where it is involved in intercellular adhesion. In other epithelial cells, such as lung, the role of Syk may be more closely aligned with its function in immune cells, since Syk activation is required for production of inflammatory molecules induced by tumor necrosis factor.

Clinical Studies of Syk Expression and Activity

Clinical pathology studies of tissues from breast and gastric cancer patients reveal that the loss of Syk is associated with poor outcome such as reduced overall survival and increased metastasis risk. Syk nuclear localization was significantly associated with improved outcome in gastric cancer. Loss of Syk protein in cancer cells is an independent prognostic marker of poorer overall survival in hepatocarcinoma cells revealed by multivariate statistical analysis but Syk has not been found to be an ► independent prognostic factor in breast or gastric cancers to date.

Loss of Syk is at least partially due to ► epigenetic gene silencing caused by the ► hypermethylation of a ► CpG island in the promoter portion of the SYK gene. This has been documented in breast, bladder, gastric, ovarian, hepatocellular, and oral squamous cell carcinoma, as well as in melanoma and in T-lineage acute lymphoblastic leukemia. Hypermethylation of the SYK gene is an independent prognostic factor of poorer overall survival in hepatocellular carcinoma. Microarray comparison of genes previously shown to be altered by hypermethylation revealed differential Syk expression in normal versus prostate cancer cells. And, Syk methylation is associated with ► histopathological grade in transitional cell carcinoma of the bladder, loss of Syk being associated with the most invasive tumors. Overall, Syk expression appears to be primarily regulated at the transcriptional level; presently, no transcription factors regulating Syk expression have been identified.

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Sympathetic Ganglia Neurons

Definition

Nerve cells that make-up the sympathetic nervous system.

Symptom Management

Definition

Treatment of the symptoms caused by cancer disease.

► Leptomeningeal Dissemination

Synaptic Vesicle Recycling

Definition

A specialized class of small vesicles (synaptic vesicles, ~50 nm diameter) in nerve cells store neurotransmitters and release them upon the arrival of an action potential at the nerve terminal. Neurons can fire in excess of a thousand times per second, which would rapidly lead to depletion of synaptic vesicles. Thus, synaptic vesicles are efficiently internalized, after their fusion with the plasma membrane by a molecular machinery that is largely overlapping that of ► endocytosis.

Synaptophysin

Definition

Synaptophysin is a Mr 38,000 integral membrane ► glycoprotein expressed by a variety of normal and neoplastic ► neuroendocrine cells. Present in virtually all neurons in the brain and spinal cord that participate in synaptic transmission. It serves as a marker for ► neuroendocrine tumors.

Synaptopodin

Definition

A gene expressed in renal podocytes and neurons and interacting with actin.

► Myopodin

Synchronizer

Definition

The regular alternation of light and darkness over 24 h, social time cues, and feeding schedules periodically reset the endogenous bodily rhythms and, hence, they are called synchronizers. The light–dark synchronization displays species specificity. Thus, mice or rats that are used for preclinical tests of cancer therapeutics rest during the light span and are active at night, while the reverse is true for humans.

► Circadian Clock Induction

Syndecans

Definition

Are a diverse group of type I transmembrane heparan sulfate ▶ proteoglycans with a wide array of ligands and distinct cell signaling capabilities. Four mammalian syndecans have been identified: syndecan-1/CD138, syndecan-2/fibroglycan, syndecan-3/N-syndecan, and syndecan-4/ryudocan. The heparan sulfate glycosaminoglycan chain of syndecans binds a number of extracellular proteins, including growth factors, ▶ chemokines, extracellular matrix components, cell adhesion molecules, proteases, and protease inhibitors. Although in some cases the family members have overlapping functions, syndecans and their heparan sulfates can bind distinct ligands and produce cellular responses unique to each syndecan. Moreover, the in vivo expression pattern of each syndecan can differ greatly from the others.

▶ Pleiotrophin

Synergism

Definition

Is opposite to antagonism, i.e., two or more agents create stronger effect than the predicted sum of their individual effects.

▶ Xenobiotics

Synergistic

Definition

Interaction of two or more agents that results in a combined effect greater than the sum of their individual effects.

▶ Nilotinib

Syngeneic

Definition

Genetically identical or closely related, so as to allow tissue transplant, immunologically compatible.

Synovial Sarcoma

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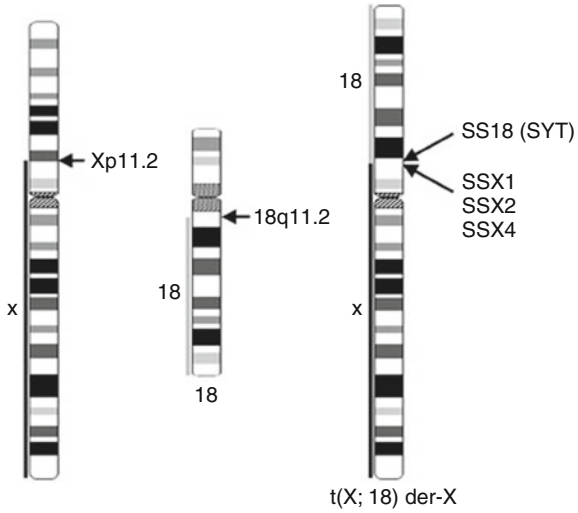
Definition

Human synovial sarcomas are soft tissue tumors that account for up to 10% of all human sarcomas and mainly affect children and young adults. These tumors display relatively high rates of local recurrences and metastases (▶ metastasis) and are therefore regarded as high-grade tumors. Five- and ten-year survival rates of 60–80% and 40–50%, respectively, have been reported in several large retrospective studies. The tumors occur most frequently in the extremities (often associated with the large joints), but may also be encountered in a wide variety of organs. The name "synovial sarcomas" is misleading since it has become clear that they do not originate from synovial tissue. Instead, synovial sarcomas are thought to be derived from progenitor cells that are capable of differentiating into mesenchymal and/or epithelial structures. A very recent study indicated that these progenitor cells may, in fact, be primary myocytes. Histopathologically, synovial sarcomas can be subdivided in four subtypes: (1) biphasic (with an epithelial and a mesenchymal component), (2) monophasic (either mesenchymal or epithelial), (3) calcifying, and (4) poorly differentiated tumors.

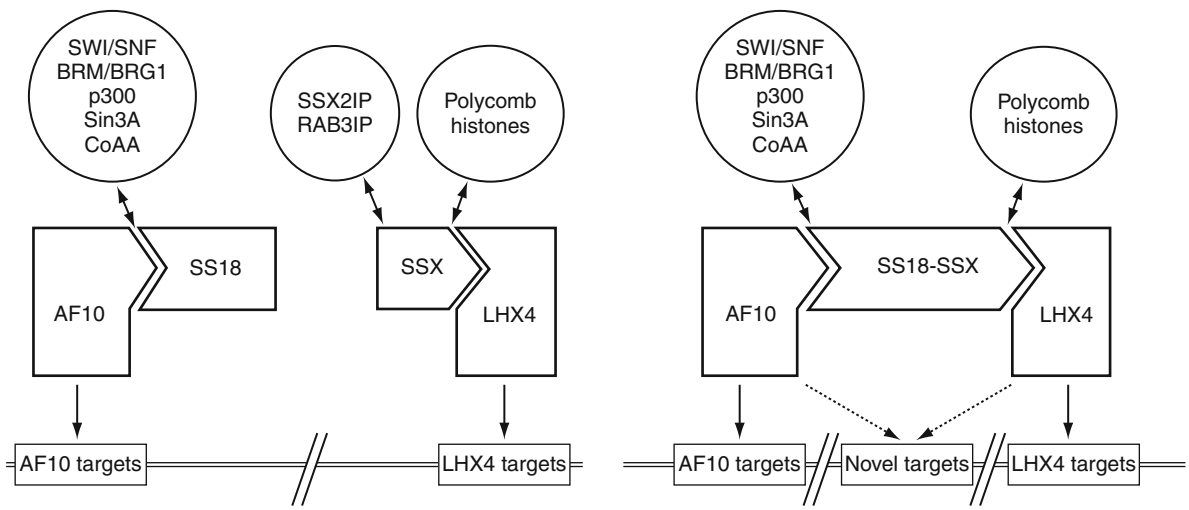
Characteristics

Cytogenetically, the tumors are characterized by a specific chromosomal translocation, t(X;18)(p11;q11) (Fig. 1), which leads to a fusion of the SS18 (previously known as SYT or SSXT) and SSX genes.



Synovial Sarcoma. Fig. 1 The synovial sarcoma specific t(X;18)(p11;q11) chromosomal translocation. The normal chromosomes X and 18 with the respective breakpoints are shown to the *left*. The derivative-X chromosome, with the breakpoint-associated genes (*SS18*, *SSX1*, *SSX2*, and *SSX4*) is shown to the *right*

In the far majority of SS18-SSX fusion proteins identified to date, the C-terminal eight amino acids of the SS18 protein are replaced by the C-terminal 78 amino acids of one of the SSX proteins. The SSX genes constitute a family of at least nine (highly related) X-chromosomal genes. Of these, three were found to be involved in the SS18 fusions: SSX1, SSX2, and SSX4. SS18-SSX fusion genes have been detected in almost all synovial sarcomas, but not in any other tumor type examined so far. Clinically, synovial sarcomas display a variable response to common treatment protocols, such as radiation and chemotherapy (▶ adjuvant Therapy). Over the years, many investigators have reported adverse prognostic factors, such as tumor size (>5 cm), high tumor grade, advanced (metastatic) disease, and (higher) patient age. Treatment with chemotherapy (ifosfamide, either alone or in combination with doxorubicin) has been reported to increase the overall survival rates, also of patients with high-grade tumors and metastases. Despite these findings, there is ample room for further improvement and optimization



Synovial Sarcoma. Fig. 2 Model depicting the synovial sarcoma associated SS18, SSX, and SS18-SSX (fusion) proteins and their respective interactions. In normal cells (*left)* the SSX proteins and their interactors SSX2IP and RAB3IP may associate with the Polycomb repressor complex and histones and in addition, through interaction with the LHX4 protein, bind to cognate DNA sites and affect target gene expression. The SS18 protein can interact with several members of the SWI/SNF chromatin remodeling complex (BRM and BRG1), but also with proteins involved in covalent chromatin modifications (p300 and Sin3A) and the co-activator CoAA. In addition, through interaction with the transcription factor AF10, SS18 may bind to cognate DNA sites and affect target gene expression. In synovial sarcoma cells (*right*), the SS18-SSX fusion proteins have lost the interaction domains for SSX2IP and RAB3IP, but have retained the interaction/association domains for both the SWI/SNF and the Polycomb complexes. Through these interactions, the SS18-SSX fusion proteins may anomalously affect the regulation of these target genes and/or affect the regulation of other (novel) target genes, through either AF10, LHX4, or both

of the (differential) diagnosis and treatment of human synovial sarcomas. To enable this, detailed information about the molecular mechanisms underlying synovial sarcoma development is of imperative importance.

The tumorigenic nature of the SS18-SSX fusion protein has been established in vitro and in vivo (▶ oncogene). Further, functional, analysis of the SS18 and SSX genes has revealed that they encode nuclear proteins that exhibit opposite transcriptional regulatory activities. The SS18 protein functions as a transcriptional co-activator which interacts directly with the transcription factor AF10, the co-activator CoAA, and several members of the epigenetic chromatin remodeling (BRM and BRG1) (▶ Chromatin remodeling in cancers) and modification machineries (p300 and SIN3A) (▶ P300/CBP Co-Activators). In contrast, the SSX proteins function as transcriptional co-repressors which interact with the RAB3IP and SSX2IP proteins and the transcription factor LHX4, and are associated with histones and several Polycomb group repressor proteins. The domains involved in these apparently opposite transcription regulatory activities are retained in the SS18-SSX fusion proteins. Therefore, these may function as "activator-repressors" of transcription, which can bind to target DNA through the AF10 and LHX4 transcription factors (Fig. 2). This notion implies that the SS18 and/or SSX protein functions may be impaired in the SS18-SSX fusion protein. Alternatively, the fusion protein may have gained novel functions. A recent functional analysis revealed that the SS18-SSX fusion protein influences the process of ▶ epithelial to mesenchymal transition (EMT) which is commonly observed in tumor cells. This example of a SS18-SSX gain-of-function may underlie the above-mentioned histologic differences among synovial sarcomas. A recent study has indicated that novel treatment options for synovial sarcoma patients may include so-called epigenetic drugs (▶ epigenetic Therapy). Synovial sarcoma cell lines were shown to be extremely sensitive to the histone de-acetylase inhibitory drug Romidepsin (also known as FK228 or depsipeptide), both in vitro and in vivo. As yet, the exact mode of action of this broad-spectrum "epigenetic" drug on synovial sarcoma growth is unknown. However, it is to be expected that detailed knowledge on the molecular mechanisms underlying synovial sarcoma pathogenesis will be instrumental for obtaining insight into its mode of action and, thus, the development of more targeted therapies.

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Syntenic

Definition

Refers to genes or genetic loci that lie on the same chromosome, i.e., are genetically linked.

▶ Amplification

Synthetic Cannabinoids

▶ Cannabinoids

Synthetic Chemoprotectants

Definition

Chemoprotectants that are artificially and chemically synthesized.

▶ Chemoprotectants

Synuclein

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Definition

Synucleins are small cytosolic proteins of uncertain function, normally expressed at high levels in the vertebrate nervous system. Increased expression of synuclein proteins, especially γ-synuclein, is associated with progression of a variety of tumors.

Characteristics

The precise function of the synuclein proteins is not well understood. All family members (α-synuclein, ▶ SNCA; β-synuclein, ▶ SNCB, and ▶ γ-synuclein, ▶ SNCG) share a conserved domain related to the lipid-binding domains of the ▶ exchangeable apolipoproteins, the major lipid transporters in blood. This conserved domain mediates reversible interactions with lipid membranes. α-Synuclein regulates the uptake and incorporation of fatty acids into phospholipids, and mutations in α-synuclein alter the composition of cellular membranes.

Each of the synuclein isoforms possesses a unique tail domain, which may mediate distinct physiological actions. α- and β-synuclein are normally expressed in the ▶ central nervous system, while γ-synuclein expression is found throughout the central and ▶ peripheral nervous systems, and at lower levels in some nonneural tissues. Synuclein family genes have so far been identified only in vertebrate species.

Synuclein Expression in Cancer

The first synuclein family member to be associated with cancer was γ-synuclein, which was originally named ▶ breast cancer-specific gene 1 (▶ BCSG1), due to its specific expression in infiltrating breast carcinoma as compared to normal breast tissue. The other synuclein proteins are also somewhat associated with certain cancers. For example, 87% of ▶ ovarian cancers display increased expression of one or more synuclein family members, while 42% express all three. The γ-synuclein isoform is particularly associated with cancer. Overexpression of γ-synuclein is observed in a large percentage of tumors from varied tissues of origin, but not in adjacent non-neoplastic tissues. Expression of γ-synuclein in cancer increases in a stage-specific manner, with moderate expression in stage I tumors, and very high expression in stages III–IV.

Increased expression of γ-synuclein in tumor cells apparently results from deregulation of normal expression, as no γ-synuclein mutations or gene amplifications have been associated with cancer. Tissue-specific expression of γ-synuclein is mediated by methylation of ▶ CpG islands in exon 1, and ▶ hypomethylation at these sites has been observed in γ-synuclein overexpressing tumor cells. The increased expression of γ-synuclein observed in advanced cancer is mostly likely the consequence of a loss of ▶ epigenetic gene silencing of γ-synuclein expression during tumor progression, rather than a primary event in tumor initiation.

Misfolding of α-synuclein protein is associated with both familial and sporadic ▶ Parkinson's disease (PD), and mutations in α-synuclein that alter its sequence or increase its expression cause early-onset PD. PD patients have an increased risk of both ▶ melanoma and breast cancer, as compared to the general population, but a decreased risk for many other cancers. This may reflect the involvement of common genes in PD and cancer.

Role in Tumor Progression

γ-Synuclein expression in breast cancer cell lines increases cell proliferation. It interacts with the mitotic spindle checkpoint control protein ▶ Burkitt lymphoma cell lines, promoting its degradation by the proteasome and overriding mitotic arrest. Loss of spindle checkpoint control can result in ▶ aneuploidy, the state of having the wrong number of chromosomes. γ-Synuclein augments proliferative signaling through the ▶ estrogen receptor pathway, by acting as a molecular chaperone to increase ▶ estradiol binding to the receptor. γ-Synuclein also alters signaling via the ▶ MAP kinase pathway, which plays a key role in the regulation of cell proliferation.

γ-Synuclein enhances cell motility and invasiveness, and its overexpression in established tumors

may drive malignant tumor progression. γ-Synuclein promotes production of ▶ matrix metalloproteinases, secreted proteases that break down the extracellular matrix and allow tumors to invade surrounding tissues and blood vessels, thereby facilitating ▶ metastasis. γ-Synuclein expression also increases resistance to certain chemotherapeutic agents that trigger ▶ apoptosis via MAP kinase and/or ▶ JNK signaling pathways, e.g., ▶ taxol, vinblastine, and ▶ paclitaxel.

Overall, γ-synuclein expression in a tumor correlates with a poor clinical prognosis. Its expression in primary tumors is associated with the presence of distant metastases. This protein may prove to be a useful ▶ Biomarker for detecting cancer, ▶ staging tumor progression, and evaluating metastatic potential.

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Synuclein α

Definition

A member of the ▶ synuclein protein family; also known as NACP; encoded by the gene SNCA.

▶ Synucleins

Synuclein β

Definition

A member of the ▶ synuclein protein family; also known as PNP14; encoded by the gene SNCB.

▶ Synucleins

Synuclein γ

Definition

A member of the ▶ synuclein protein family; also known as persyn or synoretin; encoded by the gene SNCG.

▶ Synucleins

Systemic Antibody-directed Radionuclide Therapy

▶ Radioimmunotherapy

Systemic Chemotherapy

Definition

Oral or intravenous administration of ▶ chemotherapy

Systemic Clearance

Definition

A measure of the efficiency with which a drug is removed from the body. It is proportional to the dose and inversely proportional to the area under the curve (AUC).

▶ Lead Optimization

Systemic Inflammatory Response Syndrome

Synonyms

SIRS

Definition

A syndrome proposed by American College of Chest Physicians in 1992. It is defined as a clinical response to a nonspecific insult of either infectious or noninfectious origin. SIRS is defined as two or more of the following variables:

1. Fever of more than 38°C or less than 36°C
2. Heart rate of more than 90 beats per minute
3. Respiratory rate of more than 20 breaths per minute or a $PaCO_2$ level of less than 32 mmHg
4. Abnormal white blood cell count (>12,000/μL or <4,000/μL or >10% bands)

▶ Sivelestat

Systemic Lupus Erythematosus

Definition

SLE; systemic lupus erythematosus is an autoimmune disease in which autoantibodies against DNA, RNA, and proteins associated with nucleic acids from immune complex damage small blood vessels, especially of the kidney.

Systemic Spread of Cancer

▶ Metastatic Colonization

Systemic Targeted Radionuclide Therapy

Synonyms

Systemic antibody-directed radionuclide therapy

Systemic Therapy

Definition

Application of medical therapy (usually a drug) through an artery or vein. This is followed by systemic distribution of the drug.

▶ Neoadjuvant Therapy

Systemic Treatment or Therapy

Definition

Therapy theoretically delivered to the entire body, and is distinguished from ▶ local therapy. ▶ Chemotherapy is the most common form of systemic therapy. Its purpose can be either curative or palliative.

▶ Induction Chemotherapy

Systems Biology

Definition

A scientific discipline that seeks to understand the dynamic nature of a life by quantitatively integrating the network of interactive sub-processes (metabolic pathways, gene expression, cell–cell interactions, etc.) occurring within an organismal unit in a manner that can accurately predict the net outcome(s) of perturbing or stimulating any of those sub-processes.

▶ Drug Design