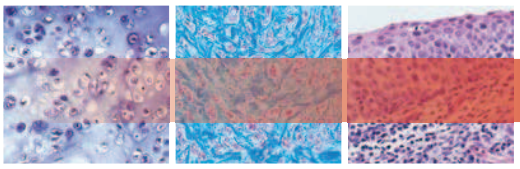

5

Neoplasia

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The Pathology of Neoplasia

A **neoplasm** (Greek, *neo*, "new," + *plasma*, "thing formed") is the autonomous growth of tissues that have escaped normal restraints on cell proliferation and accumulation and exhibit varying degrees of fidelity to their precursors. As well, some tumors (e.g., promyelocytic leukemia) involve impaired maturation, even if the tumor cells are committed to a specific line of differentiation. Neoplastic cells' resemblance to their cells of origin usually enables conclusions about tumors' sources and potential behavior. As most neoplasms occupy space, they are often called **tumors** (Gr., *swelling*). Tumors that remain localized are considered **benign,** while those that spread to distant sites are called **malignant,** or **cancer**. The neoplastic process entails not only cell proliferation but also variable modification of the differentiation of the involved cell types. Thus, in a sense, cancer may be viewed as a burlesque of normal development.

Cancer is an ancient disease. Evidence of bone tumors has been found in prehistoric remains, and the disease is mentioned in early writings from India, Egypt, Babylonia

and Greece. Hippocrates is reported to have distinguished benign from malignant growths. He also introduced the term *karkinos,* from which our term **carcinoma** is derived. In particular, Hippocrates described cancer of the breast, and in the 2nd century AD, Paul of Aegina commented on its frequency.

The incidence of neoplastic disease increases with age, and longer life spans in modern times enlarge the population at risk. In previous centuries, humans did not live long enough to develop many cancers that are particularly common in middle and old age, such as those of the prostate, colon, pancreas and kidney. If all cancer deaths caused by tobacco smoke are removed from the statistics, the overall age-adjusted cancer death rate has been decreasing. In part, this stability and decline in cancer death rates reflects improved early detection techniques (e.g., Pap smears, colonoscopy).

Neoplasms are derived from cells that normally can multiply. Thus, mature neurons and cardiac myocytes do not give rise to tumors. A tumor may mimic its tissue of origin to a variable degree. Some closely resemble their parent structures (e.g., hepatic adenomas), while others seem to be collections of cells that are so primitive that the tumor's origin cannot be identified.

BENIGN VERSUS MALIGNANT TUMORS

Although exceptions are known, benign tumors basically do not penetrate (invade) adjacent tissue borders, nor do they spread (metastasize) to distant sites. They remain as localized overgrowths in the area in which they arise. Benign tumors tend to be more differentiated than malignant ones—that is, they more closely resemble their tissue of origin. *By contrast, malignant tumors or cancers, invade contiguous tissues and metastasize to distant sites, where subpopulations of malignant cells take up residence, grow a new and again grow into surrounding areas.*

In common usage, **the terms "benign" and "malignant" refer to a tumor's overall biological behavior rather than its morphologic characteristics**. In most circumstances, malignant tumors have the capacity to kill, while benign ones spare the host. However, so-called benign tumors in critical locations can be deadly. For example, a benign intracranial tumor of the meninges (meningioma) can kill by exerting pressure on the brain. A minute benign tumor of the ependymal cells of the third ventricle (ependymoma) can block the circulation of cerebrospinal fluid, resulting in lethal hydrocephalus. A benign mesenchymal tumor of the left atrium (myxoma) may cause sudden death by blocking the mitral valve orifice. In certain locations, erosion of a benign tumor of smooth muscle can lead to serious hemorrhage—witness the peptic ulceration of a stromal tumor in the gastric wall. On rare occasions, a functioning, benign endocrine adenoma can be life-threatening, as in the case of the sudden hypoglycemia associated with an insulinoma of the pancreas or the hypertensive crisis produced by a pheochromocytoma of the adrenal medulla. Conversely, certain types of malignant tumors are so indolent that they pose no threat to life. In this category there are many cancers of the breast and prostate.

Tumors can usually be identified as benign or malignant by virtue of their microscopic morphologic characteristics. However, the biological behavior of some types of tumors may not necessarily reflect, or correlate with, pathologic appearance. Some tumors that look histologically malignant may not metastasize or be able to kill a patient. Thus, basal cell carcinomas of the skin may invade subjacent structures locally but rarely metastasize and are not life-threatening. On the other hand, some tumors showing benign histologic characteristics may be lethal. Aggressive meningiomas do not metastasize, but their local invasiveness may cause death by compromising vital structures. For many endocrine tumors (e.g., islet cell tumors of the pancreas) metastatic potential is not predictable from histology, and a tumor's benign or malignant nature can only be determined retrospectively, based on the presence or absence of metastases.

CLASSIFICATION OF NEOPLASMS

In any language, the classification of objects and concepts is pragmatic and useful only insofar as its general acceptance permits effective prognostication. Similarly, the nosology of tumors reflects historical concepts, technical jargon, location, origin, descriptive modifiers and predictors of biological behavior. Although the language of tumor classification is neither rigidly logical nor consistent, it still serves as a reasonable mode of communication.

HISTOLOGIC DIAGNOSIS OF MALIGNANCY

Essentially, labeling a tumor as benign or malignant is a prediction of its eventual biological behavior and clinical outcome. In effect, the criteria used to assess the true biological nature of any tumor are based not on scientific principles but rather on accumulated experience and historical correlations between histologic and cytologic patterns and clinical courses. In most cases, the differentiation between benign and malignant tumors poses few problems; in a few, additional study is required before an accurate diagnosis is secure. However, there will always be tumors that defy the diagnostic skills and experience of any pathologist; in these cases, the correct diagnosis must await the clinical outcome. *Remember that the definition of a benign tumor resides above all in its inability to invade adjacent tissue and to metastasize.*

The Primary Descriptors of All Tumors Are Their Cells of Origin

Although historically the suffix "oma" referred to benign tumors, current terminology is so varied that tumor names do not specify biological behavior with any precision. For example, tumors called melanomas, mesotheliomas and seminomas are all highly malignant even though they carry the suffix "oma." Growths called hamartomas are not even true neoplasms but disorganized developmental medleys of multiple structures. Nevertheless, by definition, the term "carcinoma" describes a malignant proliferation of epithelial cells and "sarcoma" refers to malignancies of mesenchymal origin. These terms are not necessarily all-inclusive, as most malignant proliferations of the blood-forming organs are called leukemias and those of the lymphoid system are lymphomas. Finally, tumors in which historically the histogenesis was poorly understood are often given an eponym—for example, Hodgkin disease or Ewing sarcoma.

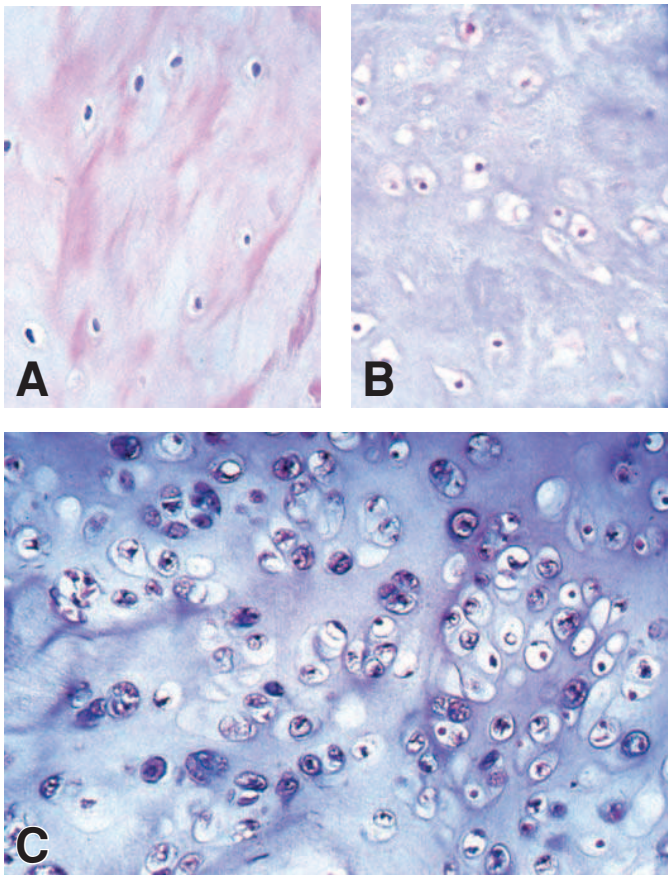


FIGURE 5-1. Cartilaginous lesions. A. Normal cartilage. **B.** A benign chondroma closely resembles normal cartilage. **C.** Chondrosarcoma of bone. The tumor is composed of malignant chondrocytes, which have bizarre shapes and irregular hyperchromatic nuclei, embedded in a cartilaginous matrix. Compare with **A** and **B**.

Some of the histologic features that are considered in distinguishing benign from malignant tumors include the following:

- **Degree of cellular atypia:** This term refers to the extent to which the tumor departs from the appearance of its normal tissue or cellular counterparts (Fig. 5-1). An example is the difference between normal cartilage, a benign chondroma and a malignant chondrosarcoma. In general, the magnitude of cellular atypia (also called **anaplasia**) correlates with the aggressiveness of the tumor. Cytologic evidence of anaplasia includes (1) variation in size and shape of cells and cell nuclei **(pleomorphism),** (2) enlarged and hyperchromatic nuclei with coarsely clumped chromatin and prominent nucleoli, (3) atypical mitoses and (4) bizarre cells, including tumor giant cells (Fig. 5-2).
- **Mitotic activity:** Many malignant tumors show high mitotic rates. For some tumors (e.g., leiomyosarcomas), a diagnosis of malignancy is based on finding even a few mitoses. Nonetheless, such obvious proliferative activity is not obligatory to consider a tumor malignant in all situations.
- **Growth pattern:** In common with many benign tumors, malignant neoplasms often exhibit disorganized growth, which may be expressed as sheets of cells, arrangements around blood vessels, papillary structures, whorls, rosettes and so forth. Malignant tumors often suffer from compromised blood supply and show ischemic necrosis.
- **Invasion:** Malignancy is proved by the demonstration of invasion, particularly of blood vessels and lymphatics. In some circumstances (e.g., squamous carcinoma of the cervix or carcinoma arising in an adenomatous polyp), the diagnosis of malignant transformation is made mainly on the basis of local invasion.
- **Metastases:** The presence of metastases identifies a tumor as malignant. If a metastatic tumor was not preceded by a diagnosed primary cancer, the site of origin may not be readily apparent from histologic characteristics alone. In such cases, electron microscopy and demonstration of specific tumor markers may establish the correct origin.

General criteria for malignancy are recognized but must be used cautiously in specific cases. For example, **nodular fasciitis,** a reactive proliferation of connective tissue cells (Fig. 5-3), appears more alarming histologically than many fibrosarcomas, and misdiagnosis can lead to unnecessary surgery. Conversely, well-differentiated endocrine adenocarcinomas may be pathologically identical to benign adenomas.

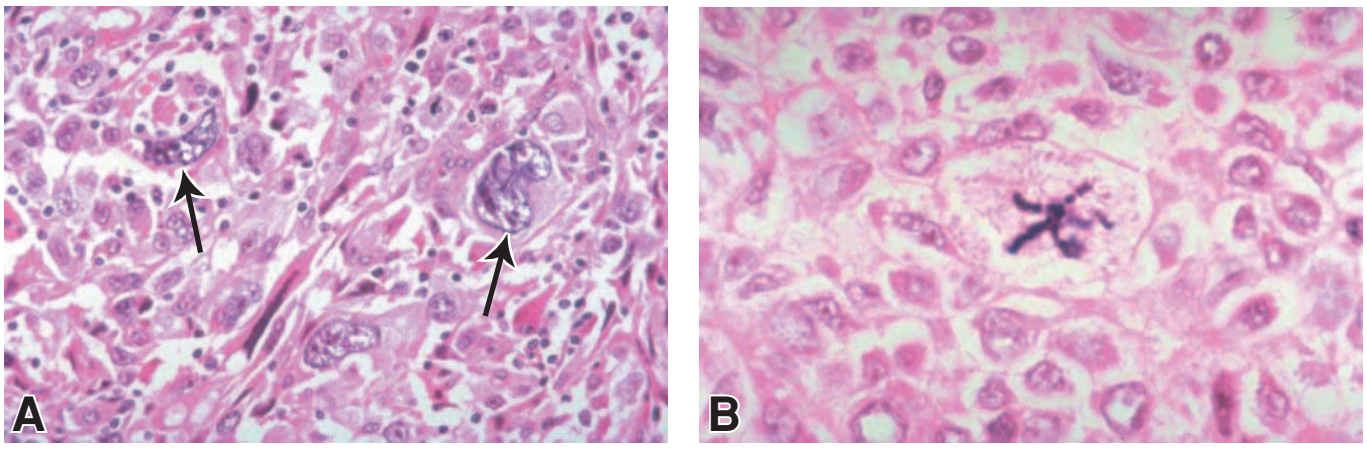


FIGURE 5-2. Anaplastic features of malignant tumors. A. The cells of this anaplastic carcinoma are highly pleomorphic (i.e., they vary in size and shape). The nuclei are hyperchromatic and are large relative to the cytoplasm. Multinucleated tumor giant cells are present (*arrows*). **B.** A malignant cell in metaphase exhibits an abnormal mitotic figure.

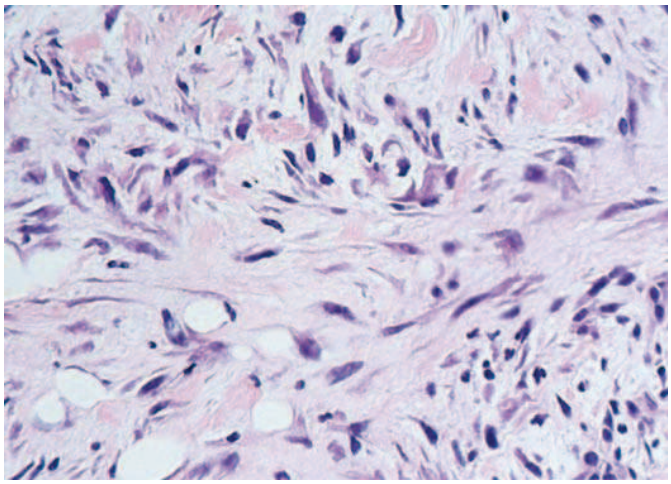


FIGURE 5-3. Nodular fasciitis. This cellular reactive lesion contains a typical and bizarre fibroblasts, which may be mistaken for a fibrosarcoma.

Marker Studies Are Used to Identify Tumor Origin

Electron Microscopy

There are no specific determinants of malignancy or even of neoplasia itself that can be detected by electron microscopy. Although this technique may aid in identifying poorly differentiated cancers whose classification is problematic by routine light microscopy, electron microscopy has largely been supplanted in tumor diagnosis by immunohistochemical staining. Nonetheless, carcinomas often contain desmosomes and specialized junctional complexes, structures that are not typical of sarcomas or lymphomas. The presence of melanosomes or premelanosomes signifies a melanoma, and small, membrane-bound granules with dense cores are features of endocrine neoplasms (Fig. 5-4). Another diagnostically useful granule is the characteristic crystal-containing granule of an insulinoma derived from the pancreatic islets.

Tumor Markers

Tumor markers are products of neoplasms that can be detected in the cells themselves or in body fluids. Some metastatic tumors may be so undifferentiated microscopically as to preclude even the distinction between epithelial and mesenchymal origin. Tumor markers rely on the preservation of characteristics of the progenitor cell or the synthesis of specialized substances by neoplastic cells to make this distinction. Determination of cell lineage of undifferentiated tumors is more than an academic exercise, because therapeutic decisions may be based on their appropriate identification. Among these diagnostically useful markers are such diverse products as immunoglobulins, fetal proteins, enzymes, hormones and cytoskeletal and junctional proteins. The ultimate tumor marker would be one that allows unequivocal distinction between benign and malignant cells. Unfortunately, no such marker exists.

Tumor markers can be detected in tissue sections by immunologic (immunohistochemistry, immunofluorescence) techniques (Fig. 5-5) and by molecular studies (in situ hybridization). Table 5-1 contains examples of marker studies that are used to identify the tissue of origin of tumors.

In addition to their use in identifying the lineages of malignancies, tumor-associated antigens are also used in

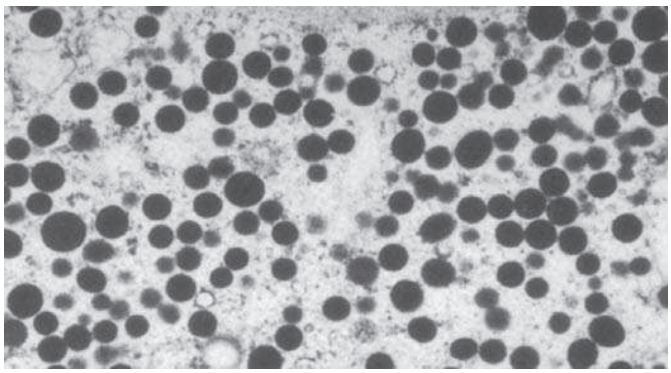


FIGURE 5-4. Electron micrograph of a metastatic cancer of the adrenal medulla (pheochromocytoma). The neuroendocrine origin of this poorly differentiated tumor was identified by the presence of characteristic cytoplasmic secretory granules.

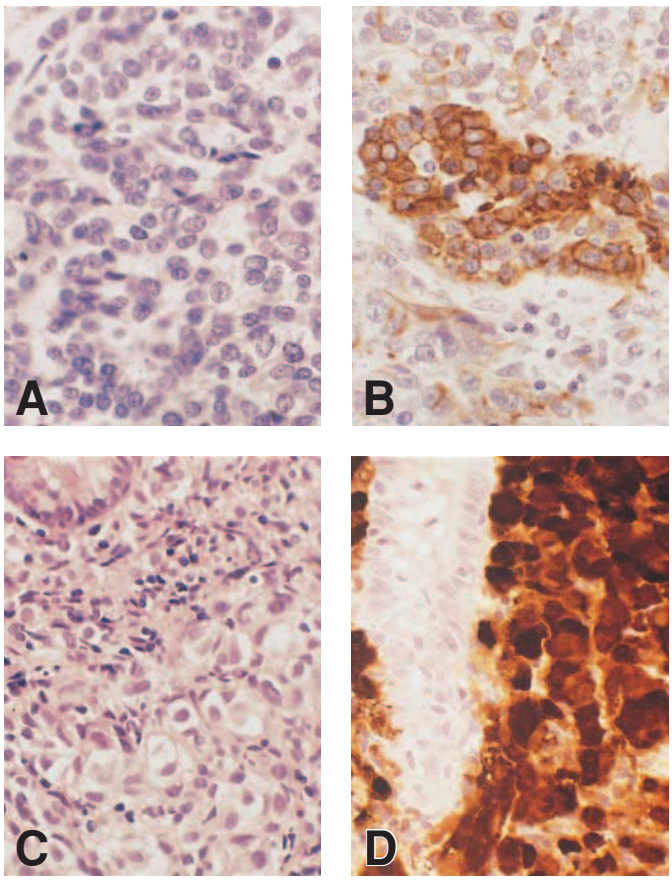


FIGURE 5-5. Tumor markers in the identification of undifferentiated neoplasms. A. A poorly differentiated metastatic bladder cancer is difficult to identify as a carcinoma with the hematoxylin and eosin stain. **B.** A section of the tumor depicted in **A** is positive for cytokeratin with an immunoperoxidase stain and is identified as carcinoma. **C.** A metastasis to the colon of an undifferentiated malignant melanoma is not pigmented, and its origin is unclear. **D.** An immunoperoxidase stain of the tumor shown in **C** reveals numerous cells positive for S-100 protein, a commonly used marker for cells of melanocytic origin.

TABLE 5-1
FREQUENTLY USED MARKERS TO IDENTIFY TUMORS

Marker	Target Cells
Epithelial Cells	
Cytokeratins (CKs)	Carcinomas, mesothelioma
CK7	Many nongastrointestinal adenocarcinomas
CK20	Gastrointestinal and ovarian carcinomas, urothelial carcinomas, Merkel cell tumor
Epithelial membrane antigen (EMA)	Carcinomas, mesothelioma, some large cell lymphomas
Ber-Ep4	Most carcinomas, but not in mesothelioma
B72.3 (tumor associated)	Many adenocarcinomas, but not in mesothelioma
Carcinoembryonic antigen (CEA)	Many adenocarcinomas of endodermal origin but not in others (e.g., renal, mesothelioma)
Mesothelial Cells	
Cytokeratins CK5/6	Mesothelioma
Vimentin	Mesothelioma
HBME	Mesothelioma, thyroid tumors
Calretinin	Mesothelioma
Melanocytes	
HMB-45	Malignant melanoma
S-100 protein	Malignant melanoma, glial cells
Mel A	Malignant melanoma
Neuroendocrine and Neural Cells	
Chromogranins, particularly chromogranin A	Neuroendocrine tumors
Synaptophysin	Neuroendocrine tumors
CD57	Neuroendocrine tumors, T and NK cells, Schwann cells
Glial Cells	
Glial fibrillary acidic protein (GFAP)	Astrocytoma and other glial tumors
Mesenchymal Cells	
Vimentin	Most sarcomas
Desmin	All types of muscle tumors
Muscle-specific actin	Muscle tumors, myofibroblast tumors
CD99	Ewing sarcoma, peripheral neuroectodermal tumors (PNETs), acute lymphoid and myeloid leukemias

Marker	Target Cells
Specific Organs	
Prostate-specific antigen (PSA)	Prostatic cancer
Prostate-specific alkaline phosphatase (PSAP)	Prostate cancer
Thyroglobulin	Thyroid cancer
α-Fetoprotein (AFP)	Hepatocellular carcinoma, yolk sac tumor
HepPar1	Hepatocellular carcinoma
WT1	Wilms tumor, some mesotheliomas
Placental alkaline phosphatase (PLAP)	Seminoma, embryonal carcinoma
Human chorionic gonadotropin (hCG)	Trophoblastic tumors
CA19-9	Pancreatic and gastrointestinal carcinomas
CA125	Ovarian carcinoma, endometrial carcinoma, some other nongynecologic tumors (pancreas, mesothelioma)
Calcitonin	Medullary carcinoma of the thyroid
CD Markers	
CD1	Some T-cell leukemias, Langerhans cell proliferations
CD2	T cells, T-cell malignancies
CD3	T cells, T-cell malignancies
CD4	T cells, T-cell malignancies, monocytes, monocytic malignancies
CD5	T cells, some B-cell malignancies
CD8	Suppressor T cells, some T-cell malignancies
CD10 (common ALL antigen, CALLA)	Acute lymphoblastic leukemia, some B-cell lymphomas, renal cell carcinomas
CD15	Reed-Sternberg cells, some T cells, some myeloid leukemias, many adenocarcinomas, but not in mesothelioma
CD19	B cells, B-cell malignancies
CD20	B cells, B-cell malignancies
CD30	Hodgkin disease, anaplastic large cell lymphoma
CD33	Myeloid leukemias

(*continued*)

TABLE 5-1
FREQUENTLY USED MARKERS TO IDENTIFY TUMORS (*Continued*)

Marker	Target Cells
CD34	Acute myeloid or lymphoblastic leukemias, some spindle cell tumors
CD117 (c-Kit)	Chronic myeloid leukemia, gastrointestinal stromal tumors, seminomas, also tumors of lung, breast, endometrium, urinary bladder
Non-CD Leukemia/Lymphoma Markers	
κ Light chain	B-cell malignancies
λ Light chain	B-cell malignancies
TdT	Acute lymphoblastic leukemia
Bcl-1 and cyclin D1	Mantle cell lymphoma
von Willebrand factor (vWF)	Vascular neoplasms
CD31	Vascular neoplasms, endothelial cells
CD34	Bone marrow stem cells, vascular neoplasms (endothelial cells)
Lectins	Vascular neoplasms
CD43	Almost all leukocytes
CD56	NK cells

CA = cancer antigen; CD = cluster designation; NK = natural killer; TdT = terminal deoxynucleotidyl transferase.

other ways. Blood levels of tumor antigens are helpful in following the development of metastases and progression of the tumor after the primary neoplasm has been treated. Representative examples include carcinoembryonic antigen (CEA) for gastrointestinal tumors, cancer antigen (CA) 125 for ovarian carcinoma and prostate-specific antigen (PSA) for prostate cancer. Tumor markers can be detected in blood using immunoassays (e.g., enzyme-linked immunosorbent assay [ELISA]) on serum, immunologic analyses of circulating blood cells (flow cytometry), electrophoretic separation of blood proteins and other methodologies. As effective as analysis of blood levels for tumor-produced substances may be in following a tumor's course, application of such analyses to screening for tumors has generally met with little success.

Some tumor antigens may also be used to make important therapeutic decisions (e.g., estrogen/progesterone receptors and HER2/neu in breast cancer, epidermal growth factor receptor [EGFR] for lung cancer and c-kit for gastrointestinal stromal tumors). Tumor antigens may also be useful therapeutic targets, as illustrated by HER2/neu for breast cancer and CD20 for B-cell lymphoma.

INVASION AND METASTASIS

Two properties that are unique to cancer cells are the ability to invade locally and the capacity to metastasize to distant sites. These characteristics are responsible for the vast majority of deaths from cancer; the primary tumor itself (e.g., breast or colon cancer) is generally amenable to surgical resection.

Direct Extension Damages Involved Organs and Adjacent Tissues

Most carcinomas begin as localized growths confined to the epithelium where they arise. As long as these early cancers do not penetrate the basement membrane on which the epithelium rests, such tumors are called ***carcinoma in situ*** (Fig. 5-6). In this stage, it is unfortunate that in situ tumors are asymptomatic, because they are invariably curable. When in situ tumors acquire invasive potential and extend through the underlying basement membrane, they can compromise neighboring tissues and metastasize. In situations in which cancer arises from cells that are not confined by a basement membrane—such as connective tissue cells, lymphoid elements and hepatocytes—an in situ stage is not defined.

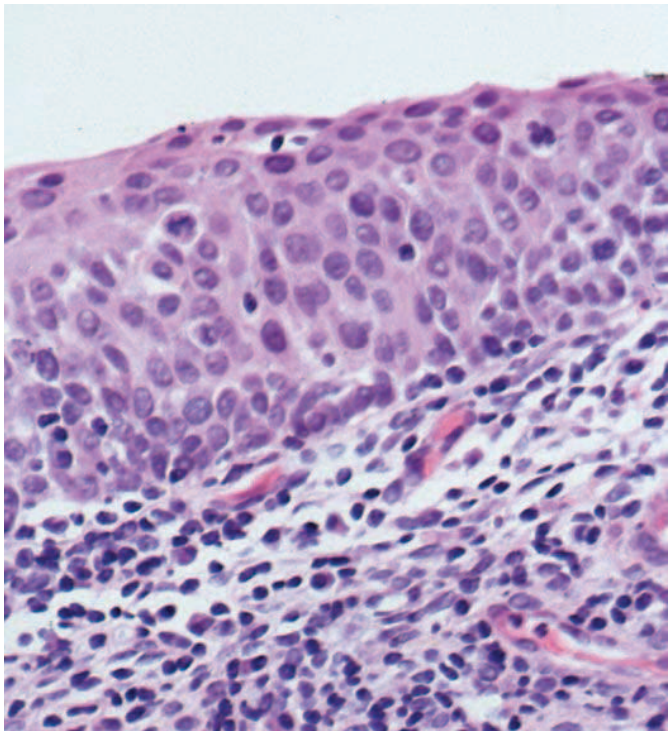


FIGURE 5-6. Carcinoma in situ. A section of the uterine cervix shows neoplastic squamous cells occupying the full thickness of the epithelium and confined to the mucosa by the underlying basement membrane.

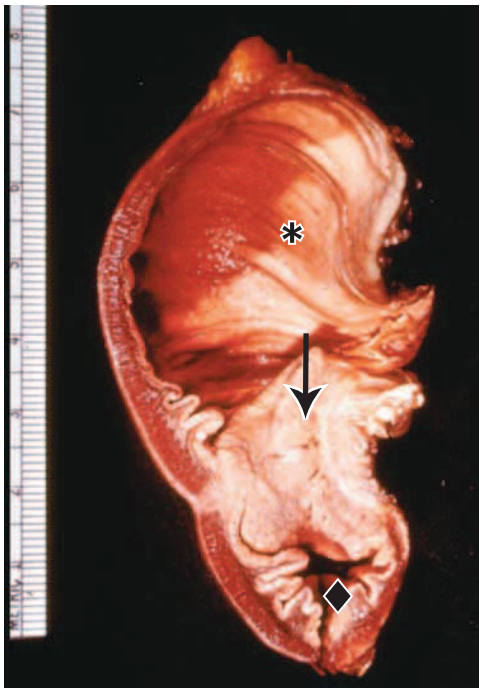


FIGURE 5-7. Adenocarcinoma of the colon with intestinal obstruction. The lumen of the colon at the site of the cancer is narrow (*arrow*). The colon above the obstruction is dilated (*). The colon distal to the stricture is normal caliber (♦).

Malignant tumors growing within the tissue of origin may also extend beyond the confines of that organ to involve adjacent tissues. The growth of the cancer is occasionally so extensive that replacement of the normal tissue results in functional insufficiency of the organ. Such a situation is not uncommon in primary liver cancer. Brain tumors, such as astrocytomas, infiltrate the brain until they compromise vital regions. The direct extension of malignant tumors within an organ may also be life-threatening because of their location. A common example is intestinal obstruction produced by colon cancer (Fig. 5-7).

The invasive growth pattern of cancers may secondarily impair the function of an adjacent organ. Squamous carcinoma of the cervix frequently grows beyond the genital tract to obstruct the ureters or produce vesicovaginal fistulas. Neglected cases of breast cancer are often complicated by extensive skin ulceration. Even small tumors can produce severe consequences when they invade vital structures. A small lung cancer can cause a bronchopleural fistula when it penetrates the bronchus or exsanguinating hemorrhage when it erodes a blood vessel. The agonizing pain of pancreatic carcinoma results from direct extension of the tumor to the celiac nerve plexus. Tumor cells that reach serous cavities (e.g., those of the peritoneum or pleura) spread easily by direct extension or can be carried by the fluid to new locations on the serous membranes. The most common example is the seeding of the peritoneal cavity by certain types of ovarian cancer (Fig. 5-8).

Metastatic Spread Is the Most Common Cause of Cancer Deaths

Metastasis (Greek, "displacement") is the migration of malignant cells from one site to another noncontiguous site. The invasive

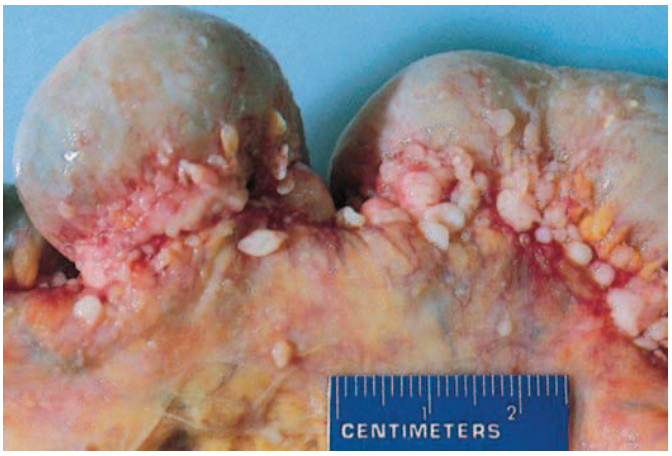


FIGURE 5-8. Peritoneal carcinomatosis. The mesentery attached to a loop of small bowel is studded with small nodules of metastatic ovarian carcinoma.

properties of malignant tumors bring them into contact with blood and lymphatic vessels, which they can also penetrate, and through which they disseminate to distant sites. They may also reach body cavities (e.g., pleural space), and so spread via those routes as well.

Hematogenous Metastases

Cancer cells often invade capillaries and venules, but thicker-walled arterioles and arteries are relatively resistant to their attack. Before they can form viable metastases, circulating tumor cells must lodge in the vascular bed of the metastatic site (Fig. 5-9). Here they attach to, and then traverse, blood vessel and lymphatic walls. Often, the location of a primary tumor with regard to blood or lymph flow determines the distribution of the initial metastases from that tumor. Thus, abdominal tumors that seed the hepatic portal system may cause liver metastases; other tumors penetrate systemic veins that eventually drain into the vena cava and hence to the lungs. Breast cancers first metastasize to regional lymph

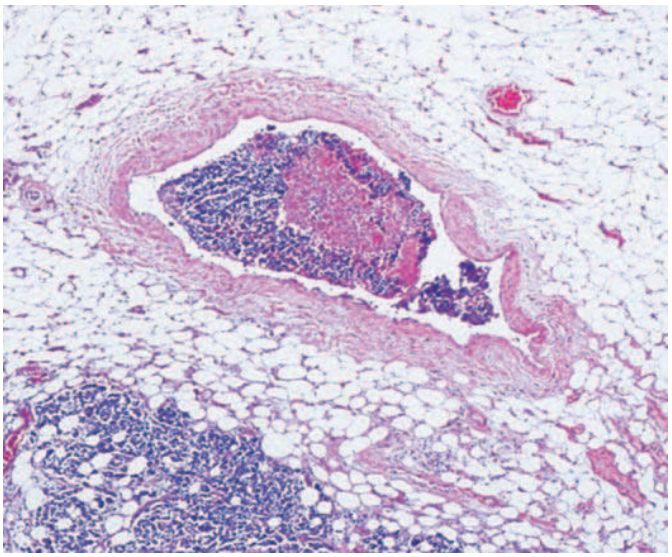


FIGURE 5-9. Hematogenous spread of cancer. A malignant tumor (*bottom*) has invaded adipose tissue and penetrated into a small vein.

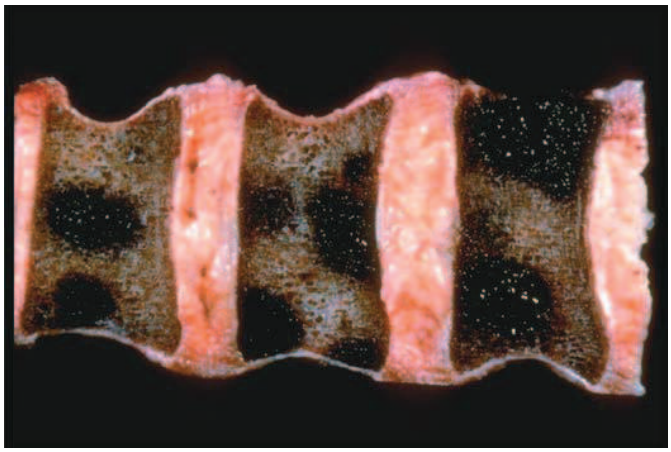


FIGURE 5-10. Multiple pigmented metastases in the vertebral bodies in a patient who died of malignant melanoma.

nodes because of the direction of lymphatic flow. More widespread metastatic disease may result from extensive early dissemination of tumor cells or from secondary spread from early metastatic foci (Fig. 5-10).

Lymphatic Metastases

Basement membranes envelop only large lymphatic channels; lymphatic capillaries lack them. Once in lymphatic vessels, itinerant tumor cells are carried to regional draining lymph nodes. There, they first lodge in the marginal sinus and then extend throughout the node. Lymph nodes bearing metastatic deposits may be enlarged to many times their normal size, often exceeding the diameter of the primary lesion (Fig. 5-11).

The regional lymphatic pattern of metastasis is most prominently exemplified by breast cancer. The initial metastases are almost always lymphatic, and these regional lymphatic metastases have considerable prognostic significance. Cancers that arise in the lateral aspect of the breast characteristically spread to axillary lymph nodes; those arising in the medial portion drain to the internal mammary thoracic lymph nodes.

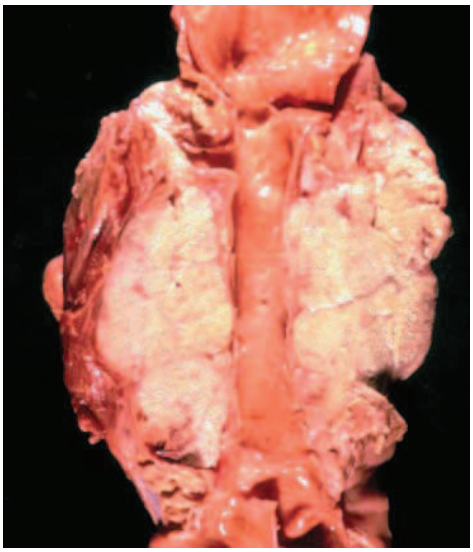


FIGURE 5-11. Metastatic carcinoma in periaortic lymph nodes. The aorta has been opened and the nodes bisected.

Lymphatic metastases are occasionally found in lymph nodes far from the primary tumor site. For example, the first sign of some abdominal cancers may be an enlarged supraclavicular node. A graphic example of the relationship of lymphatic anatomy to the spread of malignant tumors is afforded by cancers of the testis. Rather than metastasizing to inguinal nodes, as do other tumors of the male external genitalia, testicular cancers typically involve the draining abdominal periaortic nodes. The explanation lies in the descent of the testis from an intra-abdominal site to the scrotum, during which it is accompanied by its own lymphatic supply.

Seeding of Body Cavities

Malignant tumors that arise in organs adjacent to body cavities (e.g., ovaries, gastrointestinal tract and lung) may shed malignant cells into these spaces. Such body cavities principally include the peritoneal and pleural cavities, although occasional seeding of the pericardial cavity, joint space and subarachnoid space is observed. Similar to tissue culture, tumors in these sites grow in masses and often produce fluid (e.g., ascites, pleural fluid), sometimes in very large quantities. Mucinous adenocarcinoma may also secrete copious amounts of mucin in these locations.

Organ Tropisms of Metastases

It was recognized more than a century ago that the distribution of metastases in breast cancer is not random. In 1889, Paget proposed that the spread of tumor cells to specific secondary sites depends on compatibility between the tumor cells (the seed) and favorable microenvironmental factors in the secondary site (the soil). For example, cancers of the breast, prostate and thyroid metastasize to bone, a tropism that suggests a favored "soil." Conversely, despite their size and abundant blood flow, neither the spleen nor skeletal muscle is a common site of metastases. There is evidence that tumor-associated stromal cells in fact "plow the road" for tumor spread to particular sites that are suitable to the metastatic survival of implants from that tumor (see below).

STAGING AND GRADING OF CANCERS

Cancer Staging Describes the Extent of Spread

In an attempt to predict the clinical behavior of a malignant tumor and to establish criteria for therapy, many cancers are staged: they are assessed using specific protocols that help determine the detectable extent of their spread.

The choice of surgical approach, or the selection of treatment modalities, is influenced by the stage of a cancer. Moreover, most statistical data related to cancer survival are also based on this criterion. The significant criteria used for staging vary with different organs. Commonly used criteria include:

- Tumor size
- Extent of local growth, whether within or out of the organ
- Presence of lymph node metastases
- Presence of distant metastases

These patterns have been codified in the international **TNM cancer staging system,** in which "T" refers to the size and local extent of the primary tumor, "N" to the level of regional node metastases and "M" to the presence of distant metastases. Thus, for example, a breast cancer that is staged at T3N2M0 is a large primary tumor (T3) that has involved axillary lymph nodes moderately (N2) but has not detectably spread to distant sites (M0). The specific definitions of each T, N or M number vary among different tumors. As well, some tumor types, like central nervous system (CNS) tumors and hematologic malignancies, are staged according to different systems.

Cancer Grading Reflects the Architecture and Cytology of Tumors

Well-differentiated tumors are referred to as low grade, and poorly differentiated neoplasms are regarded as high grade. Cytologic and histologic grading, which are necessarily subjective and at best semiquantitative, are based on the degree of anaplasia and on the number of proliferating cells. The degree of anaplasia is determined from the shape and regularity of the cells and from the presence of distinct differentiated features, such as functioning gland-like structures in adenocarcinomas or epithelial pearls in squamous carcinomas. The presence of such characteristics identifies a tumor as well differentiated. By contrast, the cells of poorly differentiated malignancies bear little resemblance to their normal counterparts. Evidence of rapid growth is provided by (1) large numbers of mitoses, (2) atypical mitoses, (3) nuclear pleomorphism and (4) tumor giant cells. Most grading schemes classify tumors into three or four grades of increasing malignancy (Fig. 5-12). The general correlation between the cytologic grade and the biological behavior of a neoplasm is not invariable: there are many examples of tumors of low cytologic grades that exhibit substantial malignant properties. Thus, in most cases, staging is a more important criterion in predicting a tumor's course and influencing therapeutic decisions than is grading.

The Biology and Molecular Pathogenesis of Cancer

Normal cells, even those that divide the most rapidly (e.g., myelocytes, intestinal mucosal cells), are exquisitely controlled in the rate and location of their proliferation and accumulation. Cancer arises with accumulated DNA mutations within a single cell. When enough mutations have occurred, the cell escapes growth control and eventually acquires additional mutations that permit local invasion and subsequent spread through vascular and lymphatic channels.

For most of recorded history, cancer was considered to be simply due to a mysterious act of God. That is, the causation of cancer was not inherently comprehensible. However, in the late 18th century, specific causes of cancer were first identified. At that time, John Hill of London proposed that exposure to tobacco caused cancer. Shortly thereafter, in 1775, Sir Percival Pott described scrotal cancer caused by soot among chimney sweeps in London. More than a century later, bladder cancer was reported in aniline dye workers in Germany.

In modern times, major events in our understanding of oncogenesis include:

- 1911: F. Peyton Rous first described an avian cancer as being caused by a filterable agent (virus).
- 1920s: Human exposure to x-rays via fluoroscopy led to cancer.
- 1941: Berenblum first proposed the two-step (initiation/promotion) theory of chemical carcinogenesis.
- 1953: Watson and Crick identified DNA as the genetic material of cells and elucidated the structure of DNA.
- 1971: A. G. Knudson reported the involvement of two mutated alleles of the retinoblastoma (Rb) gene in the development of retinoblastomas and named these genes tumor suppressors.
- 1974: The gene responsible for defective DNA repair in the skin disease xeroderma pigmentosum was linked to visceral cancers.

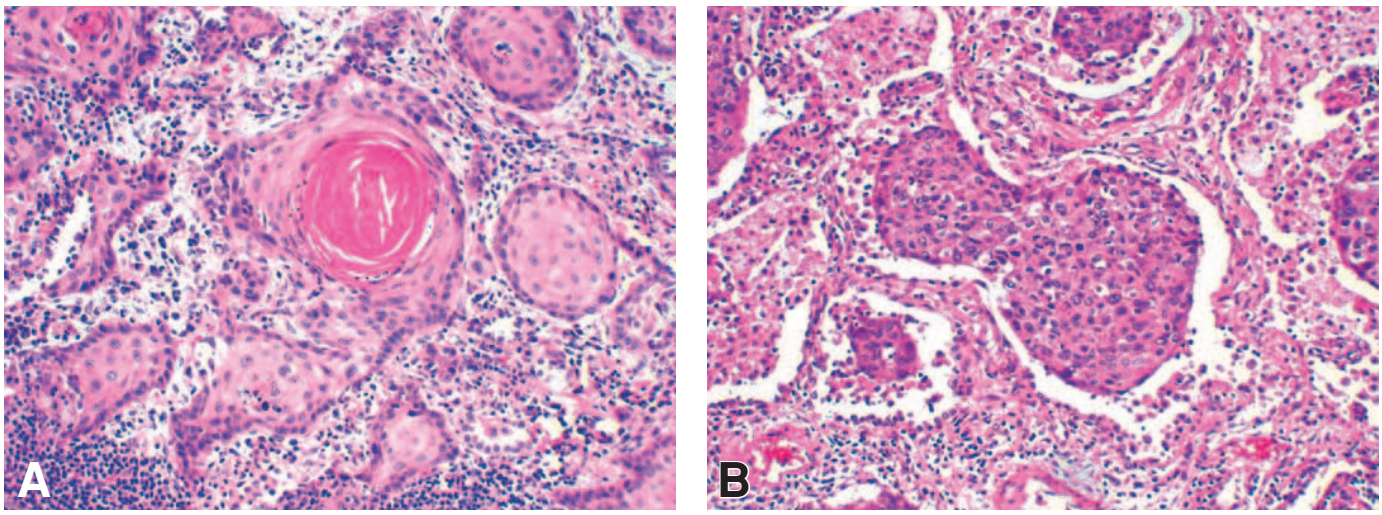


FIGURE 5-12. Cytologic grading of squamous cell carcinoma of the lung. A. Well-differentiated (grade 1) squamous cell carcinoma. The tumor cells bear a strong resemblance to normal squamous cells and synthesize keratin, as evidenced by epithelial pearls. **B.** Poorly differentiated (grade 3) squamous cell carcinoma. The malignant cells are difficult to identify as being of squamous origin.

- 1976: Bishop and Varmus demonstrated mammalian genetic homologs, called **proto-oncogenes,** of viral transforming genes **(oncogenes)**. When mutated, these cellular genes may become growth-promoting genes that can lead to cancer.

Substantial progress has been made in understanding neoplasia by the study of human tumors. Nonetheless, much of our appreciation of the processes involved in cancer development and spread has been derived from experiments in cells in culture, laboratory animals and genetically modified species. The applicability of those studies to oncogenesis in people should not always be assumed.

NORMAL PROCESSES THAT REGULATE CELLS AND INHIBIT ONCOGENESIS

As we will see shortly, cancer develops as a consequence of genetic changes in cells, leading to development, and often spread, of a tumor that is potentially fatal. Several critical cellular processes protect the organism and prevent tumor development. These processes are closely related, and intertwined: cell cycle regulation, DNA repair and telomerase activity. The next several pages describe how these cellular defenses operate and open the door to an understanding as to how they can be, and are, subverted during oncogenesis. As we will see, these defense mechanisms offer clues as to approaches to attacking tumors.

What is a gene? A century before the discovery of the structure of DNA, Gregor Mendel described discrete units of heredity that were later called genes. With the elucidation of the genetic code, the term "gene" came to signify a DNA string that defined the amino acid sequence of a protein. However, the idea of a gene is now being reexamined because regions of the genome previously thought to be "noncoding" are now known to produce new classes of RNAs that influence gene expression. Moreover, regulatory DNA sequences may be located adjacent to, at a large distance from and even within the protein-coding sequences. The contemporary notion of a gene entails interdependent structures and layers and webs of control involving DNA sequences, RNA species, regulatory proteins and a complex signaling apparatus. Thus, the precise definition of a gene remains unsettled.

Mutations and polymorphism: DNA replication is not perfect, and with each cell division about 1 nucleotide in 10^9 differs from the original. Thus, although the vast majority (99.6%) of base pairs in somatic cells are identical within the human race, two humans differ on average by about 2.4×10^7 base pairs out of 6×10^9 base pairs.

Variations in DNA sequences may result from germline changes or acquired alterations in somatic DNA as a result of single nucleotide substitutions or insertion or deletion of one or more nucleotides. **Polymorphisms** are defined as variations in DNA sequence that are not associated with known diseases. **Mutations** are comparable genetic changes that contribute to disease. (Mechanisms and types of DNA sequence variations are described in Chapter 6.)

The Normal Cell Cycle Drives Cellular Proliferation

Since most cancers are characterized by uncontrolled cellular proliferation, an understanding of the normal cell cycle is important. We focus here only on those aspects of the cell cycle that are commonly aberrant in the pathogenesis of cancers.

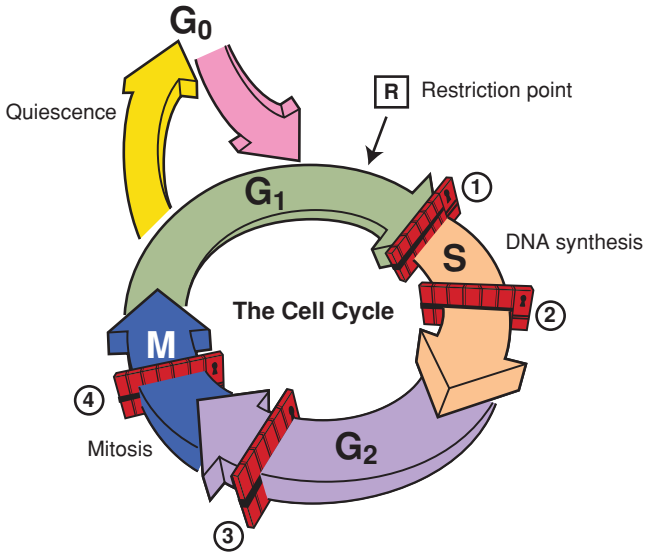


FIGURE 5-13. Normal cell cycle. The phases of the cell cycle, with key checkpoints indicated. *R* = restriction point.

Phases of the Cell Cycle

Cells may be cycling or quiescent. Those that replicate continuously (e.g., intestinal mucosa, hematopoietic progenitor cells) always transition from mitosis (M phase) to G_1, which is the antechamber to further cell division. By contrast, cells that replicate infrequently (e.g., hepatocytes) are in a quiescent phase, G_0. Cells in G_0 may be propelled to enter G_1 by various stimuli. They then replicate their DNA in S phase and proceed to G_2 and ultimately M (Fig. 5-13). When they are in an actively dividing mode, cells progress directly from M phase into G_1 by diverse stimuli. Thereafter, they initiate DNA replication, which occurs in S phase. G_2 follows S, and ultimately cells undergo mitosis (M phase). Following mitosis, cells again enter G_1 if they are in an actively dividing mode.

Cyclins and Cyclin-Dependent Kinases Driving Cell Cycle Progression

A cell's progress through the proliferative cycle is dependent on two important classes of regulatory molecules, **cyclins** and **cyclin-dependent kinases (CDKs)** (Fig. 5-14). These form a series of dimeric complexes that propel cell proliferation. Once a cell is stimulated to divide (e.g., by growth factors), D-type cyclins are activated first and form complexes of two CDKs (4 and 6). These cyclin D–CDK complexes play key roles in inactivating the retinoblastoma protein (pRb; see below). Together with cyclin E–CDK2 complexes they drive the cell into S phase. Other cyclins, A and then B, bind to CDKs (1 and 2), then push the cycle to completion.

Regulation of Cyclins and CDKs Provides Critical Protection against Tumor Development

Considering their importance in driving cell proliferation, it is not surprising that the activities of cyclins and CDKs

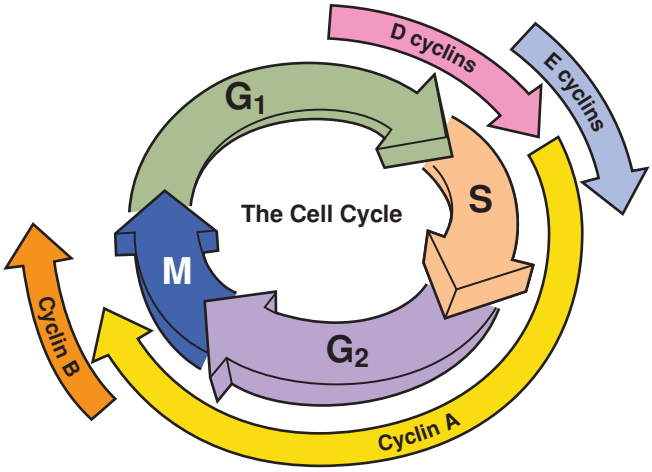


FIGURE 5-14. Activities of the cyclins and cyclin-dependent kinases (CDKs) in the cell cycle. The D cyclins mediate passage through G_1 and into early S phase. The E cyclins overlap D cyclins early in S phase. Cyclin A shepherds the cell through S and G_2 phases, into M phase. Finally, cyclin B overlaps cyclin A in late G_2 phase and directs the cell through mitosis (M phase).

are carefully controlled. Restraint of the cyclin–CDK system occurs in several ways (Fig. 5-15).

Decreasing Cyclin Availability

CDKs require activation by cyclins. Thus, the availability of cyclins limits the activity of CDKs. Levels of most cyclins fluctuate with the cell cycle: most are synthesized only when needed, and then, when the cell has traversed that part of the cycle for which a particular cyclin is required, that cyclin is ubiquitinated and degraded by the ubiquitin–proteasome system (UPS; see Chapter 1).

CDK Inhibitors

Several families of CDK inhibitors (CKIs) strongly inhibit cell proliferation and so collectively represent an important protective mechanism against oncogenesis. Not surprisingly, then, CKIs are commonly mutated in human cancers. CKIs mostly act by either binding the binary cyclin–CDK complexes or themselves binding with CDKs and preventing CDK activation by cyclins. The three major families of CKIs are:

- **INK4** proteins bind and inhibit CDKs 4 and 6 (*in*hibit cd*k4*), and prevent them from forming complexes with cyclin D. INK4 CKIs thus block cell cycle progression in G_1. There are several INK4 proteins: p15, p16, p18 and p19. As well, $p14^{ARF}$ is encoded by the same gene as $p16^{INK4a}$ but is an alternate reading frame (i.e., ARF). These proteins play important roles in regulating both CDKs and MDM2 (see Chapter 1 and below).
- **Cip/Kip** proteins bind and strongly block CDK2 and, to a lesser extent, CDK1. Thus, they take up where the INK4 family leaves off and inhibit the rest of the cell cycle. The best understood of the Cip/Kip proteins is $p21^{CIP}$ (also called $p21^{WAF1}$).
- **Rb** family members (see below) also target CDK2.

Expression of CKIs can be induced by senescence, contact inhibition, extracellular antimitogenic factors (e.g., transforming growth factor-β [TGF-β]) and the tumor suppressor protein p53 (see below).

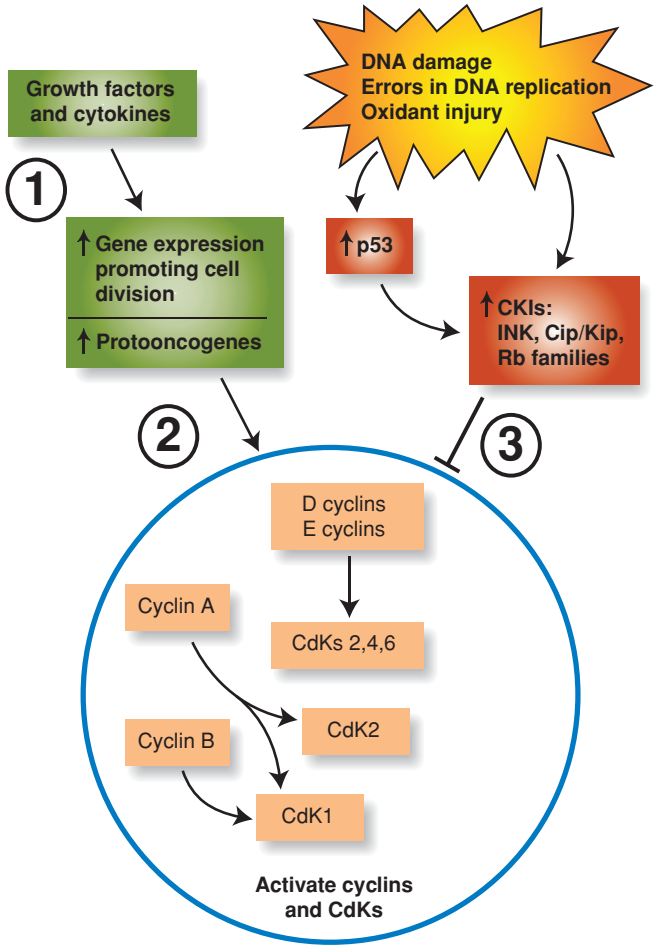


FIGURE 5-15. Activation and inhibition of the cyclins. 1. Activation. Cells receive signals via growth factors, cytokines and so forth that trigger the process of cell division. This leads to increased expression of genes that promote cell cycle progression, including a number of proto-oncogenes. **2. Actions of the early-phase cyclins.** D and E cyclins increase activity of cyclin-dependent kinases (CDKs) 2, 4 and 6. In addition, cyclin A activates CDKs 2 and 1, while cyclin B activates the latter. These CDKs drive cell cycle progression. **3. Inhibition.** Diverse stimuli, including errors in DNA replication, DNA damage and inhibitory signals of many kinds, may counteract the activating signals in **1.** These may do so by activating p53. Whether via p53 action or directly, these inhibitor stimuli increase production and activity of cyclin kinase inhibitors (CKIs), such as the INK family of proteins, CIP and KIP, as well as Rb. These families of CKIs block all the steps listed in **2.**

Other Forms of Inhibition of Cyclin–CDK Activities

In addition to mechanisms mentioned above, cells limit the ability of cyclin–CDK complexes to drive proliferation by selective posttranslational modification, largely phosphorylation, to inactivate some CDKs. Specific nuclear export systems also prevent cyclin–CDK dimers from accumulating in the nucleus, which is their site of activity. Both of these mechanisms support regulation supplied by other modalities mentioned above.

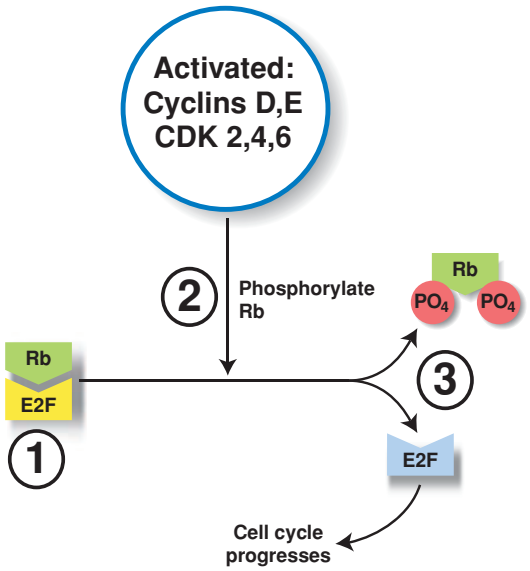


FIGURE 5-16. Role of cyclins and cyclin-dependent kinases (CDKs) in removing Rb-mediated blockade of cell cycle progression. 1. Normally Rb protein is complexed with E2F transcription factor. In this complex, E2F is inactive, yet it must be freed in order for cell division to proceed. **2.** Activated cyclins D and E, together with activated CDKs 2, 4 and 6, phosphorylate and then hyperphosphorylate Rb. **3.** This induces a conformational change that makes Rb release E2F, which is now free to direct transcription of proteins that help cell division to proceed.

Retinoblastoma Protein and Its Family

One of the most important mechanisms regulating the cell's commitment to division involves the family of retinoblastoma proteins, which principally act near the $G_1 \rightarrow S$ boundary. The prototype of these related proteins is $p105^{Rb}$ (pRb). Other members are related structurally and functionally, and for simplicity, we refer here only to pRb. pRb inhibits progression of the cell cycle by binding members of the **E2F** family of transcription factors. These E2F factors mediate cell entry into, and transit through, S phase by increasing transcription of downstream cyclins (A and E), as well as other proteins that promote DNA replication.

Binding of E2F by pRb blocks E2F activity. Phosphorylation of pRb by the complex of cyclin D–CDK4/6 (see above) alters pRb conformation, releasing E2F. E2F-mediated upregulation of other cyclins (A and E; see above), which, complexed to CDK2, again phosphorylate pRb, allows the cell cycle to progress (Fig. 5-16). The Rb system thus integrates many signals that control cell cycle progression.

Interestingly, Rb resumes its cell cycle inhibitory activity as cell division culminates. In anaphase, a protein phosphatase (PP1) dephosphorylates pRb, allowing it to bind E2F again, thus preventing further division.

Landmark Transitions in the Cell Cycle

The major cell cycle transitions are $G_1 \rightarrow S$ and $G_2 \rightarrow M$. Passage from $G_1 \rightarrow S$ occurs when the activity of complexes involving cyclins A and E overwhelm inhibition by the Cip/Kip family of inhibitors. Cells progress from $G_2 \rightarrow M$ when cyclin B–CDK1 complexes are activated by removing inhibitory phosphorylation of CDK1.

Cell Cycle Checkpoints

Transit from one phase to another of the cell cycle is regulated at **checkpoints,** namely, times when progression in the cell cycle can be arrested, should the need arise. There are checkpoints in G_1, before entry into S, during S and in G_2 before entry into M. These are activated by DNA damage. Others, during S phase and during M phase, are activated differently (see below).

Checkpoints Activated by DNA Damage

Safeguarding the integrity of the cell's DNA requires ceaseless vigilance, of which specific mechanistic details are discussed below. However, once DNA damage is sensed (by ATM and ATR proteins; see below), p53 is activated (Fig. 5-17). If the cell is in G_1, p53 increases production of Cip/Kip CKIs, blocking further progress in cell division. If the cell is in G_2, there are two means by which cycling is stopped. One involves p53-mediated downregulation of cyclin B and CDK1. The other involves two related enzymes called checkpoint kinases (**chk1, chk2;** see below), which block cell cycle progression immediately. These kinases are also responsible for blocking the cycle at the S phase DNA damage checkpoint as well.

Restriction Point

During G_1, the cell commits itself to enter S phase at the **restriction point (R)**. Here, the cell crosses the Rubicon and decides whether to proceed with mitosis or not. Past the R point, external forces driving or inhibiting mitosis no longer come into play (i.e., cell proliferation is determined only by intracellular mechanisms). The R point is activated by cyclin D–CDK4/6 phosphorylation of pRb, with consequent release of E2F (see above) to facilitate mitosis. *Loss of R-point control occurs in many cancers and deregulates progression through the cell cycle.*

Other Checkpoints

The duration of the proliferative cycle is finite. On occasion, the cell dawdles (usually because of exposure to a

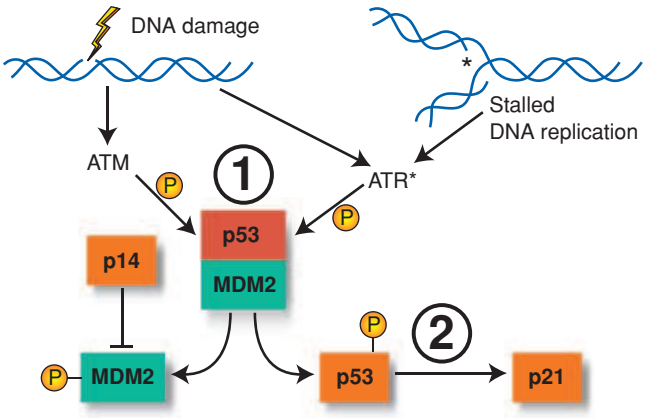


FIGURE 5-17. Linkage of DNA damage and replication stress to cell cycle arrest, via p53. 1. Both DNA damage and other interference with DNA replication activate the kinases ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3 related). These kinases phosphorylate p53, releasing it from binding to its inhibitor, MDM2. **2.** Activated p53 stimulates p21 (also called $p21^{CIIP1/WAF1}$).

toxic agent) in S phase. Should that happen, nature loses its patience and activates chk1 to block mitotic separation of incompletely duplicated chromosomes. This is called the **replication checkpoint**.

During M phase, there are elaborate controls to ensure that daughter cells each receive the correct complement of chromosomes. Thus, sensors at the points (kinetochores) where chromosomes attach to the mitotic spindle may activate a **spindle integrity checkpoint** if they sense that the segregation process is unbalanced or inaccurate. An additional mechanism involves an important enzyme, **Aurora B kinase,** which ensures that the kinetochore binds effectively and the mitotic spindle is effective, and so prevents improper segregation of chromosomes.

p53 in the Cell Cycle

As mentioned in Chapter 1, p53 has been called "the guardian of the genome." It coordinates cellular responses to DNA damage, mediates activation of G_1/S and G_2/M checkpoints and initiates apoptosis. Mechanisms underlying p53 activities include:

1. Normally, p53 is maintained at low levels by MDM2 (murine double minute), an E3 ubiquitin (Ub) ligase that conjugates p53 to Ub and triggers its proteasomal degradation (Fig. 5-18A; see Chapter 1).
2. The protein kinases **ATM** (ataxia telangiectasia mutated) and **ATR** (ATM and Rad3 related) recognize DNA damage and, in combination with chk1 and chk2 kinases, phosphorylate, and therefore activate, p53. Activation of p53 through these kinases or via telomere dysfunction (see Chapter 1 and below) contributes to cell cycle arrest (Fig. 5-17).
3. Activation of a cell cycle checkpoint requires a period of arrest to permit DNA repair. When p53 is phosphorylated, it dissociates from MDM2 and translocates to the nucleus.
4. In this location, p53 further promotes cell cycle arrest by stimulating production of $p21^{Cip1}$, one of the Cip/Kip family of CKIs (Fig. 5-18B).
5. If DNA is repaired, the block is removed and the cell cycle proceeds (Fig. 5-18B).
6. If DNA cannot be repaired, p53 triggers apoptosis (Fig. 5-18C).

It is important to appreciate that tumor cells often have mutations in genes that control cell cycle transit and that, whether these genes are mutated or not, many antineoplastic agents target cell cycle–related genes to try to limit the ability of cancer cells to divide. Mutations in cyclins or CDKs, for example, may render them impervious to the regulatory activities described above. Other alterations, in regulatory proteins like Cip/Kip CKIs or pRb, may eliminate one or another aspect of their ability to limit cell cycle progression.

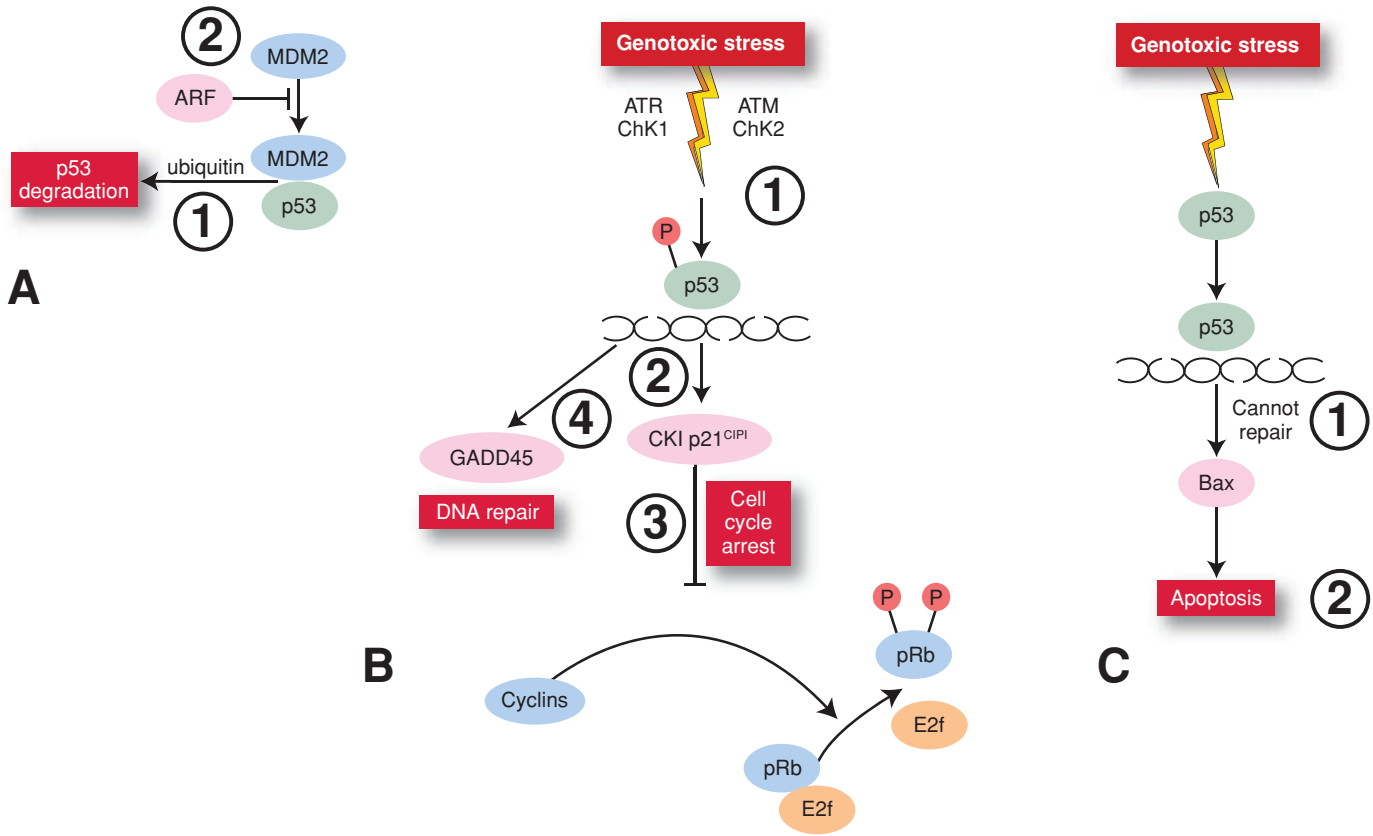


FIGURE 5-18. A. Regulation of p53 and MDM2 (murine double minute). 1. MDM2 is an E3 ubiquitin ligase that binds to and directs the inactivation of p53. **2.** MDM2 activity is inhibited by the tumor suppress $p14^{ARF}$. **B. 1.** In response to genotoxic stress (e.g., ionizing radiation, carcinogens, mutagens), ATR (ATM and Rad3 related) and ATM (ataxia telangiectasia mutated) increase p53, which does two things. **2.** It binds to DNA and upregulates transcription of several genes, including cyclin kinase inhibitor (CKI) $p21^{CIP1/WAF1}$. **3.** It also induces cell cycle arrest by preventing release of E2F from retinoblastoma protein (pRB). **4.** GADD45 promotes DNA repair. **C. 1.** Should DNA repair not be possible, p53 directs increased transcription of the proapoptotic protein Bax. **2.** Increased Bax triggers apoptosis.

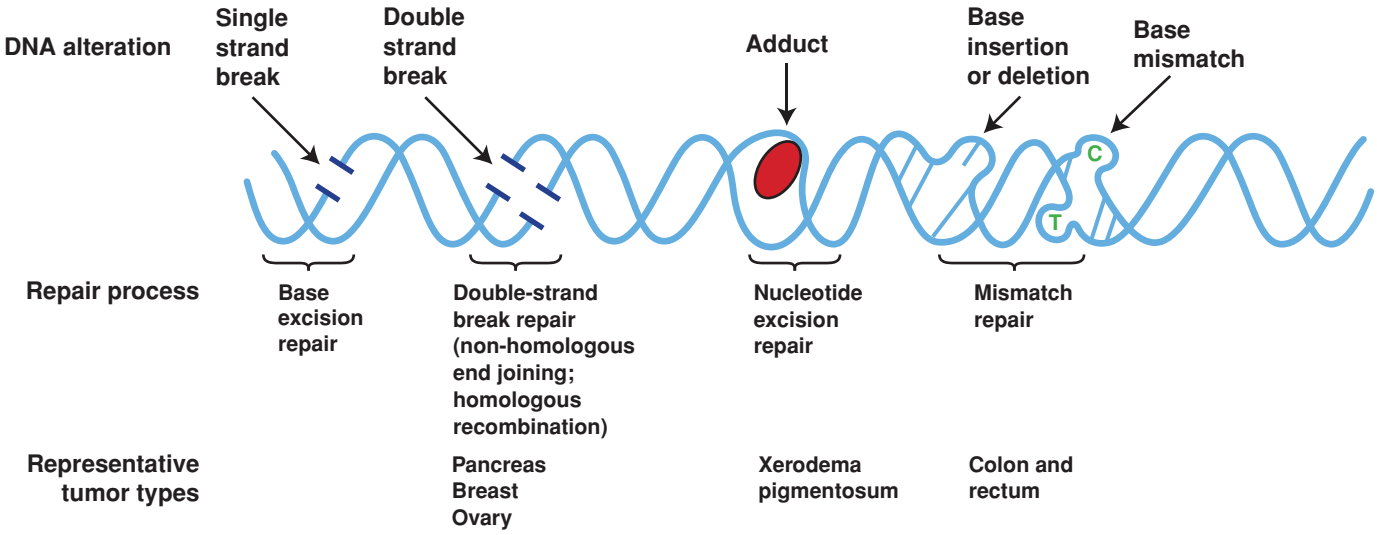


FIGURE 5-19. DNA damage and the mechanisms that repair DNA damage. Most forms of localized DNA damage are either (1) single-strand breaks, (2) double-strand breaks, (3) DNA adducts, (4) base insertions or deletions or (5) base mismatches. The mechanisms that repair these insults are, respectively, (1) base excision repair, (2) double-strand break repair, (3) nucleotide excision repair, and (4) and (5) mismatch repair. Some tumor types that arise from dysfunctional DNA repair mechanisms are indicated in conjunction with the mechanisms that tend to malfunction in those tumors.

DNA Repair Protects Cellular Genomes from Genotoxic Stresses

The DNA of the one hundred trillion (10^{14}) cells that constitute the human body is under relentless assault by both internal and exogenous stresses, including environmental chemicals, ultraviolet and ionizing radiation, reactive oxygen species (ROS) and an intracellular milieu that may break down the chemical bonds that hold DNA together. Cellular DNA is further at risk from the infidelity of DNA polymerases. Cells maintain genomic stability via diverse mechanisms that are ceaselessly vigilant in detecting and repairing such damage. In addition, these systems also communicate with cell cycle checkpoint regulators (see above) and apoptosis triggers, so that mutations are not transmitted to daughter cells. Collaborations among diverse proteins restore genomic integrity after such DNA modifications as single- and double-strand breaks, single nucleotide substitutions, base insertions and deletions of variable lengths and other perturbations of orderly and correct double-stranded DNA structure.

The importance of understanding these mechanisms extends beyond their roles in homeostasis and, if impaired, tumorigenesis. DNA repair systems may also protect tumors from genotoxic therapies. In this sense, they represent a key facet of tumor resistance to treatment and, therefore, important targets for current drug development.

DNA Repair Pathways

DNA damage may occur at different times in the cell cycle, be caused by diverse insults and reflect different types of alterations in DNA structure. Maintaining the integrity of a cell's genome thus requires multiple DNA repair processes, illustrated in Fig. 5-19.

Mismatch Repair

This pathway is mainly involved in repairing errors in DNA replication. From one somatic cell division to the next, the error rate in duplicating the genome is 1 miscopied base per 10^9 bases. In germ cells, it is even lower, 1 miscopied base in 10^{11}. The two polymerases that replicate human DNA (called Pol δ and ε) possess editing functions that have error rates of 1 base/$10^{4–5}$. The difference between the error rates of polymerase editing and the final product, 4–5 orders of magnitude, largely reflects the effectiveness of mismatch repair (MMR) processes (Fig. 5-20). MMR includes two overlapping systems, one that mainly corrects single base mismatches and another that fixes insertions and deletions caused by slippage during DNA replication.

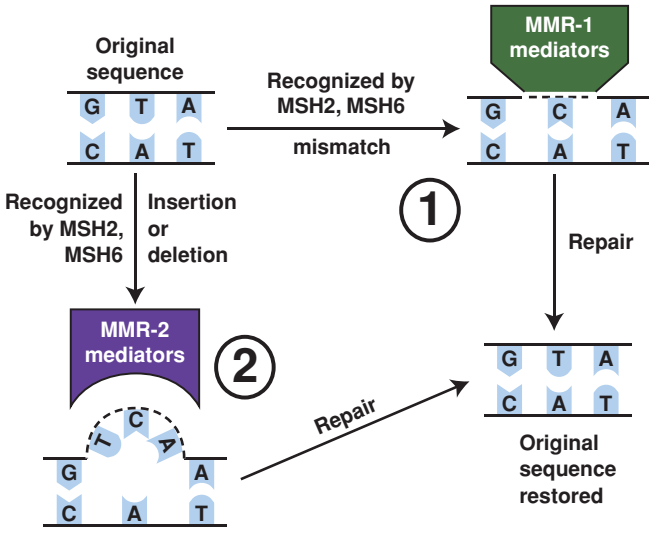


FIGURE 5-20. Mediators of mismatch repair (MMR). 1. A DNA single base mismatch (here, a C is present where a T should be) is recognized by two proteins, MSH2 and MSH6. These recruit a group of MMR repair enzymes, which correct the defect. **2.** If the mispairing is the result of a small insertion or deletion, a second group of MMR enzymes, MSH2 and MSH3, recognize the mistake and recruit another group of MMR mediators to correct the defect and restore the correct sequence.

Errors are recognized and repaired by a family of enzymes (e.g., MSH6, MSH2, MLH1; Fig. 5-20) that stop DNA replication at the G_2/M checkpoint, correct the mistake and then allow replication to continue. If the damage is irreparable, MMR enzymes activate apoptosis.

Defective MMR may be inherited or acquired. Acquired defects in MMR may reflect either mutations that develop over time in somatic cells or epigenetic silencing (see below) of a component of the MMR system. An important indicator of defective MMR is **microsatellite instability**. Microsatellites are short sequences of up to 6 base pairs that may be repeated as many as 100 times. They are common in the human genome and are inordinately prone to mutation, including changes in numbers of repeats. Microsatellite mutations are usually detected and repaired by MMR enzymes. If, however, they escape repair in germline or somatic cells, microsatellite mutations may indicate the likelihood of cancer developing (see below).

Nucleotide Excision and Repair

This process corrects distortions of DNA helical structure, such as are caused by bulky DNA adducts, by base oxidation by ROS derived from mitochondria or other sources or by ultraviolet (UV) and ionizing radiation (Table 5-2). Nucleotide excision and repair (NER) searches for DNA damage in two ways. One constantly scans the genome and the other identifies alterations that interfere with RNA transcription. Inherited defects in these NER detection systems are associated with human cancer syndromes. The first (scanning) pathway is deficient in **xeroderma pigmentosum,** and the second (transcription coupled) in **Cockayne syndrome**.

Once either NER system recognizes a defect, both pathways repair it similarly, via an enzyme called ERCC1. This enzyme is of considerable practical therapeutic importance. As mentioned above, effective DNA repair by tumor cells helps to protect them from chemotherapies that target DNA. For certain types of tumor, the activity (or lack thereof) of ERCC1 helps to predict the sensitivity of cancer cells to chemotherapy with such DNA-damaging agents as *cis*-platinum.

Base Excision Repair

Base excision repair (BER) is related to NER, and the two overlap somewhat in the types of DNA damage for which they are responsible (Table 5-2). BER mostly repairs chemical injury to DNA bases, such as hydrolysis of base–sugar bonds, single-strand breaks and small chemical changes in base structure. Such alterations occur 10^4 times/cell/day. Insults leading to BER are mostly caused by environmental and other chemicals, ROS and UV and ionizing radiation. Inherited defects in BER have not been described.

Double-Strand Break Repair

Double-strand breaks (DSBs) may be caused by ROS or ionizing radiation, or during DNA replication if replication is stalled at a single-strand break (Table 5-2). Such DNA lesions commonly lead to chromosome rearrangements, particularly when they occur, as they often do, in clusters.

Two related enzymes, ATM and ATR (see above, Fig. 5-17), are the sensors for DSBs, and each recognizes different types of DSBs. ATM identifies those that result from DNA damage (e.g., ionizing radiation). Once activated, ATM recruits chk2. ATR detects single-stranded DNA at stalled replication forks, whereupon it in turn activates chk1 (see above).

TABLE 5-2
TYPES OF DNA DAMAGE, THEIR COMMON CAUSES AND THE RESPECTIVE REPAIR PATHWAYS

Type of Damage	Causes	Repair Pathway
Base oxidation	Mitochondrial ROS Phagocyte-generated oxidants UV and ionizing radiation Smoking	NER, BER
Other base modifications	Chemotherapeutic drugs Neutrophil bactericidal enzymes Cigarette smoke Other environmental chemicals	BER
Alterations Perturbing DNA Architecture		
Additions	Errors in transcription, ROS UV light	NER
Interstrand DNA cross-links	Ionizing radiation Arrested DNA replication Environmental chemicals	Other[a]
Single-strand nicks	ROS Ionizing radiation Spontaneous loss of sugar–phosphate bonding	BER, NER
Double-strand breaks	Arrested DNA replication Ionizing radiation Chemical damage	HR, NHEJ

[a]Other mechanisms include Fanconi-related repair pathways.
BER = base excision repair; HR = homologous recombination; NER = nucleotide excision repair; NHEJ = nonhomologous end-joining; ROS = reactive oxygen species; UV = ultraviolet.

Both resulting complexes, ATM/chk2 and ATR/chk1, phosphorylate p53 and so activate cell cycle checkpoints, halting cell division until the break is fixed. DSBs are repaired either by **nonhomologous end-joining (NHEJ)** or by **homologous recombination (HR)**.

In HR, which is mostly active in S and G_2, the sister chromatid or the homologous chromosome is used as a template, to reproduce the original sequence. However, in so doing, two major types of genome alterations may result. If HR uses the homologous chromosome as a template for DNA repair, allelic differences between the two chromosomes may be lost (**loss of heterozygosity [LOH];** see below). As well, because repetitive sequences are abundant throughout the human genome, HR repair of a break that happens to occur in repetitive sequences in one chromosome may lead to use of an identical repetitive sequence on a nonhomologous chromosome as a template for recombination, thus generating a translocation. Several genes (e.g., BRCA1, BRCA2, PALB2) whose protein products are important for HR are often mutated or inactivated in many human tumors. This

may occur (see below) either as part of inherited cancer susceptibility syndromes or epigenetically during oncogenesis.

By contrast, NHEJ repairs DSBs by rejoining the broken ends. NHEJ thus restores DNA integrity but may not reproduce the original sequence (depending on the nature of the break). Furthermore, there is no guarantee that the ends that are so joined are in fact the broken ends of the same chromatid, in which case, translocations may result.

Unlike HR, NHEJ operates throughout the cell cycle. It is more efficient than HR and uses the Ku proteins (Ku70, Ku80), which are among the most abundant protein species within cells. NHEJ is the mechanism behind V(D)J recombination to generate diversity in B lymphocytes.

NHEJ generally functions well, and without many errors. However, it does not easily account for the original base order of the DNA sequences it joins. In settings in which there are multiple, clustered DSBs, NHEJ may link non-contiguous DNAs. It should be noted that defective NHEJ is not associated with any known inherited predispositions to developing cancer, nor is it commonly associated with oncogenesis.

HALLMARKS OF CANCER

Tumor development generally requires accumulation of changes in cellular behavior, many of which occur via mutation. Extensive and ongoing analyses of human tumors have uncovered a great many mutations. Not all changes so identified are equally important for tumor development and spread: some are more equal than others.

A relatively small number of genetic changes are fundamental to oncogenesis. These are called **"drivers."** It should be stressed that **driver mutations** may affect both DNA sequences that encode proteins and so-called noncoding sequences, since altered sequences and levels of untranslated RNAs (see below) and of regulatory regions may drive oncogenesis. Thus, the idea of driver mutations should be inclusive and should accommodate the complexities of regulation of gene expression and action.

Other mutations—"passengers" or "hitchhikers"—seem to be along for the ride. How they affect tumor development and progression, if at all, is unknown.

What is clear is that there are several basic activities that distinguish cells of solid malignancies from their normal counterparts. (Hematologic cancers develop and spread differently, and so share some, though not all, of these characteristics.) To understand oncogenesis and how cancer-related processes are being targeted therapeutically, the following hallmarks or attributes of malignant tumors should be appreciated:

- **Unregulated cellular proliferation:** In normal tissues, progression through the cell cycle (see above) is carefully regulated. However, cancer cells have acquired the ability to determine their own destinies, independently of normal restraints to multiplication.
- **Cellular immortalization:** While normal cells in culture have a limited potential for replication, cancer cells can multiply indefinitely. Thus, malignant cells circumvent the process of senescence and retain youthful vigor and ability to reproduce.
- **Evasion of programmed cell death (PCD):** Programmed cell death (see Chapter 1) can be activated by such factors as genomic instability and inhospitable cellular microenvironments. Cancer cells often develop strategies that circumvent destruction via such suicide programs.
- **Stimulation of vascular proliferation:** Expansion of solid tumors requires increased supplies of nutrients and oxygen. This, in turn, necessitates proliferation of blood vessels. Thus, tumor cells secrete signaling molecules that stimulate **angiogenesis** (i.e., formation of new blood vessels).
- **Invasion and metastasis:** Death from cancers is usually caused by tumor dissemination (metastasis). To accomplish such spread, tumor cells must be able to surmount anatomic barriers such as basement membrane, traverse intervening connective tissues, enter blood vessels and lymphatics, identify fertile sites for implantation, exit the vasculature and then establish colonies far from their origin.
- **Inactivation of tumor suppressors:** Many strong influences normally limit cell cycle transit, maintain genomic stability and regulate other key functions. These restraints affect most of the key attributes mentioned above, as well as associated facilitating processes described below. If a tumor is to be successful, these endogenous tumor suppressors must be evaded or inactivated.

Additional Processes Facilitate Tumor Cell Growth and Spread

Some mechanisms play supporting roles in developing and maintaining many cancers but are not yet generally accepted as being obligatory to tumor development.

- **Genomic instability:** Most human cancer cells show increased susceptibility to random mutation. This allows tumor cells to evolve quickly and to achieve genotypes that favor cancer maintenance and progression.
- **Altered epigenetic regulation:** Epigenetics refers to management of gene function by mechanisms independent of the DNA base sequences whose activities are being controlled. Among the modalities involved are covalent modifications of DNA and DNA-associated proteins (such as histones), noncoding RNAs, altered messenger RNA (mRNA) translation and posttranslational modifications of gene products.
- **Altered bioenergenetics:** Generally, cancer cells favor glycolysis over oxidative phosphorylation for adenosine triphosphate (ATP) generation. This metabolic change requires increased glucose utilization, which has many consequences for the cell's metabolic needs and products.
- **Immune avoidance:** A body of clinical and experimental evidence suggests that the immune system may protect from tumor production and progression. However, the nature of the interactions between tumors and host immunity remains uncertain.
- **Inflammation:** Inflammatory cells infiltrate most developing solid tumors and secrete diverse factors that facilitate tumor development and progression.

Most cancers develop because multiple mutations accumulate in dividing cells. Incipient tumors become malignant by undergoing serial genetic changes that lead to their acquiring hallmarks of cancers, as listed above. The order in which these changes arise may vary from tumor to tumor, but eventually a tumor amasses all of them.

These basic tumor attributes and contributing factors are interdependent mechanistically, and a single altered protein or gene may contribute to more than one characteristic, and no one trait operates in isolation. Interrelated though they are, cancer hallmarks are conceptually distinct. Further, every tumor evolves differently. Thus, certain attributes and genes may contribute to one tumor but not to others, or may suppress oncogenesis in one context but encourage it in another. The discussion of neoplasia that ensues follows the sequence of essential tumor characteristics and contributing factors listed above.

Tumor Cells Elude Processes That Normally Regulate Proliferation

Tumor cell escape from controlled proliferation occurs as a result of mutations that have the effects of both activating and inactivating certain genes. Generally, activating mutations stimulate passage through the cell cycle. The genes affected by such mutations have traditionally been called oncogenes. Inactivating mutations, by contrast, usually prevent the inhibitory influences of tumor suppressor genes (see below).

The Concept of Oncogenes

Early research on transforming retroviruses showed that some viral genes could impart a neoplastic phenotype to normal cells. It was later found that transfer of specific genes from human cancer cells **(oncogenes)** in vitro could also impart a transformed phenotype to normal recipient cells. Some such transforming human tumor genes were found to be mutant versions of normal genes **(proto-oncogenes)** that stimulated cellular proliferation. Transforming retroviral genes were designated with a *v-* (e.g., *v-myb*), and their cellular counterparts with a *c-* (e.g., *c-myb*).

Mechanisms by Which Cell Proliferation Is Driven

Among the key genes often altered during oncogenesis are those that stimulate cell multiplication. They are in the biochemical pathways that guide entry into the cell cycle. These include the following (Fig. 5-21):

- Growth factors
- Cell surface receptors
- Intracellular signal transduction pathways
- DNA-binding nuclear proteins (transcription factors)

Growth Factor–Related Signaling and Oncogenesis

Cell proliferation normally reflects a balance between forces driving cells to divide and, on the other hand, the cell cycle regulators discussed above. In acquiring the

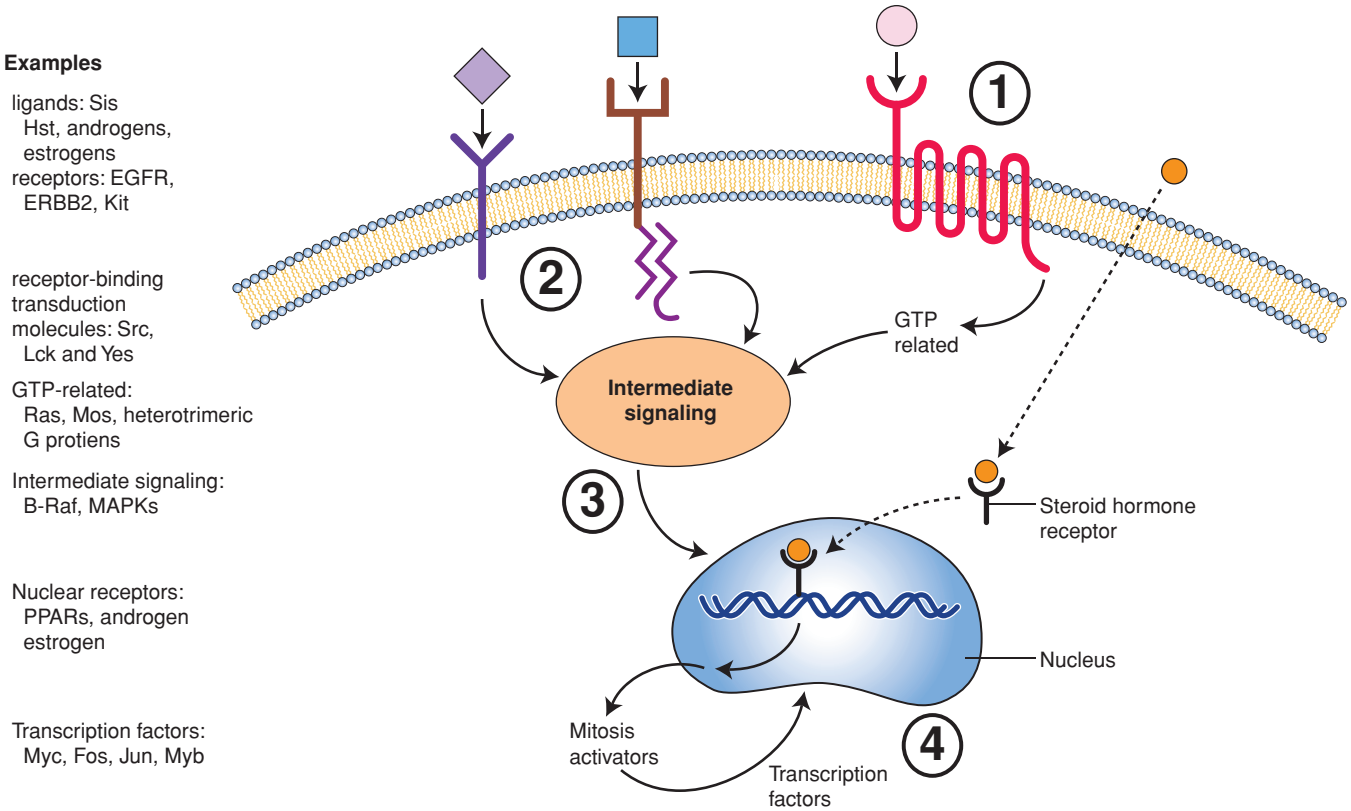


FIGURE 5-21. Signaling paradigms in cellular transformation. 1. Extracellular ligands bind to cell membrane receptors. **2.** One of several pathways of signaling is then activated. The receptor itself can activate intracellular signaling (*left*). A protein that binds to the activated receptor may trigger intracellular signaling (*center*). The receptor may be a G-protein–coupled receptor, which stimulates guanine nucleotide–related signaling. Or, the ligand may traverse the cell membrane to activate receptors within the cytosol directly, without a cell membrane intermediate (*far right*). **3.** In the first three cases, cellular intermediates of many types are activated. **4.** The end result for all pathways is activation of transcription, particularly of proteins that help take the cell through the cell cycle. Shown at the *left* are examples of proto-oncogenes and other cellular products that act in each capacity.

TABLE 5-3
COMMON PROTEINS THAT DRIVE CELL PROLIFERATION, THEIR ACTIVITIES AND ACTIVATION

Activity	Name of Protein	Nature of Mutation	Explanation
Ligand	Hst	Amplification	Growth factor in FGF family
	Sis	Derepression (autocrine stimulation)	PDGF, β subunit
	FGF3	Amplification	
RTK	Kit	Activating point mutation	Receptor for stem cell factor
	Her2/neu (ErbB2)	Amplification	Constitutively activated
	EGFR	Mutations, amplification	Constitutively activated
	Met	Translocation	HGF receptor
	Ret	Point mutation, translocation	Constitutively activated
Intracellular signaling intermediate	Ras (K-Ras, N-Ras, H-Ras)	Point mutation	GTP binding protein, three different *RAS* genes, activated in different settings
	B-Raf	Point mutation	Tyrosine kinase
	Src	Point mutation	Tyrosine kinase
	Abl	Translocation	Mutant protein, Bcr-Abl
Transcription factor	Myc (c-Myc, N-Myc, L-Myc)	Amplification, translocation	Directs transcription of up to 15% of human genes
	Fos	Amplification	Part of AP-1, with Jun
	Myb	Point mutations	Promotes hematopoietic stem cell proliferation
	Rel	Amplification, point mutations	Member of NFκB family, expressed mainly in lymphocytes
	Ets	Translocation	Large family; fusion products may drive tumorigenesis

AP-1 = activation protein-1; EGFR = epidermal growth factor receptor; FGF = fibroblast growth factor; GTP = guanosine triphosphate; HGF = human growth factor; NF = nuclear factor; PDGF = platelet-derived growth factor; RTK = receptor tyrosine kinase.

ability to multiply without restraint, cancer cells must be able to circumvent dependence on outside stimulatory influences. They usually do this by mimicking those influences. To understand how this occurs, one must first review how receptor–ligand interactions drive cells into mitosis. A general schematic relating the roles of ligand–receptor interactions in tumor development is shown in Fig. 5-21.

Tumor-driving mutations may occur at any step of this process. The consequence of such mutations is that proteins are produced that drive cellular proliferation without the normal restraints that match cell numbers to the body's needs.

Ligands, Their Receptors and Cell Proliferation

Ligands. In general, the role of external ligands reflects an ongoing need of normal cells and, often, developing tumors for exogenous stimulation that activates and maintains proliferation. Some ligands may drive cell multiplication in early stages of oncogenesis, with tumor cells eventually becoming independent of those ligands, owing to changes in, for example, receptors or other molecules. Sometimes, the developing (or developed) tumor cell itself undertakes production of these ligands, which amounts to an autocrine trigger to cell division. Some such stimulatory molecules occasionally act as oncoproteins being overexpressed, mostly by virtue of gene amplification (Table 5-3).

Receptors. There are several basic classes of receptors that may stimulate or inhibit cell proliferation. These are listed in Table 5-4. Except for the steroid hormone receptors, these are cell membrane molecules that respond to ligands produced by other cells. Usually, receptor–ligand interactions cause changes in the receptors, leading to their serving as docking sites for one or more intracellular signaling networks.

TABLE 5-4
TYPES OF SIGNAL-TRANSDUCING RECEPTORS IMPORTANT IN TUMORIGENESIS

Receptor Category	Prototypical Ligands
Tyrosine kinase (RTK)	EGF, IGF-I, insulin
G-protein–coupled receptor (GPCR)	Prostaglandins, RANTES, SDF-1
Nuclear receptors	Androgens, estrogens, other steroid hormones
Serine/threonine kinases	TGF-β
Kinase-associated receptors	GH, TCR, IL-2
Extracellular matrix receptors	Fibronectin, collagen, laminin

EGF = epidermal growth factor; GH = growth hormone; IGF-I = insulin-like growth factor-1; IL-2 = interleukin-2; RANTES = CCL5, a ligand for CCR5; SDF-1 = CXCL12, stromal-derived factor-1 (CXCR4 ligand); TCR = T-cell receptor; TGF-β = transforming growth factor-β.

The changes that ligands elicit in receptors reflect the specifics of the receptor:

- **Receptor tyrosine kinases (RTKs)** possess intrinsic tyrosine kinase activity that causes the receptor to phosphorylate itself, and perhaps other molecules as well, after recognizing its ligand.
- **Nonkinase receptors** include many that undergo structural rearrangements, making them receptive to initiating downstream signaling (see below). These types of receptors often associate with nonreceptor tyrosine kinases (**NRTKs;** see below), which mediate further signaling.
- **G-protein–coupled receptors (GPCRs)** are the most common type of membrane receptors. Upon binding their ligands—which include very diverse types of molecules—GPCRs change conformation. In so doing, they activate guanosine triphosphate (GTP)-related nucleotide exchange factors (GEFs; see below). Some GPCRs transduce mitogenic signals triggered by such ligands as prostaglandins, endothelin and thrombin. GPCRs may be amplified in cancers or they may mediate stimulatory autocrine or paracrine signals.

The nature of signaling elicited by receptor–ligand interaction varies. Many activated RTKs and nonkinase receptors serve as platforms for other proteins, including NRTKs. The latter often phosphorylate the nonkinase receptor, as well as the NRTKs themselves. In these settings, a cell membrane complex is formed, which recruits signaling intermediates and activates diverse downstream signaling pathways.

Receptor proteins are among the most important transforming proteins. They are widely implicated in oncogenesis (Table 5-3), *often driving tumor formation via mutations that render them constitutively active, independently of their ligands.*

Signaling after Receptor Activation

Once a receptor binds to its ligand, downstream signaling pathways are stimulated. If an RTK or NRTK is involved, the phosphorylated tyrosine is recognized by the next cadre of signaling intermediates via specialized structures, called SH2 (for Src-homology-2) domains, on these intermediates. These domains bind phosphotyrosines. They also are specific for individual proteins bearing the phosphotyrosines.

What follows depends on many factors, including the type of receptor activated, if its activation entails tyrosine kinase activity and the molecular species that immediately follow. Pathways that may be set in motion include:

- **Ras:** The three members of the Ras family (K-Ras, N-Ras, H-Ras) are small guanine nucleotide-binding proteins that may be activated by tyrosine kinases via a linker protein, usually Grb2. To understand activated Ras and Ras-related oncogenesis, the Ras cycle should be appreciated (Fig. 5-22). Ras binds guanosine diphosphate (GDP) and GTP. In the GTP-bound state, Ras is active. GTP binding is catalyzed by a GEF (see above), which is in turn activated when Grb2 recognizes phosphorylated tyrosines (see above). A GTPase activating protein (GAP) directs the GTPase activity of Ras, thus activating downstream signaling, converting Ras–GTP to Ras–GDP and returning Ras to its quiescent resting state.

  Many malignant tumors possess a mutated form of Ras. Such mutated Ras does not undergo the deactivation step and is constitutively turned on.

  Activation of many GPCRs stimulates a similar type of response, but by a different group of proteins, called heterotrimeric G proteins. Unlike Ras, these G proteins tend not to be mutated in cancers. Rather, they may be overexpressed, achieving a comparable effect (i.e., constitutive activation of downstream signaling).
- **Phosphatidylinositol-3 (PI3) kinase:** This family of enzymes is generally activated by RTKs and GPCRs. Family members add a phosphate group to a phosphatidylinositol lipid to create the small molecule phasphatidylinositol-3-phosphate [PI(3)P], as well as more heavily phosphorylated derivatives such as PI(3,4,5)P_3. These

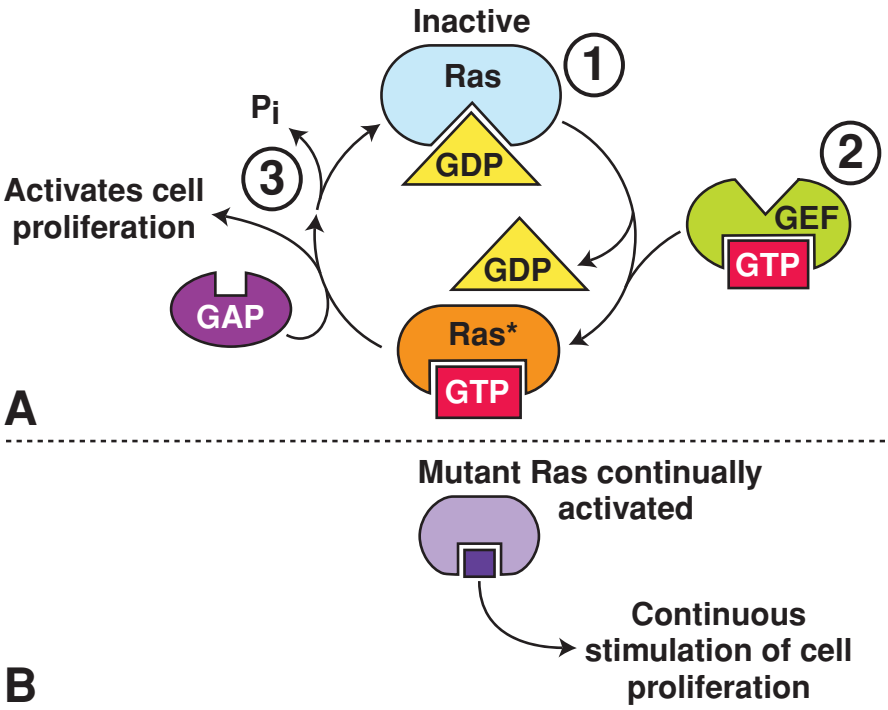


FIGURE 5-22. Mechanism of action of Ras. A (upper). Normal. The Ras protein, $p21^{Ras}$, exists in two conformational states, determined by the binding of either guanosine diphosphate (GDP) or guanosine triphosphate (GTP). **1.** Normally, most of the $p21^{Ras}$ is in the inactive GDP-bound state. **2.** An external stimulus, or signal, triggers the exchange of GTP for GDP, an event that converts Ras to the active state. **3.** Activated $p21^{Ras}$, which is associated with the plasma membrane, binds GTPase-activating protein (GAP) from the cytosol. The binding of GAP has two consequences. In association with other plasma membrane constituents, it initiates the effector response. At the same time, the binding of GAP to Ras GTP stimulates by about 100-fold the intrinsic GTPase activity of Ras, promoting hydrolysis of GTP to GDP and the return of Ras to its inactive state. **B (lower).** Mutated Ras protein is locked into the active GTP-bound state because of an insensitivity of its intrinsic GTPase to GAP or because of a lack of the GTPase activity itself. As a result, the effector response is exaggerated, and the cell is transformed.

mediate many proliferation-related (see below) and cell survival reactions.

- **Phospholipase C:** This family of enzymes is commonly activated by diverse types of receptors, especially GPCRs, but also others. They cleave certain phospholipids and so participate in generation of inositol phosphate signaling intermediates and diacylglycerol. These both may drive cellular multiplication via (respectively) calcium signaling pathways and protein kinase C.
- **Mitogen-activated protein kinases (MAPKs):** These enzymes mediate many different types of signaling reactions, leading to cell proliferation. MAPKs may be triggered by upstream proteins such as Ras (after RTK activation), GPCRs or other mechanisms. Some very important driver mutations for malignancy (e.g., b-Raf) occur among these proteins, frequently leading to constitutive activation. Typically, MAPK cascades involve three sequential species, one activating the next. The consequences of MAPK stimulation are diverse, and there is extensive cross-talk between these and other signaling intermediates.

Transforming Growth Factor-β and Other Cytokines

TGF-β, an extracellular cytokine in the microenvironment of cancer cells that triggers important regulatory pathways, is an example of a cell communication mediator that strongly influences the pathogenesis of tumors. Its role in the genesis of cancer appears to be important, although cell and tissue responses to this cytokine are highly contextual. Normally, TGF-β tends mainly to suppress tumor development by modulating cell proliferation, survival, adhesion and differentiation. It also inhibits mitogenesis induced by constituents of the extracellular matrix (see above).

However, frankly malignant cells often acquire the capacity to evade or even to manipulate TGF-β pathways for their own wicked ends. Abnormal signaling in the TGF-β pathway can actually stimulate proliferation of tumor cells, facilitate their evasion of host defense mechanisms (see below) and foster invasion and metastasis.

Cancer cells may develop the ability to circumvent TGF-β–related suppressive activity via mutations in genes for TGF-β receptors or by interfering with downstream signaling by mutation or by promoter methylation of key proteins. Under these circumstances, cancer cells can hijack the regulatory activities of TGF-β to further their needs, such as tumor growth, invasion and metastasis. The loss of the tumor suppressor function of TGF-β through inactivating mutations of genes in its core pathway has been described in cancers of the colon, prostate, stomach, breast, pancreas and ovary and many others. A summary of the effects of TGF-β in cancer is presented in Table 5-5.

Other cytokines (e.g., granulocyte/monocyte colony-stimulating factor [GM-CSF] and interleukin-3 [IL-3]) may contribute to tumor development simply by overexpression, especially for hematopoietic malignancies.

TABLE 5-5
TRANSFORMING GROWTH FACTOR-β (TGF-β) AND CANCER

Promotes	Inhibits
Normal Tumor-Suppressive Effects	
Apoptosis	Inflammation
Differentiation	Mitogenesis induced by extracellular matrix
Maintenance of cell number	
Failure of Tumor Suppression	
Autocrine mitogens	Immune surveillance
Motility	
Invasion and Metastasis	
Recruitment of myofibroblasts	
Malignant cell extravasation	
Modification of microenvironment	
Mobilization of osteoclasts	

Normally, TGF-β compels homeostasis and exerts tumor-suppressive activity through effects on the target cells themselves or the extracellular matrix.

Failure of this activity by TGF-β permits production of growth factors, evasion of immune surveillance and establishment of factors that facilitate tumor cell invasion and metastasis.

Steroid Hormones

Some three centuries ago, the Italian physician Ramazzini observed that nuns had a particularly high incidence of breast cancer. This curiosity is now recognized to reflect the unopposed estrogen stimulation of breast epithelium, uninterrupted by pregnancy and lactation. Both estrogens and progesterone bind to specific cytoplasmic receptors. The resulting hormone–receptor complexes are then translocated to the nucleus, where they act as transcription factors that foster proliferation of responsive cells. Antiestrogen therapy for hormone receptor–positive tumors reduces the risk of recurrence after surgery. Other nuclear receptors have been identified in breast cancer, including those that bind androgens, corticosteroids, vitamins A and D, fatty acids and some dietary lipids. The interactions of these signaling pathways with each other and with other signaling pathways are highly complicated and not well understood.

The influence of androgens is most conspicuous in the case of prostate cancer, in which they stimulate growth by binding to the androgen receptor. This receptor pathway engages in cross-talk with other important pathways that affect the cell cycle, apoptosis and differentiation. Such interactions involve EGF, insulin-like growth factor-I (IGF-I), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), TGF-β and other important signaling species. Removing androgen stimulation, whether by surgical or pharmacologic means, inhibits the growth of prostate cancer, although in most cases the tumors eventually become androgen insensitive.

Membrane-Bound Mucins

Traditionally, mucins have been thought to be exclusively extracellular molecules charged with establishing an interface between many epithelial surfaces and the exterior. It is now recognized that membrane-bound mucins comprise a large family of glycoproteins that are frequently

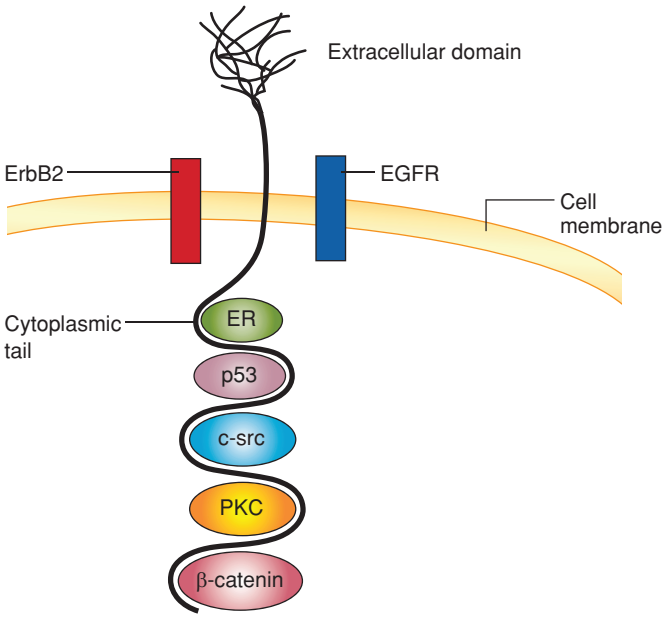


FIGURE 5-23. Membrane-bound mucins with important signaling molecules. *ER* = estrogen receptor; *PKC* = protein kinase C.

overproduced in a variety of cancers. Extracellular domains of these membrane-bound mucins (MUCs) lubricate and protect the cell surface. The cytoplasmic domains of these transmembrane glycoproteins function as scaffolds for interaction with signaling molecules that influence cell proliferation and survival (Fig. 5-23). In this context, MUC1 is overexpressed in the large majority of breast cancers, and often in malignancies of the colon, ovary, pancreas and lungs.

Interaction among Intermediate Signaling Pathways

Whether elicited by receptor–ligand interactions or by constitutively activating driver mutation, the signaling avenues discussed above, and many others, interconnect extensively. This fact endows them with baffling complexity and challenges both those seeking to understand how cells sustain proliferation and those seeking specific targets for therapy. In this context, it should be emphasized that the results of unrestrained activation of a given gene are not always predictable. A mutant protein may drive proliferation in one cell type, apoptosis in another and differentiation in a third.

Transcriptional Activation

In the end, a key element of the ability of cancer cells to proliferate without restraint is the array of genes whose transcriptional activities are turned on or off. Thus, whatever upstream driver mutations there are, transcription factors sit at the end of the afferent limb of the processes that push cancer cells to undergo uncontrolled mitosis. When transcription factors drive oncogenesis, the genetic changes responsible usually entail increased production of wild-type proteins. Thus, driver mutations of transcription factors generally entail, for example, translocations that place them under the control of more vigorous promoters. Many transcription factors are implicated in oncogenesis. Among the best known and most often inculpated are:

- **Myc:** A ubiquitous transcription factor that may control transcription of as many as 10%–15% of all human genes, c-Myc and its cousins, N-Myc and L-Myc, are key to development of many tumors. Among its functions, Myc pushes cellular proliferation, favors stemness (see Cancer Stem Cells, below), increases energy production and facilitates tumor cell invasiveness. It is of interest that Myc may also activate cell death programs in cells with intact p53 and other cell death effectors.
- **Fos and Jun:** Together, these proteins form the **AP-1** (activation protein-1) transcription factor. Increased AP-1 activity promotes cellular proliferation and survival (Fig. 5-24) and is generally a result of increased signaling via several pathways, including MAPK and the protein kinase C family (PKC; see above). AP-1 also represses expression of such tumor proteins as p53 (see below).
- **Androgen and estrogen receptors:** These cytoplasmic receptor proteins act both as receptors and as transcription factors. They translocate to the nucleus upon binding their cognate ligands. Once in the nucleus, they act as transcription factors. Depending on the cell type, these steroid sex hormone receptors may stimulate cell proliferation. Thus, estrogen receptors stimulate mammary epithelial cell proliferation and are important in the progression of many breast cancers. In many prostate cancers, similarly, androgens cause prostatic tumor cells to proliferate.

As noted above, however, cell proliferation mediated by these and similar receptors need not necessarily require exogenous hormones. Autocrine stimulation may occur when the tumor cells themselves produce the requisite androgen or estrogen. The ability of the tumor to progress thus becomes independent of exogenous sources of the stimulatory hormone and the tumor is resistant to hormone antagonist therapies.

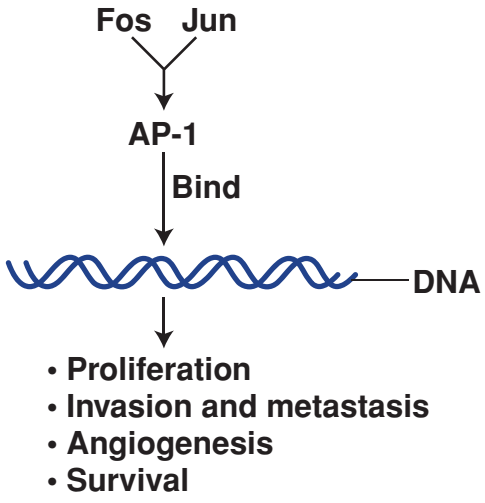


FIGURE 5-24. Activating protein-1 (AP-1) complex. The AP-1 transcription factor complex is formed by the protein products of two proto-oncogenes, Fos and Jun. When these factors form a heterodimer, they bind DNA and direct transcription of genes whose products are involved in cell proliferation, tumor cell invasion and metastasis, angiogenesis and inhibiting apoptosis.

Thus, whether by gene amplification, point mutation, translocation or other mechanisms (see genomic instability, below), tumors are characterized by cellular proliferation that is unshackled by the regulatory chains that limit normal cells. They must still, however, escape other restraints.

Cellular Senescence Helps Prevent Cancer

Senescence is a process that maintains cell viability when a cell can no longer contribute by cell division to continued homeostasis. Senescent cells are growth arrested and viable but remain unable to proliferate.

This state was reported initially in cultured normal human fibroblasts. After a certain number of mitoses (usually 40–45), they stopped dividing but remained alive. The upper limit of the number of mitoses is called the Hayflick limit, after its original discoverer. It largely reflects the effects of telomere depletion in preventing cell cycling when a cell's telomeres become very short (see below).

Mediators of Cellular Senescence

Clearly, a mechanism that limits the number of mitoses a cell may undergo must be neutralized for malignant cells to indulge in endless proliferation. The senescent phenotype entails increased formation of heterochromatin (senescence-associated heterochromatin formation [SAHF]), in which certain proteins are modified, leading them to bind chromosomal DNA and impede transcription of E2F-activated genes such as mediate cell multiplication (see above). Several effectors of senescence are involved:

- **The DNA damage response (DDR):** As noted above, once telomeres have shortened to a certain point, they elicit DDR. DNA damage-sensing proteins activate p53 and cdc25, and the cell stops dividing until either the offending DNA damage is fixed or the cell is directed into senescence or apoptosis pathways. This mechanism is critical to oncogene-induced senescence (OIS; see below).
- **Tumor suppressors:** The intimacy between several critical proteins and cell cycle blockage is important in forcing cells into a senescent phenotype. Key among these proteins are $p16^{INK4a}$ and Rb, which induce certain proteins to associate with the cell's DNA. This results in SAHF and gene silencing.
- **Oxidative stress:** In cultured cells, senescence can be delayed by decreasing ambient oxygen. Conversely, it can be hastened by adding oxidants like H_2O_2 to the culture. Activation of some oncogenes, like RAS, increases oxidative stress. Resulting increases in ROS may trigger p38 MAPK signaling and activate ATM.
- **Cytokines:** Cells secrete factors, including IL-6 and IL-8, that help trigger the senescent phenotype. Together with their receptors, they help to establish and maintain the senescence. Their participation led to the descriptive designation, secretion-associated senescent phenotype (SASP). Transcriptional regulation that these cytokines elicit inhibits cellular proliferation and promotes senescence.

Telomere Stability and Tumorigenesis

Telomeres are tandem repeats of the sequence TTAGGG, at the 3′ ends of each DNA strand. They serve as docks for protein caps that bind the ends of DNA sequences and prevent them from being used in NHEJ (see above) reactions. Because DNA polymerases "fall off" as they approach the ends of chromosomes, telomere lengths decrease with each cell division. When telomeres are reduced beyond a critical size, they become uncapped and are subject to NHEJ. Resulting NHEJ may fuse the ends of either two sister chromatids or nonhomologous chromosomes. As the cell cycle progresses, this fusion generates a chromosome "bridge" as the fused DNA strands are pulled apart during anaphase. The force of chromosome separation may then lead to chromosomal breakage, which can lead to further recombination (Fig. 5-25).

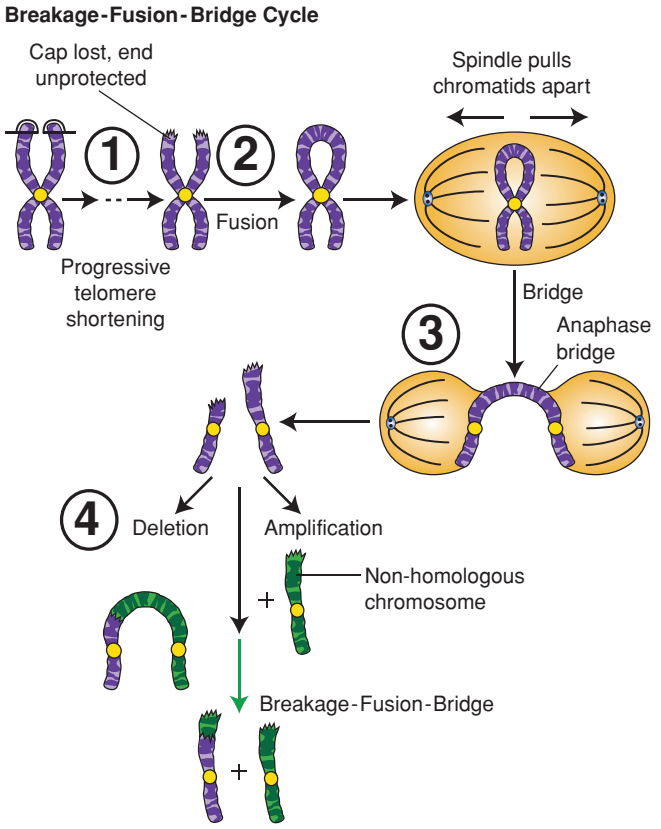


FIGURE 5-25. The genesis of telomere-related chromosome breakage. **1.** In the absence of telomerase, extensive cell proliferation leads to unprotected telomere ends. **2.** These are "repaired" by fusion of telomeres between sister chromatids, creating a bridged structure like a tongs. **3.** During anaphase, the spindles attached to the two centromeres pull the now-attached chromatids apart, resulting in abnormal chromosomes. **4.** Further production of chromosome ends without telomeres may cause the cycle to repeat itself.

This process can be prevented in several ways. In normal cells, where p53 and pRb pathways are intact, shortened telomeres activate cell cycle checkpoints and cell division ceases. This is called replicative senescence (Fig. 5-26). The cell then remains in G_0 or dies. Alternatively, many cells that replicate frequently (e.g., colon crypt epithelium) express **telomerase,** a ribonucleoprotein enzyme that lengthens telomeres and so maintains genomic stability in the face of continuing cell proliferation.

Oncogene-Induced Cellular Senescence

The vast majority of cells sustaining oncogenic mutations never become cancers. In the human body, such mutations occur several times per minute, which contrasts with

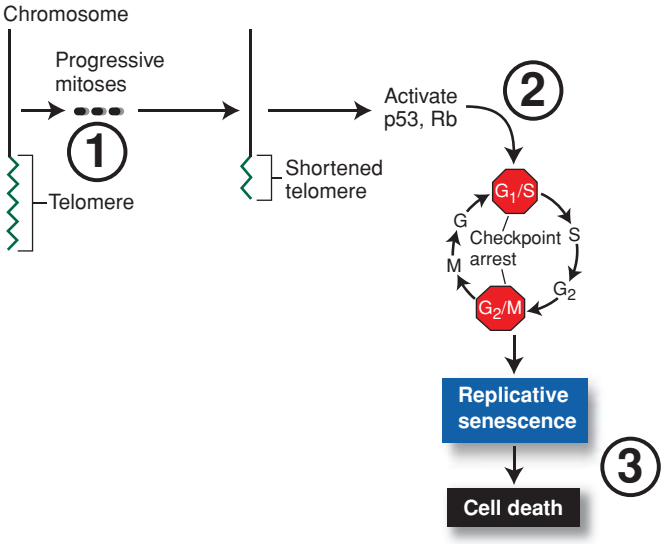


FIGURE 5-26. The sequence of events resulting from DNA instability as a result of telomere shortening and leading to cell death. This sequence occurs when the tumor suppressors p53 and Rb are intact. **1.** Progressive telomere shortening activates p53 and Rb. **2.** This leads to cell cycle arrest at the G_1/S and G_2/M checkpoints. **3.** Consequent replicative senescence triggers cell death programs.

the relative infrequency of cancer in a human life span. A major barrier to the development of cancer appears to be OIS (Fig. 5-27). OIS has been shown to constrain tumor development for many types of cancer. Consistent with this role for OIS, oncogene activation stimulates tumor suppressors such as p53 and Rb.

Earlier studies showed that after activated Ras enhanced proliferation of affected cells, an irreversible growth arrest (i.e., senescence) occurred. This senescence could be avoided by crippling p53 and Rb pathways, suggesting that such OIS actually evolved as a means to prevent tumors (Fig. 5-27). Activating alterations in other oncogenes also induce OIS in vivo. The ability to undergo OIS is a major distinction between many benign and malignant tumors, as well as between "premalignant" cell proliferations and frankly malignant ones. That is, benign tumors can senesce, but advanced malignant tumors do not. For example, benign tumors may show heightened responsiveness to IL-8 (see above), which is lost in malignancy. Thus, OIS prevents benign cell proliferations from progressing to cancers.

Evading Senescence

If critical shortening and uncapping of telomeres occur in the context of acquired loss of cell cycle checkpoint regulation (e.g., mutations in $p16^{INK4a}$ or p53), chromosomes become susceptible to instability resulting from the types of rearrangements mentioned above (breakpoint–fusion–bridge cycles). Such instability promotes carcinogenesis by facilitating chromosomal rearrangements, which in turn can lead to aneuploidy, translocations, amplifications and deletions. Thus, telomere attrition leads to sufficient genomic instability to set the stage for the development of mutations in sufficient numbers for cells to cross the borderline from benign to malignant.

Cancer cells may solve this problem, as indicated above, via telomerase. In normal human cells, levels of this enzyme are insufficient to maintain telomere length, and each cell division therefore leads to progressive telomere attrition. However, in normal cells that divide frequently, telomerase makes a DNA copy of an RNA telomere template and affixes it to the 3′ end of the replicating DNA strand. In this way, the

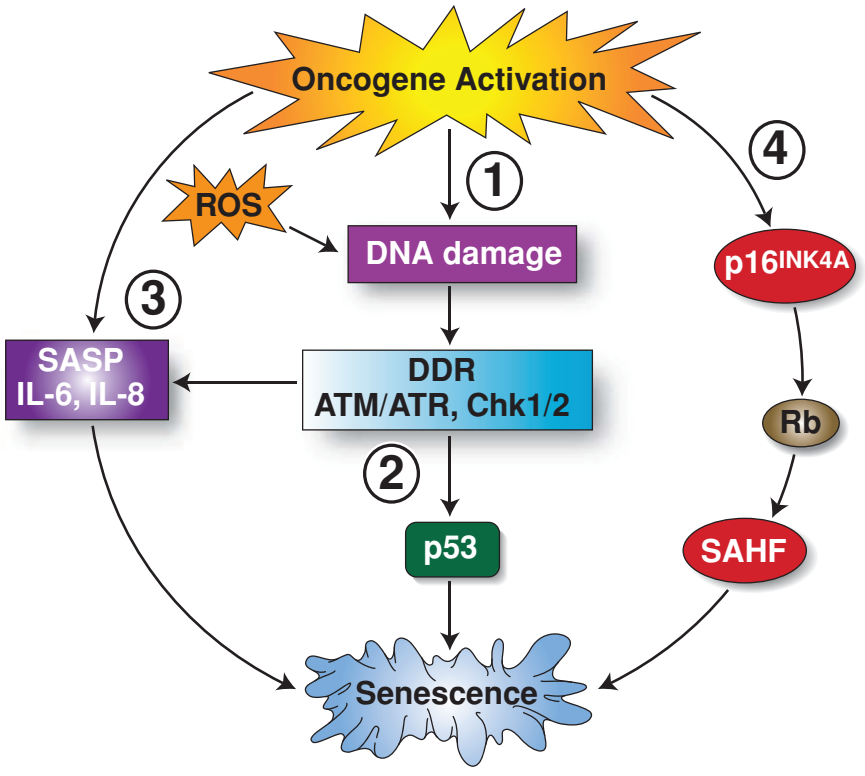


FIGURE 5-27. Oncogene-induced senescence (OIS). Oncogenic stress can elicit cellular responses that eventuate in cellular senescence. **1.** Excessive cell division, a result of oncogene activation, for example, causes oxidative stress and DNA damage to accumulate. **2.** As a consequence, the DNA damage response (DDR) is activated, with p53 expression blocking cell cycle progression. **3.** The same DDR may also activate the senescence-associated secretory phenotype (SASP), which leads affected cells to secrete cytokines that maintain the senescent state (interleukin-6 [IL-6], IL-8). SASP can also be activated directly by the excessive oncogene activity. **4.** Oncogene activation may directly activate the tumor suppressor $p16^{INK4A}$, which in turn activates Rb. This leads to the formation of senescence-associated heterochromatin (SAHF), which restricts expression of cell cycle drivers.

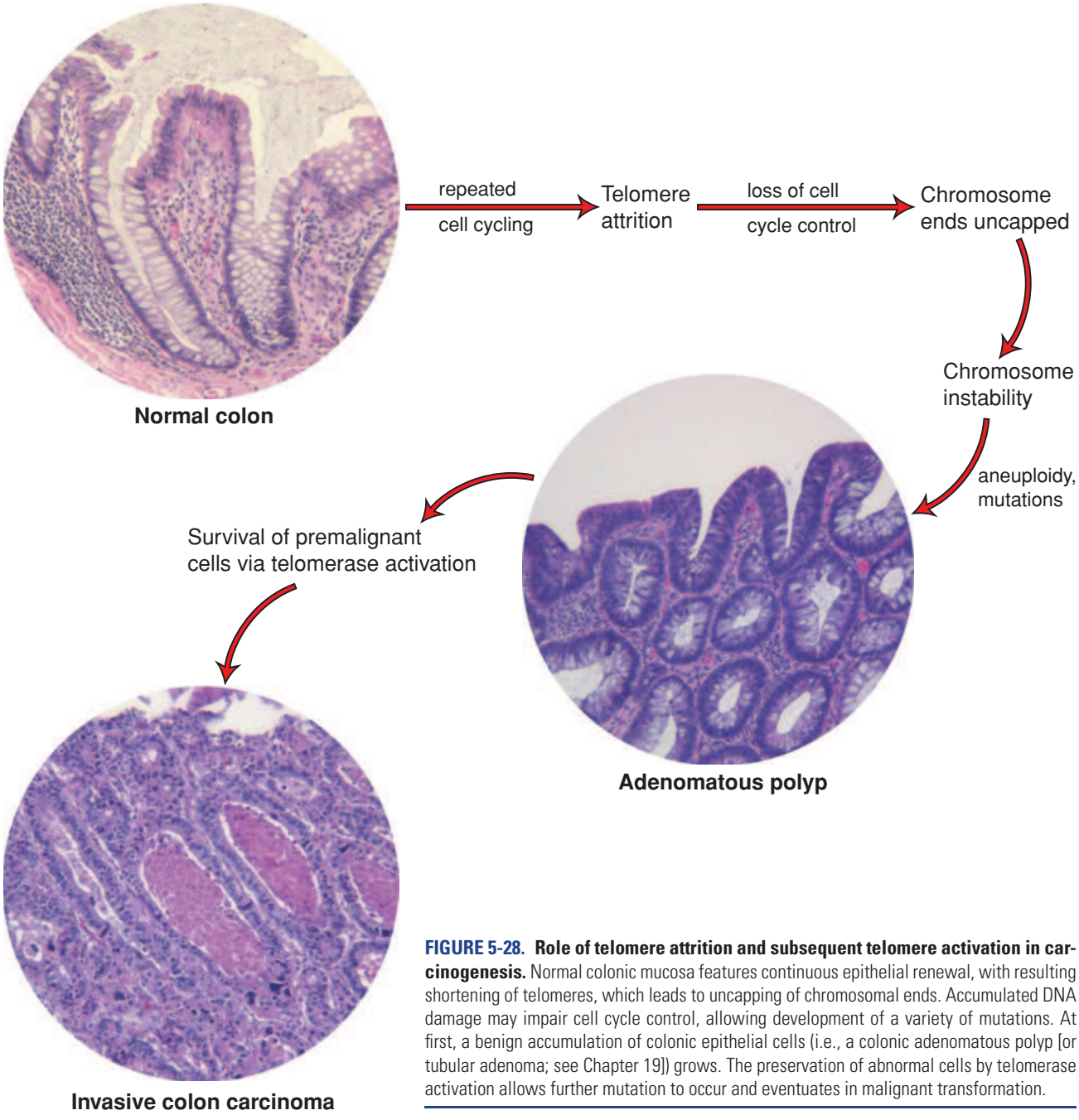


FIGURE 5-28. Role of telomere attrition and subsequent telomere activation in carcinogenesis. Normal colonic mucosa features continuous epithelial renewal, with resulting shortening of telomeres, which leads to uncapping of chromosomal ends. Accumulated DNA damage may impair cell cycle control, allowing development of a variety of mutations. At first, a benign accumulation of colonic epithelial cells (i.e., a colonic adenomatous polyp [or tubular adenoma; see Chapter 19]) grows. The preservation of abnormal cells by telomerase activation allows further mutation to occur and eventuates in malignant transformation.

enzyme preserves telomere length and chromosomal integrity despite continuing cell division. Not surprisingly, then, normal cells that express telomerase include those that need to continue to divide for the lifetime of the individual (e.g., hematopoietic progenitor cells, gastrointestinal epithelium and germ cells).

The cancer cell takes a page from that book. Presumably as a protective adaptation to genomic instability, tumor cells reactivate telomerase: 80% of human tumors show increased telomerase activity. A high level of this activity actually protects the cancer cell by suppressing the development of further, potentially lethal, chromosomal instability. *Thus, telomerase activation permits—but does not directly cause—the emergence of cancer* (Fig. 5-28).

If a cell manages to avoid senescence after oncogene activation, however it does that, the cell can continue to proliferate and may be said to have been immortalized. It has been shown in some cases that benign human tumors may retain the ability to senesce, while their malignant counterparts have lost that capacity. Thus, loss of tumor suppressor activities that promote senescence is important for the emerging cancer cell.

Programmed Cell Death Prevents Oncogenesis

The total number of cells in any organ reflects a balance between cell division and cell death. Interference with this intricate equilibrium can lead to tumor development. Cell

death programs encompass several different pathways (see Chapter 1), dysfunction of which is often a fundamental requirement for tumor development. The best understood of these is apoptosis and its cousin, anoikis.

Apoptosis as an Inhibitor of Cancer

As mentioned above and in Chapter 1, apoptosis eliminates damaged or abnormal cells. Apoptotic pathways are activated by errors in DNA replication or repair, detected genetic or metabolic instability, loss of anchoring connections to the extracellular matrix (ECM) **(anoikis)** and other stimuli. Since many triggers for apoptosis are among the attributes of tumor cells, it is not surprising that those cells often evolve mechanisms to disable it. There are many known pro- and antiapoptotic proteins that interact in a head-spinning number of ways. To make this topic understandable, we present illustrative examples. Thus, cancers may avoid PCD by impairing proapoptotic activities and/or by augmenting prosurvival functions.

Fighting for Survival Against the Forces of Death

There are many participants in the programs of cell death. The best known is p53, and it is no surprise that the gene for this protein, *TP53*, is mutated in over half of human cancers (see below). As the key angel of cell death, p53 is activated when oncogenic danger is sensed, for example, if damage to cellular DNA cannot be repaired (see Chapter 1). Wearing several of its many hats, activated p53 upregulates transcription of proapoptotic Bcl-2–like proteins and downregulates their prosurvival cousins. In addition, it is a BH3-only protein (see Chapter 1) and so may meddle directly in Bcl-2 family affairs by binding, for example, prosurvival Bcl-2 or Bcl-X_L to force them to release proapoptotic Bad and Bax. The latter activate effector caspases and cause the cell to die. It is worth recalling that apoptosis does not elicit florid, cytokine-rich inflammatory responses, but rather that apoptotic cells pass from the world not with a bang but with a whimper—they are quietly removed by macrophages.

Along similar lines, anoikis is form of apoptosis that is activated with epithelial cell membrane integrins that no longer bind their appropriate ECM partners. Integrins mediate prosurvival signals. Their detachment from their extracellular ligands leaves cells excessively susceptible to all manners of proapoptotic stimuli. Also, unligated integrins may activate caspase-8 directly. However, in some cancer cells, unbound integrins can maintain survival signaling and so protect from PCD.

The prototypical example of the effectiveness of inhibiting apoptosis in human cancer is follicular lymphoma (see Chapter 26). There, the prosurvival protein, Bcl-2, is constitutively activated by a translocation [t(14:8)] that places its expression under the control of the immunoglobulin heavy-chain promoter. As a result, the normal equilibrium between the life and death of B lymphocytes is altered in favor of the former, thus allowing accumulation—or, perhaps, more to the point, insufficient elimination—of excess neoplastic B cells.

Some other tumor types, including lung cancer and non-Hodgkin lymphoma, also express excess Bcl-2. Chromosomal translocation is not the only mechanism by which tumor cells increase Bcl-2 expression. They may show methylation and suppression of microRNAs (miRNAs) that repress Bcl-2 expression. Similarly, any impairment of p53 function can increase Bcl-2 production and decrease expression of proapoptotic Bcl-2 binding partners (see Chapter 1) and, in so doing, promote tumor formation.

The issue of PCD and cancer is further complicated by **oncogene-mediated apoptosis**. For example, although the transcription factor Myc is generally considered to be oncogenic, if PCD pathways are intact, Myc overproduction induces a default apoptosis pathway. Thus, promotion of cell proliferation by deregulated production of Myc is usually balanced by increased apoptosis. Induction of apoptosis by Myc acts as a molecular safety valve to block cancer development. If Myc-stimulated tumors are to develop, some cells overproducing Myc must also inactivate PCD, whether by overexpressing antiapoptotic proteins or by inactivating apoptosis mediators like p53.

This example illustrates the complexity inherent in the control of the on/off switch of apoptosis in cancer development.

Tumors Stimulate New Blood Vessel Formation (Angiogenesis)

Angiogenesis is the formation of new blood vessels from preexisting small blood vessels. In order to grow beyond about 2 mm in diameter, tumors need more nutrient and oxygen supply than preexisting blood vessels can provide. Most tumors experience hypoxia, which induces expression of **hypoxia-induced factors (HIFs),** especially **HIF-1α**. In turn, HIF-1α elicits production of angiogenic growth factors, which stimulate formation of new tumor-associated blood vessels. This process is obligatory for a primary tumor to grow and metastasize.

Under homeostatic conditions, there is a fine equilibrium between factors favoring new blood vessel formation and those impeding it. Consequently, endothelial cells turn over slowly, renewing themselves over the course of months or years. Solid tumors often disrupt this equilibrium in favor of new blood vessel formation.

Steps in the Formation of New Blood Vessels

Tumor angiogenesis begins when existing cells (e.g., tumor cells or attendant stromal cells) secrete substances (see below) that stimulate new blood vessel formation. The process triggered by these chemicals resembles vasculogenesis in embryonic development and follows several steps:

1. Proteolytic enzymes perforate postcapillary venule basement membranes.
2. Endothelial cells in the area of the interrupted basement membrane proliferate and migrate toward the source of the angiogenic cytokines.
3. A lumen develops in the advancing cell mass.
4. These immature capillaries are invested with a basement membrane.
5. The cells in the vanguard of the developing structure ("tip cells") join to other similar tip cells to produce a capillary network.

Under some circumstances, and for some tumor types, tumor vessels may form differently—directly from the tumor cells themselves. The end result, however the blood vessels are generated, is not always well-formed vasculature. Tumor-associated vessels may differ from their non-tumor-associated counterparts in several ways.

Tumor-associated capillaries may be variably invested by pericytes and basement membranes. They may vary in size and shape, leak blood components excessively, display excessive tortuosity and be distributed inhomogeneously. Excessive leakiness of tumor blood vessels may increase tissue hydrostatic pressure and so retard diffusion of soluble materials into tissues. Consequently, some areas of tumors may be richly supplied with oxygen and nutrients while other areas may go begging.

Mediators of Tumor Angiogenesis

Three main groups of factors may mediate tumor angiogenesis: (1) the family of VEGFs, (2) angiopoietins and (3) other agents that stimulate tumor-associated blood vessels to proliferate. These are assisted by a bevy of associated helpers including TGF-β (see below), several interleukins (IL-6, IL-8) and steroid sex hormones. The multiplicity—some might suggest redundancy—of stimulating and interacting factors has important implications for the efficacy of tumor therapies that preferentially target one or another.

VEGF

This term actually represents a family of related proteins, the best understood of which is VEGF-A, which has several isoforms. The family will be referred to collectively as VEGF, but the multiplicity of VEGFs should be borne in mind. VEGF is a major mediator of tumor angiogenesis and is made by the cells of most tumors. However, quantities of VEGF capable of stimulating tumor angiogenesis may also be produced by other cells, chiefly platelets and connective tissue cells. A related family of mediators, platelet-derived growth factors (PDGFs), has a similar spectrum of activities. In addition to stimulating new blood vessel formation, VEGF also enhances capillary permeability, promotes endothelial cell survival and mobilizes progenitor cells (e.g., from the bone marrow) to participate in angiogenesis.

VEGF Mechanisms of Action

As noted above, tumor cells produce HIF-1α when they sense insufficient oxygen. HIF-1α is a transcription factor that upregulates diverse genes, including the VEGFs. The family of VEGFs bind a family of receptors on endothelial cells, the most important of which is VEGFR2. VEGF–VEGFR2 interaction activates several signaling pathways (Fig. 5-29). Consequences of VEGFR2 activation include endothelial cell proliferation, protection from PCD, enhanced cell migration and increased vascular permeability. The latter function leads to leakage of such blood components as fibrinogen into the area. Once outside blood vessels, fibrinogen generates a fibrin matrix that facilitates endothelial cell migration and angiogenesis. VEGFR2-positive progenitors from the blood are also recruited to the site. Several members of the VEGF family also mediate proliferation of lymphatic vessels.

Angiopoietins

Of the several known angiopoietins, angiopoietin-2 contributes the most to tumor blood vessel formation. It acts principally to stabilize growing blood vessels and stimulate pericytes to surround the developing structures.

Other Angiogenic Factors

Other important stimuli include cytokines (e.g., IL-6), androgens and estrogens, as well as diverse growth factors. These

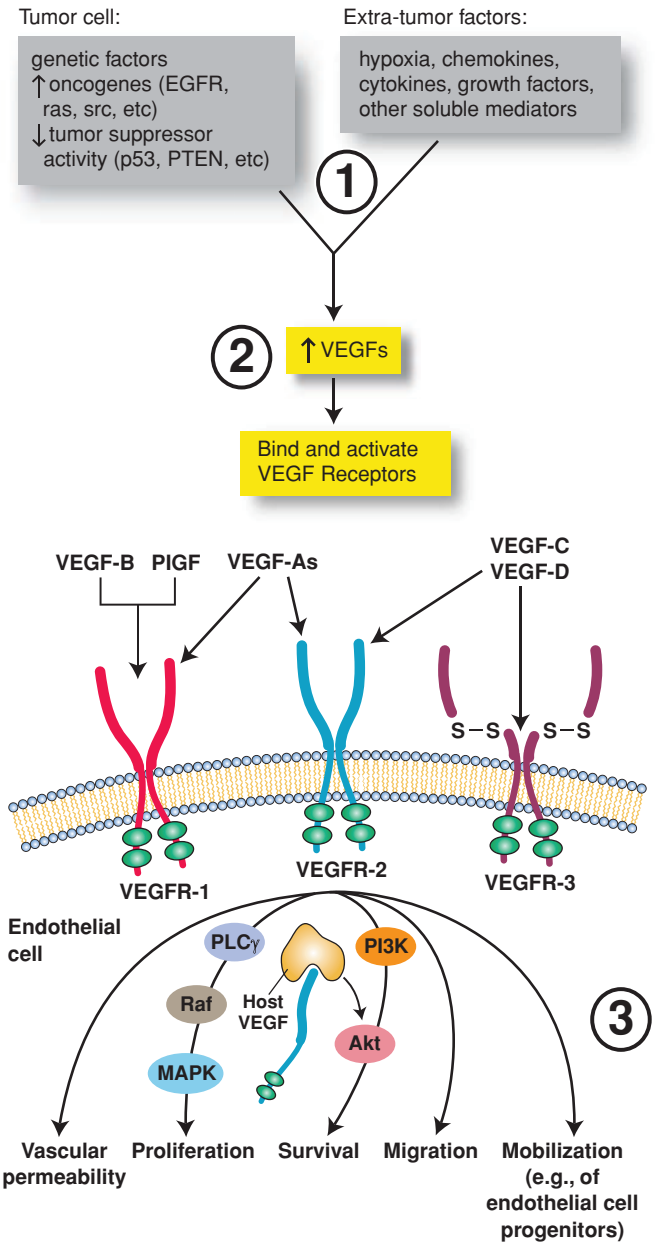


FIGURE 5-29. The vascular endothelial growth factor (VEGF) system and its effects. 1. Under the influence of factors generated by tumor cells (*left;* increased expression of certain oncogenes or decreased activity or tumor suppressors) or coming from other sources (tumor-related stroma, external environment, etc.), several VEGFs are produced. **2.** These bind the several VEGF receptors (VEGFRs), the principal of which is VEGFR-2. **3.** Downstream signaling from these receptors has diverse effects on vascular endothelium, including increasing vascular permeability, activating cell proliferation and survival mechanisms, inducing in-migration of endothelial cells and mobilizing progenitor cells to the area, to help form new blood vessels. PlGF = placental growth factor.

serve diverse functions, including eliciting VEGF production. Mutations in several important oncogenes, including *src, EGFR* and *ras,* may increase VEGF secretion by tumor cells, as may impairment of activities of certain tumor suppressor genes (see below).

The multiplicity of proangiogenic activities includes such factors as basic FGF (bFGF), PDGF, VEGF homologs and isoforms and others. These may augment blood vessel growth

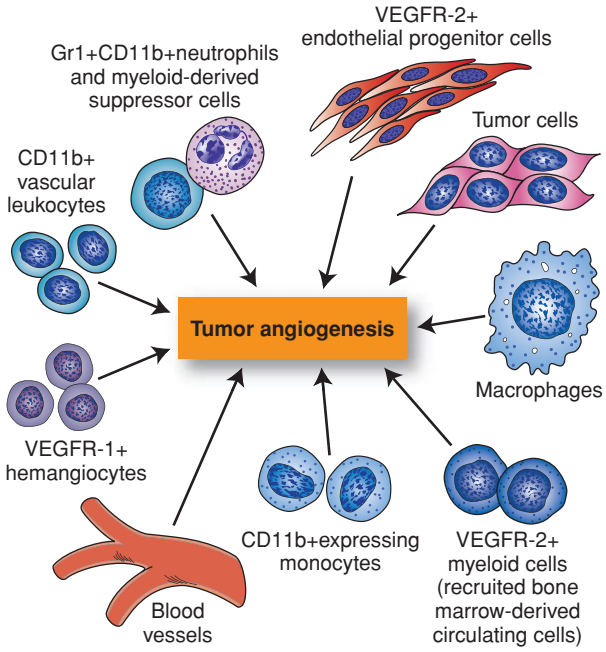


FIGURE 5-30. Diverse populations of bone marrow–derived cells that participate in angiogenesis. Circulating cells derived from bone marrow progenitors contribute to the development of tumor-related blood vessels. These include macrophages, early cells in the myeloid (neutrophil) series, neutrophils and myeloid-derived suppressor cells, endothelial progenitor cells and tumor cells themselves.

that is triggered by primary factors such as FGF2 or angiopoietin. This breadth of tumor angiogenic factors, combined with the likelihood that at least some tumor cells may differentiate into tumor blood vessels, frustrates attempts at effective tumor treatment by targeting individual VEGFs or VEGFRs.

Inflammatory Cytokines and Chemokines

Bone marrow–derived immune and inflammatory cells, including macrophages, neutrophils, natural killer cells, dendritic cells and myeloid precursor cells, all produce numerous soluble angiogenic factors and stimulators, and some may even differentiate into endothelial cells (Fig. 5-30). Equally important are tumor-associated stromal fibroblasts. The contributions of these cells to tumor blood vessel growth reflects the context of the tumor. In some settings, these cells assume a Dr. Jekyll–like antineoplastic phenotype and produce antitumor and antiangiogenic activities. In other settings, they become Mr. Hyde and generate proangiogenic and protumor microenvironments. As if this were not sufficiently complex, cells such as dendritic cells and myeloid-derived suppressor cells are capable of trans-differentiating into endothelial cells.

Invasion and Metastasis Are Multistep Events

The lethality of cancer resides in its ability to spread. Over 90% of patients who die of cancer succumb to metastatic disease. While we have accumulated considerable understanding of tumorigenesis, our appreciation of the basic principles of metastasis remains rudimentary. What is clear is that tumor spread is a multistep process, with each step potentially representing major genetic and epigenetic modifications in tumor cells and their behavior.

Malignant cells go through several steps to establish a metastasis (Fig. 5-31):

1. Invasion of the basement membrane underlying the tumor
2. Movement through extracellular matrix
3. Penetration of vascular or lymphatic channels
4. Survival within circulating blood or lymph
5. Homing to a new site and exiting from the circulation there
6. Establishment of a micrometastasis

A final step, growth of micrometastatic foci into sizeable tumor masses, culminates this progression and represents the ultimate lethality of almost all malignancies.

Cancer cells develop at a given location, comfortable in their native environments, owing in part to their interactions with tumor-associated matrix constituents and stromal and inflammatory cells (see below). The latter may either be present in the extracellular matrix or be recruited from the bone marrow. In order to metastasize, cancer cells must also establish a comparably commodious ecosystem at a distant site. This is no small undertaking.

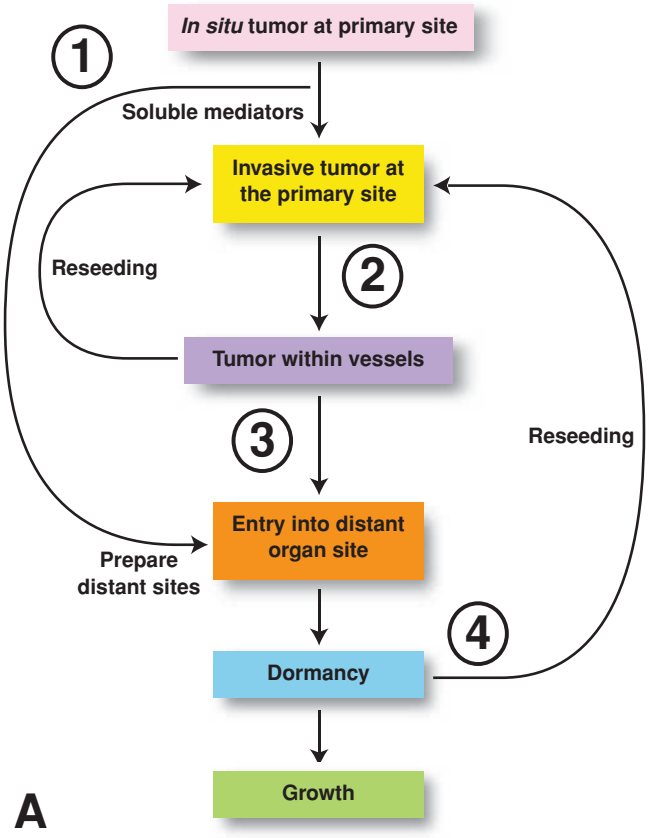
As the discussion below will demonstrate, some—perhaps most—of the traits necessary for tumors to metastasize are quite distinct from those needed for tumors to establish themselves at their sites of origin. It bears mention that it is not clear how the traits required for metastatic behavior are selected in primary tumors. While some traits needed for tumorigenesis may overlap the needs of metastases, other do not. Further, as one climbs the ladder of steps needed for metastasis, it is not known how cells that carry a phenotype that allows them to succeed at one rung are in a position to select for characteristics that facilitate success at the next rung.

The practical upshot of these philosophical ruminations is that *once a primary tumor has been removed, metastatic deposits are already in place.* The goal of subsequent therapy is to limit the growth of metastases that already exist.

Tumor cells start to disseminate early in oncogenesis. Many tumor cells enter the circulation daily (it is estimated to be 10^6 cells per gram of tumor per day). Taking out a gross primary tumor does not, therefore, "get it all out": micrometastases already exist.

Invasion and Tumor Cell Motility

For solid tumors, invasion requires that the previously stationary cell become motile. It must also become capable of disrupting and penetrating the underlying basement membrane, and then passing through the ECM. Orchestrating movement through the ECM requires proteases secreted by cancer cells and nonmalignant tumor-associated cells (see below). Cancer cells develop protrusions that contain an actin core and integrins. These projections, called **invadopodia,** express **matrix metalloproteinases (MMPs;** see below) and other proteases. Integrins in the ECM may not only act as mechanical anchors but also promote development of invadopodia. The invadopodia help to degrade the ECM and offer a guide to the perplexed cell in navigating its microenvironment via exploration of cell–cell and cell–matrix adhesions and by sensing chemoattractive molecules.



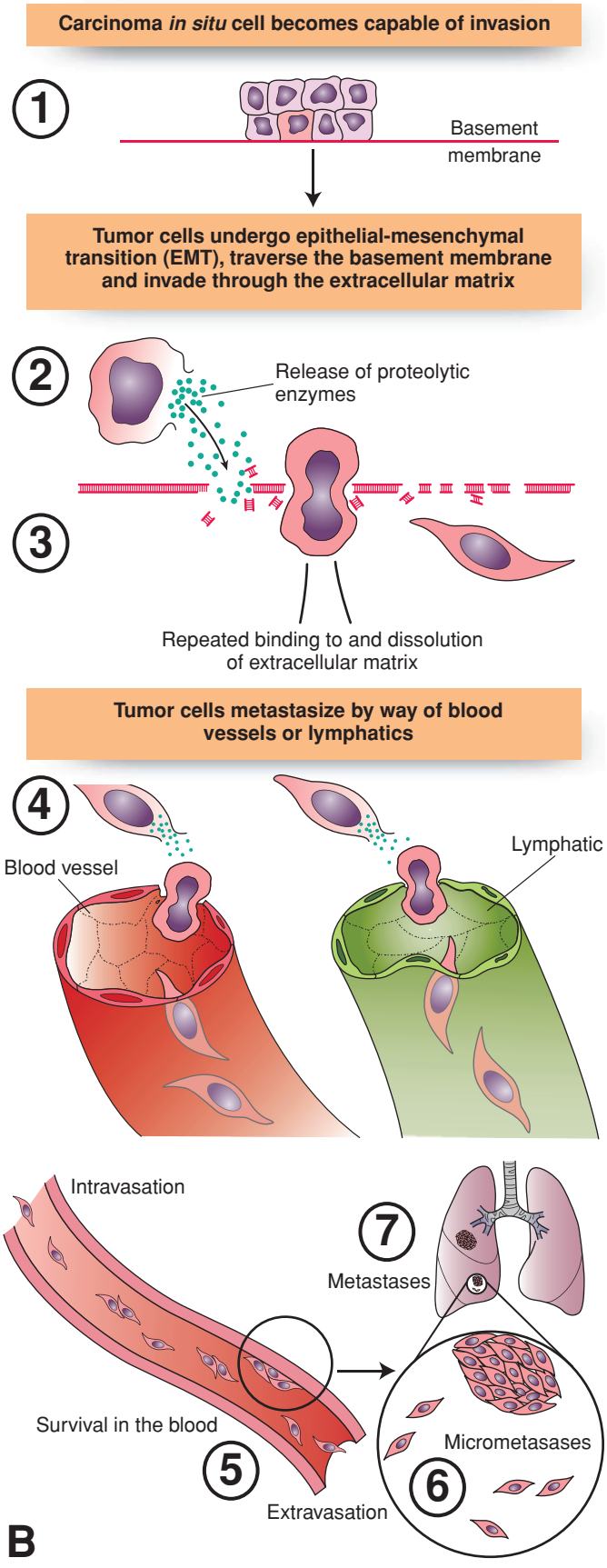


FIGURE 5-31. Mechanisms of tumor invasion and metastasis. The mechanism by which a malignant tumor initially penetrates a confining basement membrane and then invades the surrounding extracellular environment involves several steps. **1.** The tumor first acquires the ability to bind components of the extracellular matrix. These interactions are mediated by the expression of a number of adhesion molecules. **2.** The tumor undergoes epithelial–mesenchymal transition (EMT) and traverses the basement membrane. **3.** Proteolytic enzymes are then released from the tumor cells, and the extracellular matrix is degraded. **4.** After moving through the extracellular environment, the invading cancer penetrates blood vessels and lymphatics by the same mechanisms. **5.** After survival in blood vessels or lymphatics, the tumor exits the vascular system. **6.** It establishes micrometastases at the site where it leaves the vasculature. **7.** These micrometastases grow into gross masses of metastatic tumor.

Epithelial–Mesenchymal Transition

To escape the confines of the mucosa in which they originate, the epithelial cancer cells assume a phenotype that permits enhanced motility. The tumor cells, which are nonmotile and are encased in cell collectives via cell–cell tight junctions, disrupt these bonds and assume a new guise as single, nonpolarized mobile mesenchymal cells. This chameleon-like **epithelial–mesenchymal transition (EMT)** is reversible and temporary.

What Elicits EMT?

Many factors contribute to EMT (see below), but diverse stimuli probably can trigger this function. Among these, hypoxia is felt to be critical. Since the diffusion radii of glucose and oxygen are limited to 100–150 μ, tumors growing in situ (e.g., comedo-type breast intraductal carcinomas; see Chapter 25) may show central necrosis. Hypoxia induces HIF-1α (see above), which itself regulates many genes. Among these are the proteases, MMP1 and MMP2, and lysyl oxidase (LOX). (Other HIF-1α functions are discussed below.) MMP2 degrades basement membrane, and MMP1 and LOX help to digest ECM to clear a path for the tumor cell.

EMT also entails the activation of a series of transcription factors that promote a mesenchymal phenotype. These factors—colorfully named Slug, Snail, Twist and ZEB1—were once active in mediating cell mobility during embryogenesis. They are reactivated to make adult epithelial cells act like their mesenchymal embryonic forbearers. Snail and Twist downregulate expression of E-cadherin (see below), a glycoprotein that anchors epithelial cells to each other and suppresses motility. In addition, Twist and ZEB1 downregulate the antiproliferative proteins $p16^{INK4a}$ and $p21^{CIP1}$. Freed of their E-cadherin shackle, epithelial cells can then invade. Proteases clear the way.

Tumor-Associated Cells

Nonneoplastic cells associated with tumors constitute about half of all cells within tumor masses. They include macrophages, leukocytes, fibroblasts, vascular endothelial cells, neuronal cells and fat cells (Fig. 5-32). Many of these resided originally in the ECM, but others are of bone marrow origin and are recruited to the site of the expanding tumor. All of these nontumor cells can influence the behavior of the cancer, both at its site of origin and at locations of metastases.

The Contributions of Tumor Stroma

Stimulation of tumor cell invasiveness by nearby stromal elements plays an important role in the ability of cancer cells to breach the basement membrane and traverse underlying connective tissues. Tumors co-opt normal stromal cell functions, trigger inflammatory reactions and recruit additional cells to the area of the developing malignancy to further subvert anatomic and other barriers to invasion. Perversely, components of inflammatory and wound repair processes (see Chapters 2, 3 and 4) that protect against, for example, pathogens are then brought to bear to render the individual susceptible to cancer cell invasiveness. It is important to appreciate that *the players in inflammatory and wound healing that are observed in nearby tumors are orchestrated by the developing cancers themselves* (Fig. 5-33) *and should not be misconstrued as protecting the host:*

- **MMPs:** The MMPs are a family of endopeptidases that are normally regulated by tissue inhibitors of MMPs (TIMPs). MMPs are synthesized and secreted by normal cells during physiologic tissue remodeling, at which times the balance between MMPs and TIMPs is strictly regulated. By contrast, invasive and metastatic phenotypes of cancer cells are characterized by dysregulation of the MMP–TIMP balance.

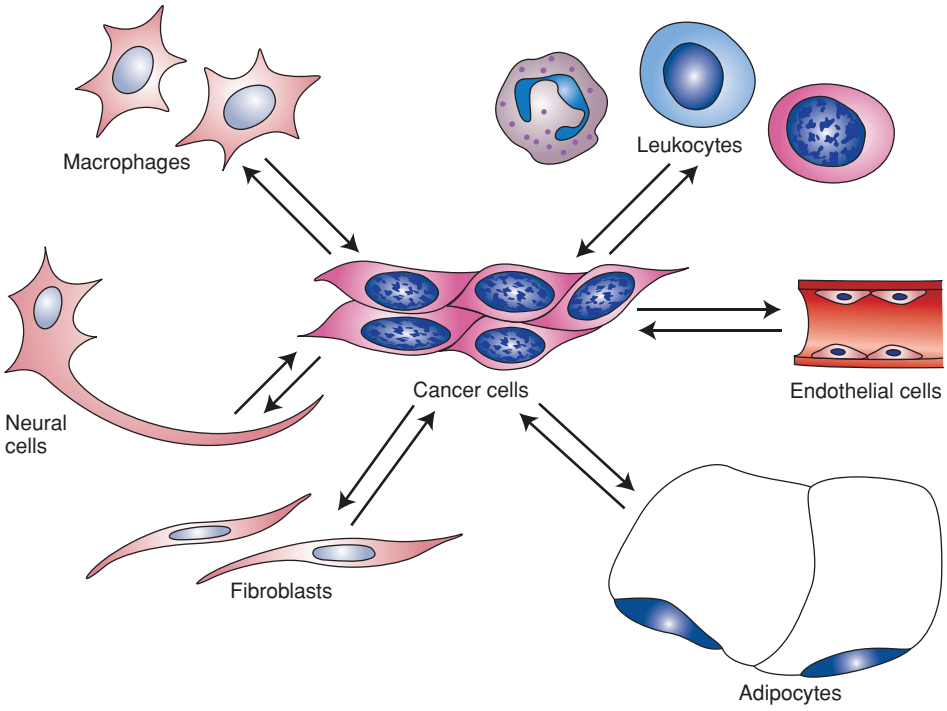


FIGURE 5-32. The cancer cell ecosystem. The developing tumor cells interact with the nonmalignant cells in their environment, via production of soluble and other mediators.

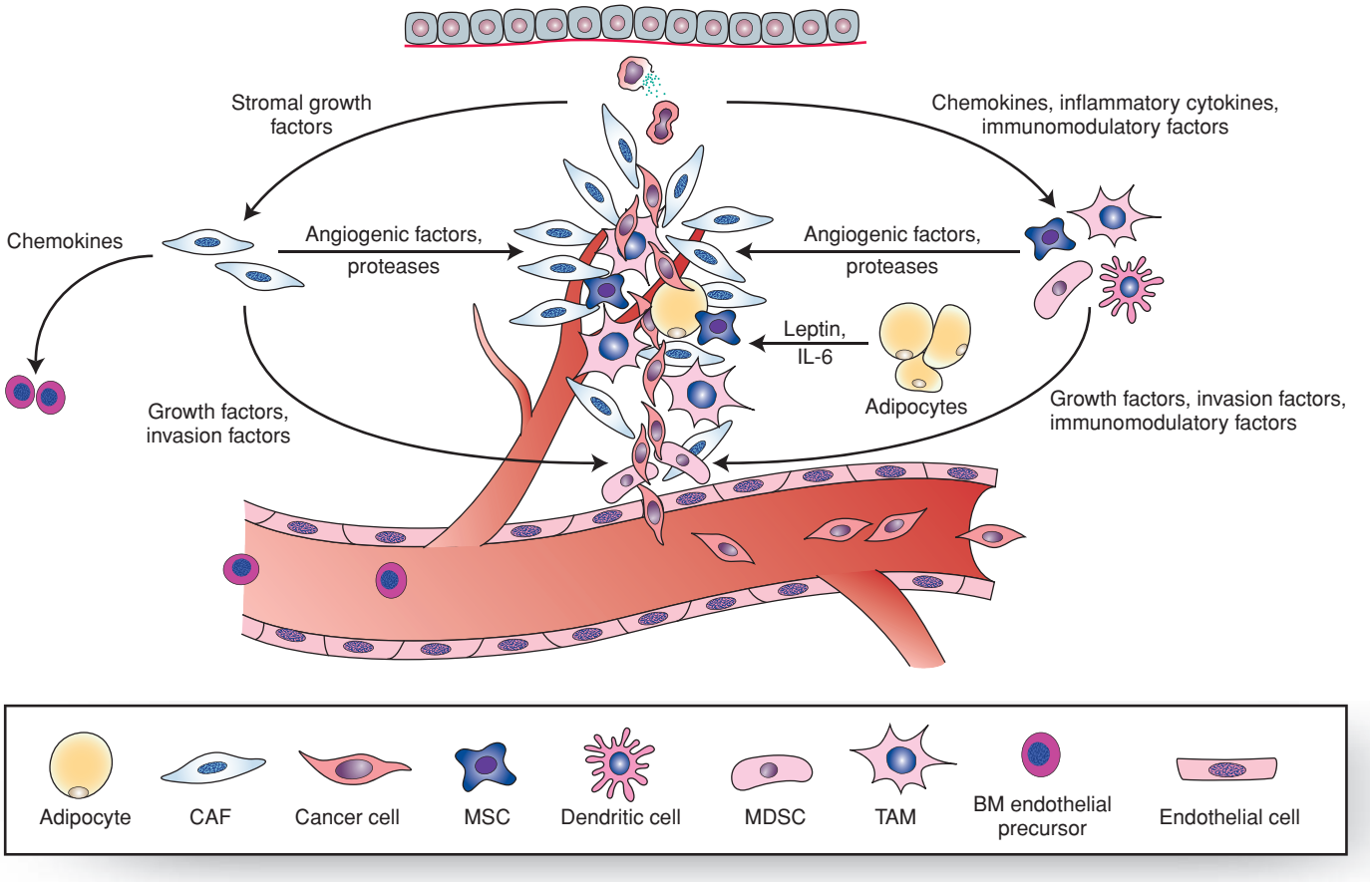


FIGURE 5-33. Tumor cell–stromal interactions involved in invasion and metastasis. Stroma adjacent to tumor is critical to the survival of tumor cells in place and to their dissemination. Such "cancerized stroma" contains bone marrow–derived elements (Fig. 5-30), including myeloid-derived suppressor cells (MDSCs), dendritic cells, tumor-associated macrophages (TAMs), fibroblasts, adipocytes and endothelial cells. Cytokines, chemokines and other mediators produced by tumor cells, as well as influences of tissue destruction and hypoxia, recruit TAMs, MDSCs, cancer-associated fibroblasts (CAFs) and mesenchymal stem cells (MSCs). MDSCs and TAMs are present at the invading tumor front—points where the basement membrane is being broken down and the tumor cells are infiltrating the stroma. These cells produce angiogenic factors, proteases and other factors that promote tumor invasion. CAFs produce similar facilitators and bring marrow–derived blood vessel precursor cells to generate new blood vessels.

In many tumors, invasiveness correlates directly with increased MMP expression. In many of these same tumors, TIMPs are decreased. MMPs in invading cancers may be produced by the tumor cells themselves, by surrounding stromal cells or both, depending on the particular neoplasm. MMPs secreted by stromal cells may be bound to integrins on the surface of the tumor cells, thus providing particularly high local concentrations of protease activity precisely where the tumor needs it in order to invade. Deregulated MMP activity permits cancer cells to enter and traverse the ECM.

Tumor cell motility is enhanced by upregulation of CXCR4 chemokine receptors in cancer cells at the invasion front. Interestingly, the invading cells induce nearby stromal cells to secrete SDF-1 (also called CXCL12), the ligand for this receptor.

- **Marrow-derived suppressor cells (MDSCs):** Tumors recruit these cells from the blood and bone marrow. They are at the edges of developing tumors and affect host responses to tumors (see below). MDSCs also secrete MMPs, which help to degrade basement membrane and ECM. They stimulate angiogenesis by secreting VEGF and PDGF.
- **Tumor-associated macrophages (TAMs):** These cells congregate at areas in which the basement membrane is breaking down. Like MDSCs, they secrete proteases, particularly urokinase plasminogen activator (uPA), which converts plasminogen to plasmin. The latter, in turn, helps digest type IV collagen in basement membranes. TAMs also produce cathepsin proteases in response to IL-4 made by tumor cells, further augmenting tumor invasiveness.
- **Carcinoma-associated fibroblasts (CAFs):** Like TAMs, CAFs produce proteases that facilitate tumor cell invasion. They also synthesize growth factors and angiogenic factors, and recruit precursor cells from the marrow, to become vascular endothelium.
- **Adipocytes:** The stroma in which many tumors arise contains adipocytes. Cross-talk between these cells and tumor cells facilitates early stromal invasion by the malignant cells. Fat cells near tumors often express a particular MMP that assists cancer cells in traversing surrounding connective tissues. Adipocyte-derived IL-6 stimulates tumor cell invasiveness. Leptin produced by adipocytes (see Chapter 13) induces macrophages to secrete proinflammatory cytokines, which, in turn, promote invasion and metastasis.

- **Lymphocytes:** T cells may facilitate tumor invasiveness via TAMs. $CD4^+$ lymphocyte-activated TAMs can elicit EGFR-related activation in some types of cancers.

The combination of these and other elements by developing tumors is sometimes called cancerized stroma. It should be noted that many interactions between invading cancer cells and their stromal accessories in crime constitute a positive feedback loop: tumors recruit and activate stromal cells, which repay the favor by magnifying the tumor's invasive tendencies.

Following their invasion of surrounding tissue, malignant cells may spread to distant sites by a process that includes a number of steps.

Invasion of the Circulation

Malignant cells penetrate lymphatic or vascular channels. Solitary cells that have already undergone EMT represent only a small component of the entire primary tumor. As lone travelers, however, these cells move more rapidly than do cell clusters and **intravasate** (penetrate) into blood vessels, which provide a route for migration to faraway body sites. Tumor-associated capillaries stimulated by tumor-produced VEGF and related angiogenic factors (see above) are not completely invested by pericytes. Tumor blood vessels are constantly being remodeled, and so are less well formed than their physiologic cousins. The angiogenesis initiator, VEGF, increases vascular permeability. In addition, TAMs produce EGF and tumor cells secrete colony stimulating factor-1 (CSF-1), both enhancing intravasation. MMP1 and MMP2, as well as other tumor and stromal cell products, increase the leakiness of tumor-induced blood vessels and facilitate their invasion by cancer cells.

By contrast, compact cell collections preferentially transfer to the lymph nodes, where they generally remain in place. Collective cell migration to lymph nodes appears to be independent of spread through blood vessels, and each may be the preferred mode of dissemination for specific tumors. However, metastases may in turn metastasize and single cells may exfiltrate and disseminate widely via the bloodstream. This phenomenon is the basis of currently used assays to quantitate single tumor cells in the peripheral blood, the results of which are used both as prognostic indicators and to guide choice of chemotherapy.

In lymph nodes, communications between lymphatics and venous tributaries allow the cells access to the systemic circulation. Most tumor cells do not survive their journey in the bloodstream, and a tiny percentage remain to establish a new colony.

Survival in the Vascular System

Once in the circulation, tumor cells have two main tasks: to survive and to find a new home. **Circulating tumor cells (CTCs)** are unlikely to spend long in the vascular system. Their size (20–30 μ on average) far exceeds the diameter of pulmonary capillaries (about 8 μ). A single passage through the pulmonary circulation should filter the vast majority from the blood.

To remain viable when detached from their native ECM, CTCs must be able to avoid anoikis (see Chapter 1), a form of apoptosis that is triggered by loss of the ECM anchors that constitute a cell's native environment. In some cases, this is achieved by activating TrkB, a suppressor of anoikis.

Preparing the Soil

It has been known for many years that metastasis is not random, that is, that certain types of tumors specifically tend to colonize particular organs. The molecular basis for some of these patterns of cancer spread is beginning to be understood.

There are certain general mechanisms that, even before a tumor begins to disseminate, help to prepare distant sites in ways that make the wandering tumor comfortable. Secretion of angiogenesis factors, VEGF and its cousin, placental growth factor (PlGF), with variable cocktails of other growth factors generated by tumor cells and their stroma causes hematopoietic progenitor myeloid cells (HPCs) to leave the bone marrow. These cells home to specific organs and set up a microenvironment there that accommodates the needs of the soon-to-arrive metastatic tumor cells. These so-called premetastatic niches undergo repeated remodeling by cytokines and enzymes produced by the marrow-derived cells. Inflammatory cells recruited from organ stroma and from the blood produce cytokines and enzymes (such as MMPs and LOX) that make the distant environment even more homey for metastatic colonization. The result is like a prefabricated house, just waiting for its future occupants.

Tumor Cell Arrest in the Circulation

While circulating, tumor cells associate with diverse formed constituents in the blood, including neutrophils, immune cells and platelets. These blood cells protect tumor cells from shear, immune and other stresses in the circulation. Such associations are mediated by adhesion molecules such as integrins, selectins (see Chapter 2) and a glycoprotein (CD44) at the surface of tumor cells. Tumor cells produce a factor that activates platelets via production of thrombin, and including fibrin and von Willebrand factor as bridging proteins. The binding of tumor cells to platelets requires P-selectin and platelet integrins, which recognize tumor cell CD44 via von Willebrand factor and fibrin. Similar interactions mediate tumor cell recognition and tethering to endothelial cells and to endothelial-bound leukocytes (Figs. 5-34A,B).

Leaving the Circulation

After arrest in the bloodstream, tumor cells and their fellow travelers mediate the process of extravasation. This is a bit more complex than its obverse—intravasation—earlier in the cancer cells' odyssey, because blood vessels at the tumor cells' destination are well constructed and anatomically complete, rather than new, poorly formed tumor-induced blood vessels. Extravasation appears to be relatively organ specific, so that the factors that are involved in extravasation of one tumor type into the lung, for example, differ from those that help the same or other tumor types exit the circulation elsewhere.

With that caveat in mind, the ability of tumor cells to enter the lung has been ascribed to a small number of proteins that increase vascular permeability, degrade tight junctions between endothelial cells and promote tumor cell escape from the circulation. These include several MMPs and VEGFs, cyclooxygenase-2 (COX-2), the EGFR ligand epiregulin and a protein called **angiopoietin-like 4 (Angptl4)**. This latter cytokine is particularly important for dissolving tight junctions between endothelial cells, thus facilitating tumor cell migration through vascular walls.

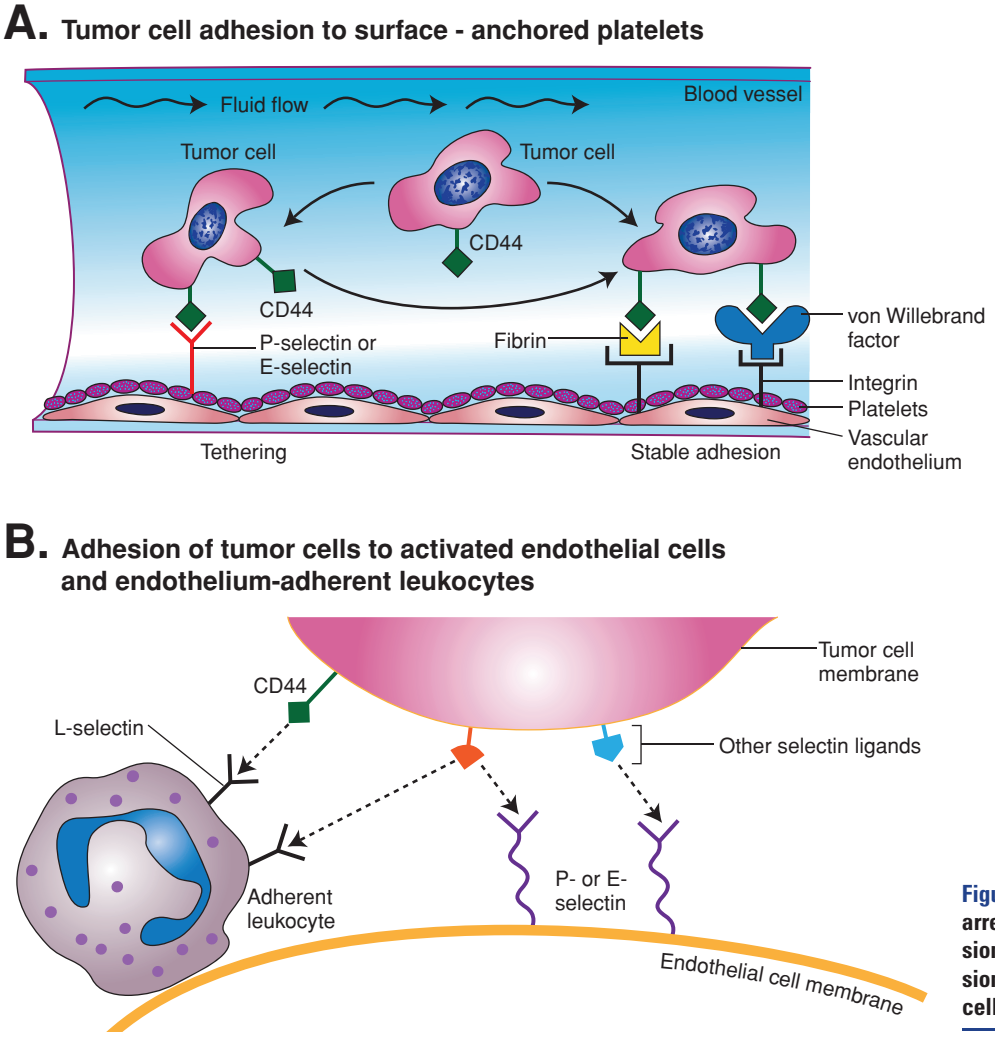


Figure 5-34. Mechanisms of tumor cell arrest in the circulation. A. Tumor cell adhesion to surface-anchored platelets. B. Adhesion of tumor cells to activated endothelial cells and endothelium-adherent leukocytes.

Establishing Micrometastases

The process of establishing colonies distant from primary tumors requires complex synchronization of the biochemistry, matrix and cellular composition of the soon-to-be metastatic site with the circulating tumor cells. Micrometastases may survive but never grow. Or they may not survive. Prospective micrometastatic foci must cope with issues relating to the suitability of the ECM, blood supply and stromal cells for tumor growth. More often than not, individual tumor cells or small clusters of tumor cells either become dormant in distant sites (see below) or die. For example, extravasated carcinoma cells may be unable to connect adequately with adhesion processes at foreign locations. Among the factors that enable micrometastatic foci to persist is the recruitment of bone marrow–derived HPCs to help guide tumor cells and stimulate their growth. SDF-1, derived from such cells, binds its receptor, CXCR4, on the tumor cells and stimulates proliferation. Active signaling through Src (see above) is important in ensuring continued survival of nascent micrometastases.

The Inefficiency of Metastasis

Tumor cells circulate in large numbers in cancer patients. It has been estimated that for every gram of tumor mass, 10^6 tumor cells are released into the circulation daily. CTCs can be detected even before the primary tumor becomes pathologically invasive. Furthermore, patients who inadvertently developed very high CTC levels because of a peritoneal-vascular shunt developed metastatic disease only infrequently.

What accounts for the inefficiency of metastasis is not clear. Mechanisms that suppress it (see below), both inherent to and outside tumor cells, undoubtedly contribute. Until we better understand these mechanisms and how tumors evade them, our effectiveness in treating metastatic solid tumors is likely to be limited.

Tumor Dormancy and Evolution of Micrometastases

What happens to all those CTCs that never become gross metastatic deposits? Some surely die, whether by apoptosis, anoikis, immune elimination or some other mechanism. Many, though, establish a dormant state. It is well known that metastases may become clinically apparent many years, even decades, after a primary tumor mass is removed. This is particularly the case in breast cancers and malignant melanomas. Such deposits were clearly derived from the original tumor yet remained below the clinical radar for long periods of time.

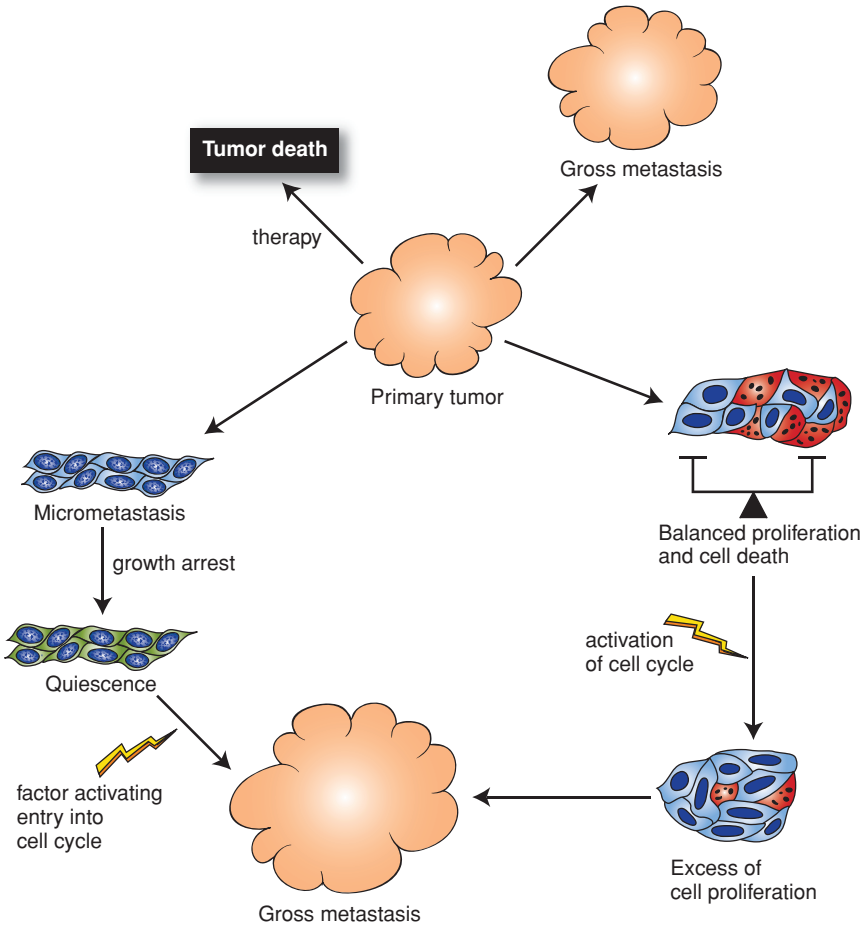


FIGURE 5-35. The fate of foci of cancer micrometastases. A primary cancer may be killed by therapies, such as radiation or chemotherapy, or it may be surgically removed. The tumor may produce a grossly evident metastasis. A number of factors may cause minute, clinically inapparent, metastatic foci of tumor cells to enter G_0 (*green*), but may be reactivated to enter the cell cycle (*blue*) and form a clinically detectable metastasis. Micrometastasis may also entail a balance between cell proliferation (*blue*) and cell death (*red*). If this equilibrium is disturbed in favor of tumor cell proliferation, the result may be a grossly evident mass of metastatic tumor.

It is also clear that some primary tumors may exist for many years before they are detected clinically. The presence of cancers, whether primary or metastatic, that do not enlarge to the point of being clinically detectable is **tumor dormancy**.

Growth of both the primary tumor and metastases is not necessarily exponential. Rather, it is often interrupted by quiescent intervals. As a result, the time needed for a tumor to grow into a clinically detectable mass is highly variable, likely reflecting fluctuations in stimulating or permissive signals on the one hand, the actions of inhibitory factors on the other or both.

Although tumor dormancy is a well-established observation, the mechanisms underlying this phenomenon are poorly understood. Factors that have been implicated in tumor dormancy and in escape from somnolence include angiogenesis, immune surveillance, apoptosis after oncogene inactivation (in cells that are oncogene addicted; see above), local processes such as inflammation, activation of cancer stem cells and adhesion molecules such as integrins. Sometimes, dormant tumor foci may represent a highly dynamic state—an equilibrium between cell proliferation and cell loss. At other times, the tumor cells are hypnotized into a somnolent state, in which they remain in the G_0 phase of the cell cycle (Fig. 5-35).

There are many **metastasis suppressor genes** that prevent various segments of the metastatic pathway. Thus, tumor dormancy has many faces, as reflected by the panoply of pathways by which tumor cells may be blocked from propagating and may be subsequently awakened by a still unrecognized Prince Charming (Fig. 5-35).

Currently, resection of many tumors in patients without evidence of distant metastases is often followed by adjuvant therapies that are directed against undetected micrometastases. Such treatments are usually of short duration and are often aimed at rapidly proliferating tumor cells. If metastases are dormant, these types of therapies may not affect somnolent tumor foci. Future cancer therapies may need to be able to exploit the characteristics of tumor dormancy (e.g., by altering the equilibrium that sustains dormancy in favor of cell death or by forcing dormant foci into more active cycling to facilitate their targeting by more conventional chemotherapy).

TUMOR SUPPRESSORS

Tumor suppressors are a very large and diverse group of cellular functions, carried out via many different pathways and mediators. Suppressors exist for all the cancer attributes mentioned above (immortalization, evading PCD, etc.) and those companion tumor characteristics (genomic instability, altered metabolism, etc.) described below. This section highlights how tumor suppression works and the nature of many different molecules, and the diverse types of molecules, that carry the burden of these functions. It is organized according to the tumor attributes listed above and illustrates how some processes counteract those attributes.

Tumor suppression is an amalgam of processes. Some of these processes are inherent in particular cellular molecules (e.g., cell cycle regulation and Rb, or the intrinsic pathway of apoptosis and Bax). It is tempting to confuse the function with the mediators of that function, a logical jump that is made all the time. But, just like that extra scoop of ice cream, this temptation should be resisted, and the student would do well to remember that tumor suppression is defined by function, not by structure. One tumor suppressor function may be executed by several different molecules, and any one molecule may have multiple functions.

There are almost infinite variations on the themes of tumor suppression. Many tumor suppressors only inhibit development and spread of some types of tumors. Still others (e.g., WT1) are tumor suppressors in some circumstances but act as oncogenes in others. Some molecules may execute their duties in some settings, but not always. Further, some mutated tumor suppressors (like p53) not only fail to inhibit tumor development but also may actively facilitate it and inactivate other tumor suppressors. This fluidity should be kept in mind. We present tumor suppressors as static structures (e.g., PTEN, VHL, etc.) as a means to aid in understanding how they work, the processes they antagonize and what goes awry when they are mutated or inactivated.

The student should be mindful of this complexity, as it may come in handy when dealing with one of nature's most vexing principles, the law of unintended consequences. That is, it may help in appreciating that, for example, therapeutic manipulations conceived with ironclad theoretical logic so as to produce a particular result may yield consequences quite different from expectations.

Tumor Suppressor Mechanisms Protect from Oncogenesis by Inhibiting Every Tumor Attribute

Cells possess complex mechanisms that guard against tumor development. The molecular guardians responsible for this protection are called tumor suppressors, and the genes that encode them, tumor suppressor genes (TSGs). Major activities of tumor suppressors are illustrated in Fig. 5-36. If an incipient tumor is to develop successfully, it must generally inactivate one or more TSGs or their products.

There are many TSGs, with diverse targets, functions and mechanisms of action. Some tumor suppressors have multiple functions and targets. Some are not proteins, but may be untranslated RNA species (see below). And some are part-time tumor suppressors and part-time oncogenes. In light of this considerable complexity, we focus here on key concepts

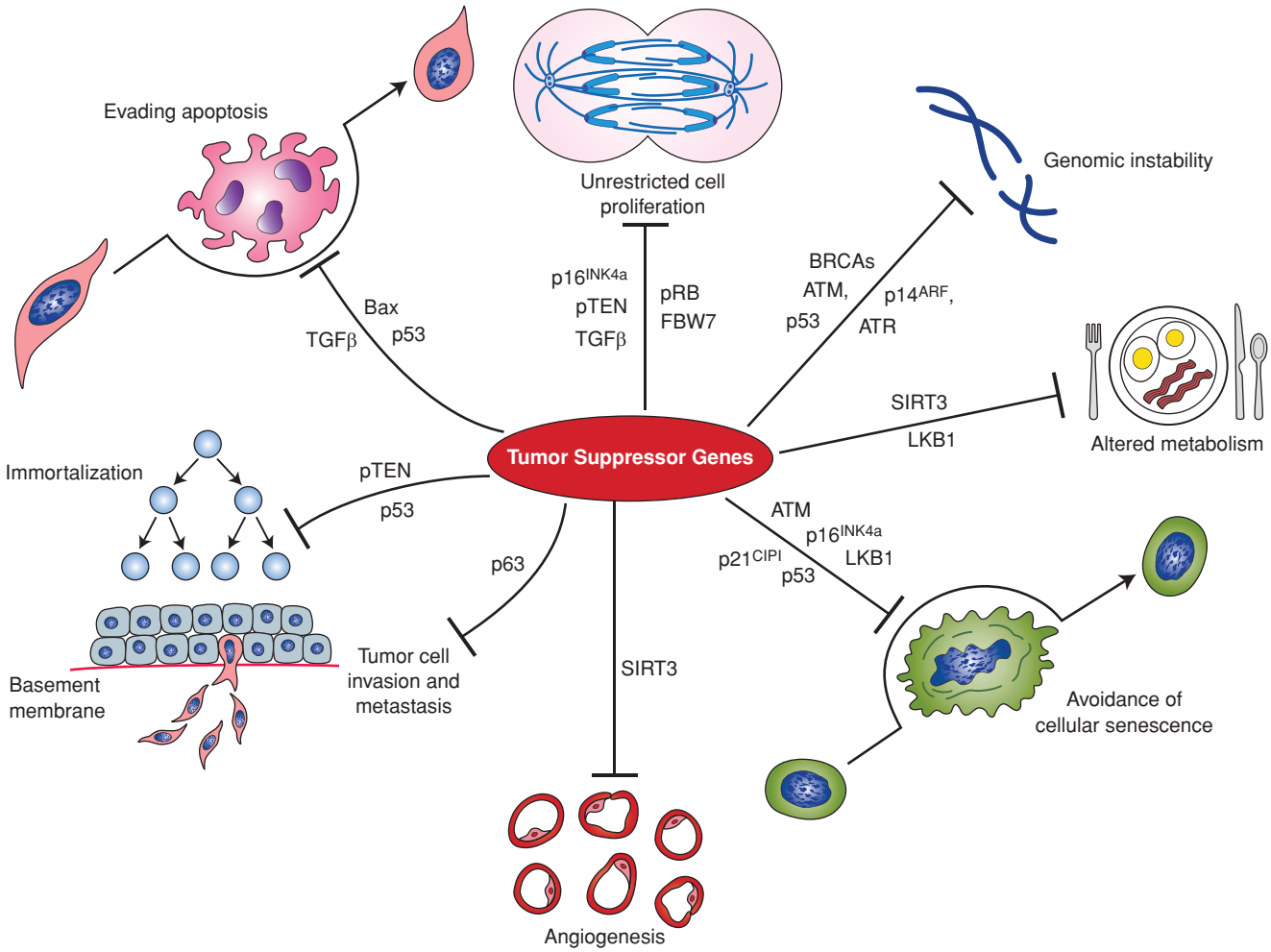


FIGURE 5-36. Tumor-related activities that are targeted by important tumor suppressor genes and representative tumor suppressors involved. The major hallmarks of malignant tumors each is antagonized by multiple tumor suppressor gene products. Those hallmarks, and the tumor suppressor activities that work against them, are illustrated here.

in understanding how important aspects of tumor suppression work, the ways in which tumor suppressors are circumvented and how tumors arise once TSG activities go awry. The protective activities of tumor suppressors are illustrated below for each of the major cancer attributes (see above).

Tumor Suppressors Regulate Cellular Proliferation

In normal settings, there are several important mechanisms that limit cell division. As noted previously, interactions between extracellular molecules and their cell membrane receptors trigger intracellular signaling via multiple pathways (Fig. 5-37). These include activation of PI-3 kinase (PI-3K; see above), which phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to produce phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 then activates downstream signaling via Akt and mTOR to drive cell division. A key tumor suppressor protein, PTEN, dephosphorylates PIP3, and so impedes cell activation initiated by mitogenic extracellular signaling (Fig. 5-38). PTEN is a major tumor suppressor, second only to p53 in the frequency with which loss of a tumor suppressor function is observed in human cancers.

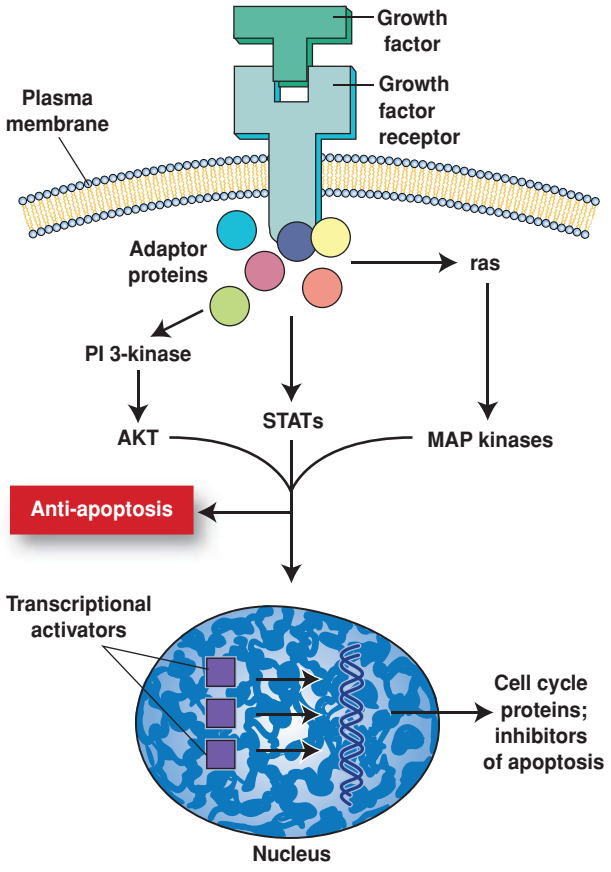


FIGURE 5-37. Signaling pathways controlling proliferation and apoptosis. The activation of growth factor receptors by their ligands causes the binding of adaptor proteins and the activation of a series of intracellular signaling molecules, leading to transcriptional activation, the induction of cell cycle proteins and inhibition of apoptosis. Key targets include *ras*, mitogen-activated protein (MAP) kinases, signal transducer and activator transcription factors (STATs), phosphatidylinositol-3-kinase (PI3-kinase) and the serine/threonine kinase Akt.

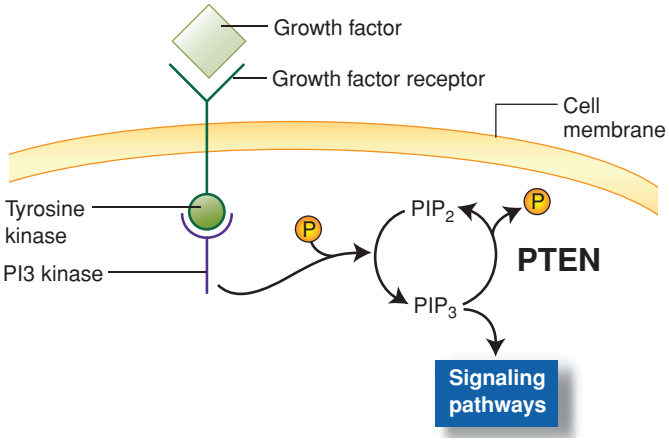


FIGURE 5-38. Signaling function of PTEN. Normal binding of a growth factor to its receptor leads to phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) to produce the important signaling molecule phosphatidylinositol-3,4,5-trisphosphate (PIP3). The level of PIP3 is regulated by its dephosphorylation by PTEN.

Many tumor suppressors act downstream from receptor–ligand interactions. Key tumor suppressor targets include the various transitions in the cell cycle (see above) and activation/inactivation of gene transcription. Thus, pRb blocks cell cycle transit, unless it is hyperphosphorylated, to release the E2F transcription factor that drives cell division. The enzymes that phosphorylate pRb (CDKs 2, 4 and 6, complexed with various cyclins; see above) are inhibited by the tumor suppressors $p16^{INK4a}$ and $p21^{WAF1}$. Once pRb is phosphorylated, however, it may be dephosphorylated. Thus (see above), pRb can be dephosphorylated to restore its ability to inhibit E2F.

E2F-mediated transcription of many genes that drive cell division (e.g., *c-myc*) is powerfully inhibited by TGF-β. TGF-β binds its receptor to activate a series of intermediary signaling molecules called Smads (Fig. 5-39A). Smad4 is a key effector of TGF-β–induced transcriptional activity. It first blocks transcription of the *c-myc* proto-oncogene, thus inhibiting cell cycle transit and allowing Smad4 to upregulate expression of genes that block cell division, $p16^{INK4a}$ and $p21^{WAF1}$ (Fig. 5-39B). TGF-β signaling is among the most potent endogenous mechanisms that inhibit cell division and is commonly mutated in human cancers.

Tumor suppressors also inhibit cell division at stages that follow transcriptional activation or repression. Thus, TGF-β signaling activates molecules that impede translation of mRNAs for proteins that drive cell cycle progression. Another tumor suppressor, FBW7, is part of a ubiquitin ligase complex that eliminates many proteins that drive cell division, such as Myc, cyclin E and Jun.

Many other tumor suppressors, far too numerous to mention, also regulate cell division. The above descriptions illustrate the diversity of mechanisms that protect the organism from runaway cell proliferation.

Programmed Cell Death Destroys Cells at the Cusp of Becoming Dangerous

The several signaling networks that culminate in cell death have been described in Chapter 1. They are all relevant to

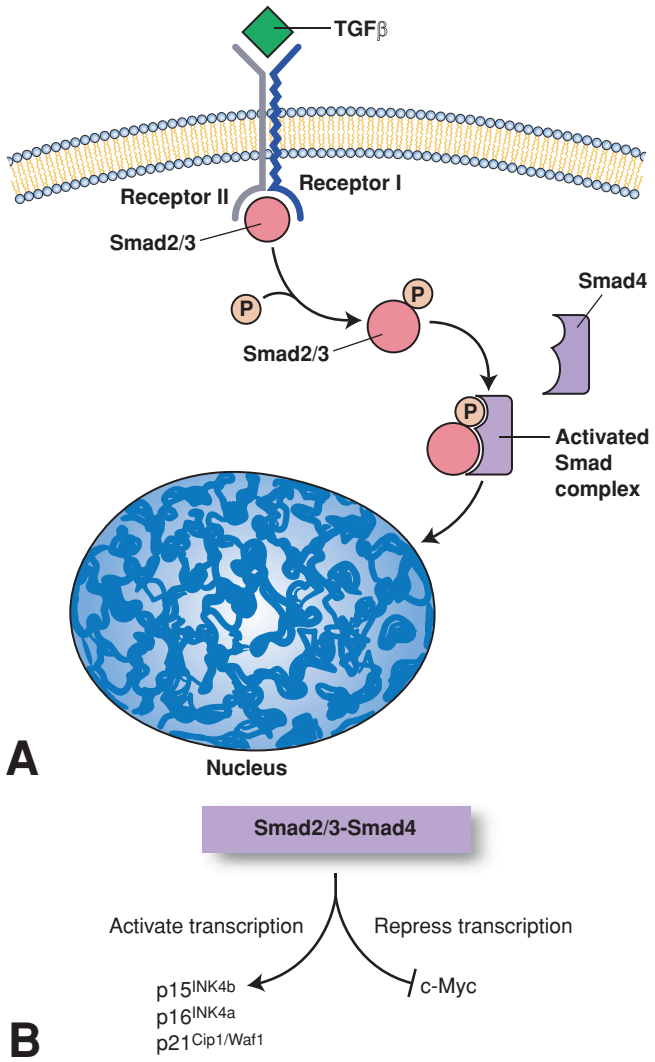


FIGURE 5-39. Transforming growth factor-β (TGF-β) as a tumor suppressor. A. Signaling. TGF-β binds its heteromeric receptor to phosphorylate and so activates Smads 2 and 3. These bind Smad4, to form an activated Smad complex that translocates to the nucleus to mediate transcriptional activation and repression. **B. Consequences.** The Smad2/3–Smad4 complex activates transcription of cell cycle suppressors, as shown, and represses transcription of the proliferation activator *c-Myc*.

protecting from tumor development, but the most critical of these is the PCD pathway that is activated by altered DNA structure. This pathway, in which there are several key participants—including ATM and ATR (see above) and, most critically, p53—is illustrated in Figs. 5-17 and 5-18.

The p53 tumor suppressor is a principal mediator of growth arrest, senescence and apoptosis (Figs. 5-17 and 5-18). In response to DNA damage, oncogenic activation of other proteins and other stresses (e.g., hypoxia), p53 levels rise and prevent cells from entering the S phase of the cell cycle, thus allowing time for DNA repair to take place. p53 thus acts as a "guardian of the genome" by restricting uncontrolled cellular proliferation under circumstances in which cells with abnormal DNA might propagate.

Acquired mismatches in DNA bases are detected by ATM if they occur in resting cells damaged by, for example, radiation or by ATR if they occur during DNA replication (see above). These proteins then activate one of two kinases, Chk2 or Chk1, respectively. The latter phosphorylates p53 (see above and Fig. 5-17), causing it to dissociate from its inhibitor, MDM2, and activating the p53 damage response.

p53 protein is a transcription factor that promotes expression of other genes involved in controlling cell cycle progression and apoptosis. DNA damage and other stresses (e.g., hypoxia) upregulate the expression of *p53*, which in turn enhances the synthesis of CKIs. The latter inactivates cyclin/CDK complexes, thus leading to cell arrest at the G_1/S checkpoint. Cells arrested at this checkpoint may either repair the DNA damage and then reenter the cycle, or undergo apoptosis. The stimulation of gene transcription by p53 results in the synthesis of proteins (CIP1, GADD45) (Fig. 5-18B) that enhance DNA repair by binding to proliferating cell nuclear antigen (PCNA; see above). Thus, *upregulation of p53 as a tumor suppressor has two important and related consequences: arrest of cell cycle progression and promotion of DNA repair.*

If it is not possible to return the cell's DNA to its correct sequence, p53 may then trigger cell death. It may do this in several ways (see Chapter 1). Largely, p53 induces apoptosis by activating the intrinsic apoptosis pathway. It does this in the following ways:

- As a transcription factor, it increases production of proapoptotic proteins (e.g., Bad, Bax, PUMA and others) and represses transcription of prosurvival proteins (e.g., Bcl-2, Bcl-xL, Mcl-1).
- It may directly activate cytosolic Bax, which in turn moves into mitochondria and triggers release of cytochrome C (CytC).
- p53 may act as a BH3-only (see Chapter 1), proapoptotic Bcl-2 family member by directly binding to Mcl-1, therefore freeing Bak to release CytC and other proapoptotic mitochondrial proteins.

By whatever means p53 activates apoptosis, the cell death program is executed by caspases, especially caspases-3, -6 and -7 (see Chapter 1).

The issue of apoptosis and protection from cancer is further complicated by the phenomenon of **oncogene-mediated apoptosis**. Myc transcription factor drives cell proliferation. However, activated Myc can be a blessing in disguise. It also induces a default apoptosis pathway. That is, deregulated production of Myc promotes cell proliferation but is usually balanced by increased apoptosis. Myc-induced apoptosis acts as a "molecular safety valve" that blocks cancer development. If Myc-stimulated tumor development is to occur, cells producing Myc at high levels must also overcome PCD-inducing mechanisms by overexpressing Bcl-2 or other antiapoptotic proteins.

Tumor Suppressors and Senescence

No single paradigm explains all of OIS. The centrality of DNA damage response (DDR) via Rb and p53 is generally accepted, but senescence entails complex signaling (Fig. 5-27), and perturbation of any member of this could facilitate development of malignancy.

As described above, ongoing telomere shortening in normal cells eventually leads to senescence. Tumor suppressor activities that elicit senescence are critical defenses against oncogenesis. They include components of the DDR system, such as ATM, ATR, Chk 1 and 2, the cell cycle regulators p53 and Rb and many others.

Inhibitors of Tumor Angiogenesis Limit Tumor Growth

There are many potent endogenous suppressors of tumor-related blood vessel growth:

- **VHL:** This protein is part of a ubiquitin ligase that targets transcription factors, called HIFs (see Chapter 1), for degradation. Inactivation of the *VHL* gene leads to a defect in Ub conjugation, which leads to increased HIF-1α (see above), an angiogenic factor that activates transcription of genes important in cellular responses to low oxygen environments. These include those that (1) increase cellular intake of glucose for anaerobic glycolysis, (2) stimulate angiogenesis (VEGF; see Chapter 16) and (3) activate several critical growth factors.

  The carcinogenicity associated with the inactivation of VHL is caused in large part by the action of HIF-1α in promoting tumor growth. Interestingly, similar activation of HIF-1α occurs in the often oxygen-starved cores of many tumors, even in the absence of *VHL* mutation. In those settings, HIF-1α degradation is impaired by decreased activity of a cofactor for the ubiquitination reaction.

  The normal VHL protein has additional tumor suppressor activities independent of HIF-1α. These include (1) promoting apoptosis, (2) increasing cellular immobilization by adherence to matrix proteins and (3) repressing certain cell activation responses.
- **NOTCH:** Although it is an important stimulator of embryonic blood vessel development, the NOTCH family of endothelial cell receptors, together with their cognate cell surface–bound ligands (especially DLL4), inhibits tumor angiogenesis. In fact, VEGF stimulation elicits DLL4 production as a negative feedback mechanism. Its conversion to angiogenesis inhibitor in postembryonic life notwithstanding, the NOTCH/DLL4 system is thought to represent a mechanism by which tumors outwit VEGF-targeted antiangiogenic therapies.
- **ECM and other angiogenesis inhibitors:** ECM constituents and clotting factors, and their breakdown products, all suppress tumor angiogenesis. **Thrombospondin** (TSP-1), derived from a large ECM glycoprotein, is a powerful inhibitor of blood vessel formation. **Angiostatin,** a breakdown product of plasminogen, and numerous fragments of ECM constituents (endostatin, inhibin and many others) restrain tumor-related blood vessel growth as well.
- **p53:** While p53 is not known to interfere with tumor angiogenesis itself, it upregulates TSP-1 expression, as noted above, and so has a strong antitumor angiogenic function. TSP-1 inhibition of tumor angiogenesis is a casualty of loss of p53.
- **SIRT:** Sirtuin deacetylases are important in stress responses and longevity. One of the sirtuins, SIRT3, increases the mitochondrial antioxidant, manganese superoxide dismutase (MnSOD; see Chapter 1). As a result, mitochondria produce less reactive oxygen species (ROS), causing decreased HIF-1 activity and thus less angiogenesis.

For Each Step of Invasion and Metastasis, There Are Antagonists That Hinder the Ability of Tumors to Spread

Just as the body arrays its defenses to prevent cancers from arising, it has developed mechanisms to impede every step of the process of invasion and metastasis. Metastasis inhibitors are conceptually distinct from tumor suppressors. To qualify as a metastasis suppressor, a molecule must impede one of the invasion- or metastasis-related processes without necessarily affecting growth and survival of the primary lesion. To date, about 30 metastasis suppressor proteins are known, plus an increasing family of miRNAs that show metastasis suppressor activity. Some suppressors act at multiple steps, while others are known to act at only one. Also, some molecules may inhibit certain processes in some tumors or tumor types but have the opposite effects in others. Finally, there are some metastasis suppressors that have additional, separate activities directed against the primary tumor (e.g., proapoptotic, antiproliferative).

Impairing EMT

Cadherins are a family of cell–cell adhesion molecules, the best characterized of which is E-cadherin. It is expressed on the surface of all epithelia and mediates cell–cell adhesion by mutual **zipper** interactions. Catenins (α, β and γ) are proteins that interact with the intracellular domain of E-cadherin and create a mechanical linkage between that molecule and the cytoskeleton, which is essential for effective epithelial cell interactions. Overall, cadherins and catenins are paramount in the suppression of invasion and metastasis. Expression of both E-cadherin and catenins is reduced or lost in most carcinomas, owing in large part to downregulation by the transcription factors mentioned above, Snail, ZEB1 and so forth. The miRNAs, miR-101 and the miR-200 family, help maintain the epithelial phenotype. The latter does so by repressing ZEB1 and ZEB2 levels, thus relieving their repression of E-cadherin levels (see above). (Nothing, of course, is so simple: the ZEBs also downregulate miR-200.)

Not to be outdone, TGF-β, which is an inhibitor of tumorigenesis, is also a promoter of metastasis. It acts in part by downregulating miR-200s. As a result, in most carcinomas, loss of E-cadherin is associated with the development of an invasive and aggressive phenotype. Clinically, there is an inverse correlation of levels of E-cadherin with tumor grade and patient mortality. Interestingly, β-catenin also binds to the APC gene product, an effect that is independent of its interaction with E-cadherin and α-catenin. Mutations in either the APC or β-catenin gene are implicated in the development of colon cancer (see later and Chapter 19).

Inhibitors of Tumor Cell Invasiveness

Nm23-H1. This was the first metastasis suppressor discovered. Its mechanism of action is still not fully understood, but it is known to inhibit tumor cell motility. Nm23-H1 achieves this by blocking cellular mobility signaled by Ras-related cell activation pathways.

p63. This member of the p53 family of tumor suppressors (see below) helps to restrain cellular invasiveness. p63 is often expressed in some in situ carcinomas, such as those of the prostate and breast. It is often repressed or lost in aggressive, metastatic carcinomas. Furthermore, mutants of p53 (see below) may bind and inactivate p63 by forming heterotetramer aggregates. Acting as a transcriptional regulator, p63 also upregulates expression of certain genes that inhibit metastasis (e.g., miR-130B).

Movement through connective tissue is a key function of tumor cells after EMT. This passage depends on the ability of

cells to wiggle through the ECM, which in turn depends on integrin-α5 as a mediator of EMT and RhoA, which helps to direct ameboid movement. These invasive characteristics are inhibited by miR-31 (see below).

Suppressors of Intravasation

Notch is a key inhibitor of tumor angiogenesis (see above). Mechanisms that impede intravasation also involve Notch. Thus, a protein called Aes (for amino-terminal enhancer of split) helps to inhibit migration of tumor cells through vascular walls via signaling networks that include Notch activation.

Limiting Tumor Cell Survival in the Circulation

Life for a tumor cell as a vagabond is no simple matter. Anoikis (see Chapter 1) is a form of apoptotic cell death triggered by loss of cells' usual liaisons with familiar ECM constituents. To add to the dangers of a cell's metastatic pilgrimage, many endothelial cells express the Duffy blood group glycoprotein, **DARC**. Upon recognizing KAI1 on tumor cell membranes, DARC triggers senescence programs, thus condemning the wandering tumor cell to a short, sterile existence. In addition, cells of the innate immune system can trigger cell death programs via TRAIL and CD95 (see Chapter 1).

As mentioned above, tumor cells tend to be significantly larger than the caliber of many vascular spaces they encounter. This disparity can stimulate the cells lining liver sinusoids to secrete nitric oxide (NO). NO triggers apoptosis in the overly large tumor cells trying to slog their way through channels that are too small for them.

Impeding Extravasation

The versatile miR-31 (see above), which inhibits tumor cell invasiveness, also blocks extravasation. This miRNA targets both integrin-5α and RhoA in the process.

Metastatic Colonization

Colonization and subsequent growth may be the major rate-limiting process in tumor metastasis. Several documented and likely metastasis suppressors act at this point, including KISS1 and its receptor, KISS1R. This pair derives their names from their discovery in Hershey, PA, home of chocolate kisses. KISS1, made by tumor cells, binds its cell membrane receptor, KISS1R. The result of this interaction is tumor cell apoptosis.

Other suppressors of metastatic colonization include GATA3 in breast cancers, which promotes cellular differentiation and impedes multiplication, and Psap in prostate cancers, which induces stromal cell production of the antiangiogenic substance thrombospondin-1 (see above). MiR-31, which has multiple antimetastatic activities, also inhibits the ability of cancer cells to colonize distant sites effectively.

Many metastasis suppressors have documented antimetastatic function, but the mechanisms by which these properties are exerted are uncertain. Once a primary tumor is removed, almost all therapy is aimed at suppressing metastases. Thus, it is not surprising that activating endogenous metastasis suppressive functions and trying to mimic them pharmacologically represent key targets of pharmaceutical investigation.

Diverse Mechanisms Participate in Compromising the Effectiveness of Tumor Suppressors

Of course, despite the body's best efforts, tumors still develop. In order to do so, they must either inactivate or circumvent the formidable defenses described above. There are a number of mechanisms by which this treachery occurs:

- **Loss of heterozygosity**
- **Spontaneous mutation**
- **Dominant negative mutations**
- **Fragile site translocations**
- **Altered levels or activities of tumor suppressor proteins**
- **Functional blockade by other related proteins**
- **Epigenetic changes that alter tumor suppressor expression or function**

These mechanisms are described and illustrated below. Epigenetic changes in cancer are discussed in a subsequent section.

Retinoblastoma Gene and Loss of Heterozygosity

Inactivation of tumor suppression may occur in many ways and is incriminated in the pathogenesis of both hereditary and spontaneous cancers in humans. It should be kept in mind that inherited defects in tumor suppression are fortunately rare. However, they help to identify tumor suppressors, to delineate how the affected tumor suppressor gene (TSG) products act and to identify mechanisms of tumor suppressor inactivation. Acquired impairments in tumor suppression are common.

Many more TSGs are known than can be described here, and their numbers are increasing. In addition, inherited mutations in TSGs are responsible for many named tumor susceptibility syndromes, some of which are listed in Table 5-6 (see below).

Retinoblastoma Gene

Retinoblastoma is a rare childhood cancer, about 40% of which reflect a germline mutation; the remainder are not hereditary. In patients with the hereditary form, all somatic cells carry a single missing or mutated allele of a gene (the *Rb* gene) on the long arm of chromosome 13. In the retinoblastoma tumors they develop, however, both alleles of the *Rb* gene are inactive. As mentioned above, the protein product of this *Rb* gene, $p105^{Rb}$, *is a critical checkpoint in the cell cycle, and inactive* Rb *proteins permit unregulated cell proliferation.*

Knudson's Two-Hit Hypothesis

A child with hereditary retinoblastoma is heterozygous at the *Rb* locus. The child inherits one defective *Rb* allele, plus one normal allele (Fig. 5-40). This heterozygous state is not associated with any observable changes in the retina, because 50% of the *Rb* gene product in the heterozygous child is sufficient to prevent a retinoblastoma. However, heterozygosity in some TSGs is unstable, because a subsequent, randomly acquired deletion or mutation may inactivate the remaining normal *Rb* allele. If that occurs, there is no residual Rb tumor suppressor function remaining to protect from unregulated cell proliferation. The child then develops a retinoblastoma. Thus, even though the child inherits a heterozygous Rb genotype, susceptibility to retinoblastoma is inherited in a dominant fashion: it is the heterozygote who develops the tumor.

Precisely the same susceptibility to LOH occurs when there is an acquired mutation in *Rb.* Cells carrying the newly

TABLE 5-6

SELECTED HEREDITARY CONDITIONS ASSOCIATED WITH AN INCREASED RISK OF CANCER

Syndrome	Gene	Predominant Malignancies	Gene Function	Inheritance[a]
Chromosomal Instability Syndromes				
Bloom syndrome	*BLM*	Many sites	DNA repair	R
Fanconi anemia	?	Acute myelogenous leukemia	DNA repair	R
Hereditary Skin Cancer				
Familial melanoma	*CDKN2 (p16)*	Malignant melanoma	Cell cycle regulation	D
Xeroderma pigmentosum	*XP group*	Squamous cell carcinoma of skin; malignant melanoma	DNA repair	R
Endocrine System				
Hereditary paraganglioma and pheochromocytoma	*SDHD*	Paraganglioma; pheochromocytoma	Oxygen sensing and signaling	D
Multiple endocrine neoplasia (MEN) type 1	*MEN1*	Pancreatic islet cell tumors	Transcriptional regulation	D
MEN type 2	*RET*	Thyroid medullary carcinoma; pheochromocytoma (MEN type 2A)	Receptor tyrosine kinase; cell cycle regulation	D
Breast Cancer				
Breast/ovary cancer syndrome	*BRCA1*	Carcinomas of ovary, breast, fallopian tube and prostate	DNA repair	D
Site-specific breast cancer	*BRCA2*	Female and male breast carcinoma; carcinomas of prostate, pancreas and ovary	DNA repair (Fanconi pathway)	D/R
Breast cancer	*PALB2*	Breast, pancreas	DNA repair (Fanconi pathway)	D/R
Nervous System				
Retinoblastoma	*RB*	Retinoblastoma	Cell cycle regulation	D
Phacomatoses				
Neurofibromatosis type 1	*NF1*	Neurofibrosarcomas; astrocytomas; malignant melanomas	Regulator of ras-mediated signaling	D
Neurofibromatosis type 2	*NF2*	Meningiomas; schwannomas	Regulator of cytoskeleton	D
Tuberous sclerosis	*TSC1*	Renal cell carcinoma; astrocytoma	Regulator of cytoskeleton	D
Gastrointestinal System				
Familial adenomatous polyposis	*APC*	Colorectal carcinoma	Cell cycle regulation; migration and adhesion	D
Hereditary nonpolyposis colorectal carcinoma (HNPCC; Lynch syndrome)	*hMSH2, hMSH6, MLH1, hPMS1, hPMS2*	Carcinomas of colon, endometrium, ovary and bladder; malignant melanoma	DNA repair	D
Juvenile polyposis coli	*DPC4/Smad4*	Colorectal carcinoma; endometrial carcinoma	TGF-β signaling	D
Peutz-Jeghers syndrome	*LKB1/STK11*	Stomach, small bowel and colon carcinomas	Serine threonine kinase	D
Kidney				
Hereditary papillary renal cell carcinoma	*MET*	Papillary renal cell carcinoma	Receptor tyrosine kinase; cell cycle regulation	D
Wilms tumor	*WT*	Wilms tumor	Transcriptional regulation	D
Von Hippel-Lindau	*VHL*	Renal cell carcinoma	Regulator of adhesion	D
Multiple Sites				
Carney complex	*PRKARIA*	Testicular neoplasms; thyroid carcinoma	cAMP signaling	D
Cowden syndrome	*PTEN*	Colorectal, breast and thyroid carcinomas	Protein tyrosine phosphatase	D
Li-Fraumeni syndrome	*TP53*	Breast carcinoma; soft tissue sarcomas; brain tumors; leukemia	Transcriptional regulation	D
Werner syndrome	*WRN*	Soft tissue sarcomas	DNA repair	R
Ataxia-telangiectasia	*ATM*	Lymphoma; leukemia	Cell signaling and DNA repair	R

[a]D = autosomal dominant; R = autosomal recessive.

ATM = mutated AT (gene); cAMP = cyclic adenosine 3′,5′-monophosphate; PTEN = phosphatase and tensin homolog; TGF-β = transforming growth factor-β.

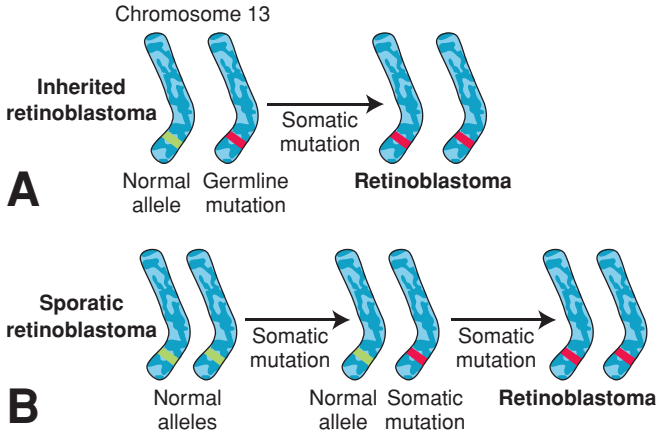


FIGURE 5-40. The "two-hit" origin of retinoblastoma. A. A child with the inherited form of retinoblastoma is born with a germline mutation in one allele of the retinoblastoma gene located on the long arm of chromosome 13. This mutation is not sufficient for tumorigenesis, but the absence of two wild-type alleles weakens protection from tumor development in the event that the remaining allele becomes altered. Then, a second somatic mutation in the retina leads to the inactivation of the functioning *RB* allele and the subsequent development of a retinoblastoma. **B.** In sporadic cases of retinoblastoma, the child is born with two normal *RB* alleles. It requires two independent somatic mutations to inactivate *RB* gene function and allow the appearance of a neoplastic clone.

acquired mutation become similarly susceptible to inactivation of the remaining *Rb* allele.

The principle, then, is that the presence of one mutant *Rb* TSG predisposes to eventual LOH and consequent development of malignancy. A mutation in one allele (whether inherited or acquired) facilitates clonal expansion of cells bearing a mutation in the other allele. This fact underscores an essential paradox of tumor suppressor genes: even if a wild-type phenotype is dominant, heterozygous cells are at high risk for LOH and becoming homozygous mutant cells, with tumor development likely to ensue.

While Rb is named for its signature tumor, an inherited *Rb* mutation affects every cell in the body and confers a more general increase in malignancies. Such patients have a 200-fold increased risk of developing mesenchymal tumors in early adult life. As well, *Rb* is not infrequently mutated in sporadic tumors, including 70% of cases of osteosarcoma and in many instances of small cell lung cancer; carcinomas of the breast, bladder and pancreas; and other organs.

Many types of *Rb* mutations have been described, including point mutations, insertions, deletions and translocations. Epigenetic events, such as promoter hypermethylation (see below), may also decrease *Rb* expression and contribute to a tumorigenic phenotype.

p53, Acquired Point Mutations and Dominant Negative Mutants

The *TP53* gene is located on the short arm of chromosome 17, and its protein product, p53, is present in virtually all normal tissues. *TP53* is deleted or mutated in 75% of human colorectal cancers and frequently in carcinomas of the breast, lung (small cell), liver, brain (astrocytomas) and many others. *In fact, mutations of* TP53 *are the most common genetic change in human cancer.* Inactivating mutations found in human cancers are largely missense mutations that impair the ability of p53 to bind to DNA (Fig. 5-41A). Affected cells may then progress through the cell cycle despite having damaged DNA. While in some cancers both *TP53* alleles are inactivated by the mechanism described above, this is not always the case. Often, one mutant *TP53* gene is sufficient.

The active form of p53 protein is a homotetramer (i.e., a composite of four individual p53 proteins) (Fig. 5-41B). For the complex to be functional, all four p53 monomers must be functional. Mutant p53 subunits therefore can inactivate the whole tetramer (Fig. 5-41C). When the protein product of a mutant allele inactivates that of the wild-type allele, the mutant is said be a **dominant negative**. A cell carrying one mutant *TP53* allele (i.e., a heterozygote) should have a growth advantage over normal cells, and so predominate in vivo with a high risk of then becoming cancerous.

Additional Mechanisms of Inactivating p53

Because p53 is so intensively studied, much of the diversity of mechanisms by which tumor suppression can be inactivated has been uncovered for this protein. Normally, p53 activity is regulated by its binding to the E3 ubiquitin ligase, MDM2. The MDM2–p53 complex prevents p53 from functioning and targets it for degradation via the ubiquitin–proteasome pathway. In turn, MDM2 is inhibited (see above) by binding $p14^{ARF}$. Any oncogenic stimulus (e.g., *myc, ras*) upregulates the $p14^{ARF}$ tumor suppressor protein, which in turn induces Rb phosphorylation.

Some cancers in which both *p53* alleles are structurally normal may overexpress MDM2, consequently increasing p53 degradation. Other tumors in which p53 is intact do not express functional $p14^{ARF}$, and so allow unopposed MDM2-mediated proteolysis of p53. As in the case of *Rb,* certain DNA viral products in tumors (e.g., human papillomavirus [HPV] E6, see below) bind to p53 and promote its degradation. In addition to numerous feedback loops, there are posttranslational modifications (phosphorylation, acetylation, etc.), natural antisense transcripts, binding proteins and small regulatory RNAs. It is no surprise, then, that *most human cancers display either inactivating mutations of p53 or abnormalities in the proteins that regulate p53 activity.*

p53 directs cell cycle arrest, apoptosis and cellular senescence, but these activities are only a part of a more complex tapestry of p53 functions. p53 also regulates responses to metabolic stress; regulation of autophagy and the redox state; production of ROS; and both promotion and limitation of longevity.

The p53 Family

Like a gathering of relations among whom one is the most boisterous, the family of p53-like proteins has largely been dominated by its most conspicuous member—that is, p53. However, there are several important cousins, p63 and p73, as well as some derivative proteins that deserve mention. Just as the region on chromosome 17 that encodes p53 is often mutated or deleted in human cancers, so are the regions on chromosomes 1 and 3 where p73 and p63 reside, respectively. If p63 and p73 are intact, they may partly compensate for loss of p53. Both are now considered to be a tumor suppressor in their own rights, with functions that partly overlap, and that are partly distinct from, those of p53. p63 is a transcriptional regulator, directly increasing levels of the proapoptotic proteins, CD95 (FasR; see Chapter 1) and Bax. It is also important for effective chemotherapy using agents like *cis*-platinum.

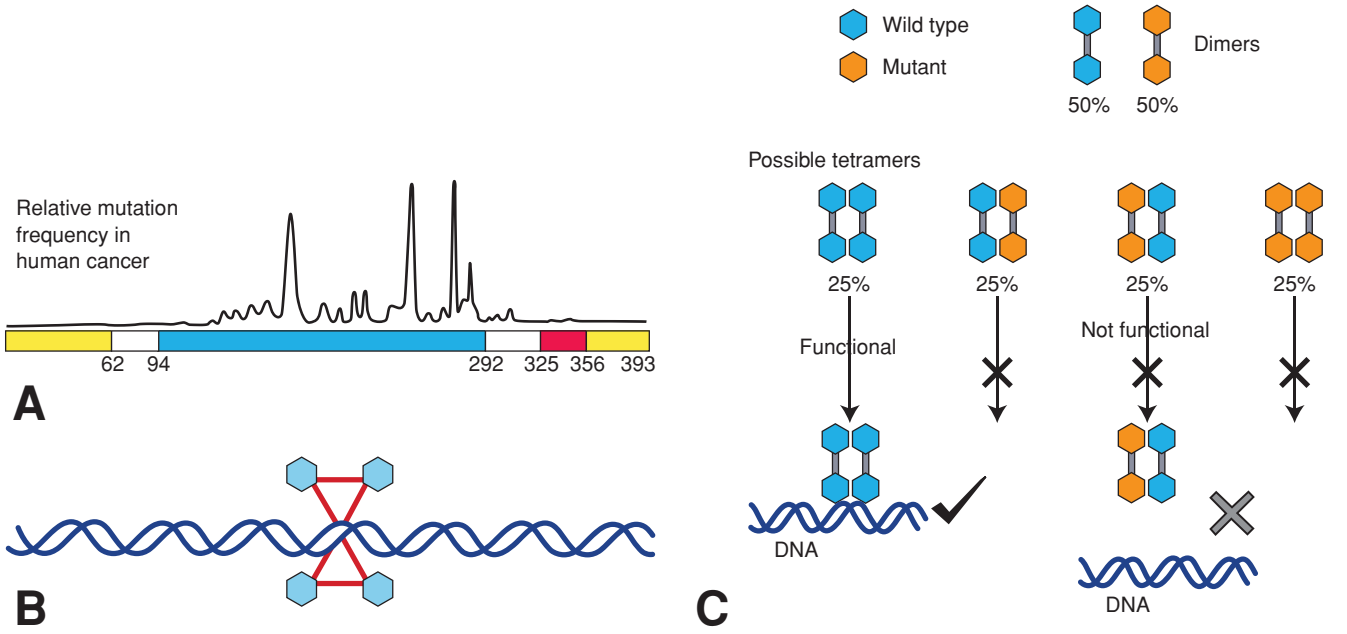


FIGURE 5-41. Mutations in TP53 and stoichiometry of impaired function of p53 tumor suppressor. A. Locations and frequency of mutations in different p53 protein regions. There are multiple domains of the p53 protein. The largest domain is the DNA-binding domain, which is where the vast majority of known mutations in p53 are located. **B. p53 binding to DNA.** To regulate transcription, p53 binds DNA as a tetramer. The tetramer is composed of two dimers. Each dimer is the product of one of the two alleles of the *TP53* gene. **C. Consequences of heterozygous mutation in *TP53* gene.** If one of the two alleles of the p53 gene is mutant, the result is that one dimer is completely mutant (and hence inactive) and the other is wild type (and hence active). However, as p53 transcriptional activity requires a fully functional tetramer, and as the sorting of the dimers into a tetramer is random, 3/4 of the resulting tetramers will be inactive, as shown. Thus, one mutant allele of p53 inactivates 3/4 of p53 activity.

There are many variants of each of these proteins. These may affect the protection afforded by these three proteins in diverse ways (see below).

Treacherous Mutant p53

Interestingly, the mischief of mutant p53 molecules extends far beyond simple inactivation of tumor suppressor function. The aberrant protein also functions as an oncogene, modulating gene transcription. In addition, it protects cells from apoptosis. Mutant p53 also activates proinflammatory cytokines and extracellular matrix modulators. It blocks ATM-mediated (see above) protection against double-stranded DNA breaks. A common denominator underlying the effects of mutant p53 is its widespread stimulation of genes involved with cell proliferation. Moreover, mutant p53 activates cellular mechanisms that are responsible for resistance to chemotherapeutic drugs. In many cases, including tumors of the hematopoietic system, breast, urinary bladder and head and neck, mutant p53 is associated with a poorer prognosis. Along these lines, it should be noted that some splice variants of p53 and p73, particularly those lacking the N-terminal domains, appear to inhibit aspects of their tumor suppressor activities and to act in part as oncogenes.

Fragile Site Translocation

The human genome contains a number of more or less universally shared **common fragile sites** (CFSs) that are inordinately structurally unstable. (Small percentages, 5% or less, of the population also possess rare fragile sites that are prone to the same fragility.) Gene amplification, chromosomal translocations, sister chromatid exchanges, deletions and other kinds of chromosomal malfunctions occur inordinately often at these sites. This instability may be implicated in tumor development, via resultant loss of TSG integrity.

The most active CFS is called FRA3B, and deletions or translocations there are associated with many human malignancies, including solid tumors and leukemias. A gene that is often inactivated or deleted in that setting is the **fragile histidine triad (FHIT)** tumor suppressor. It encodes a protein that cleaves certain nucleotides into adenosine monophosphate (AMP) and adenosine diphosphate (ADP), but it is not clear how much its tumor suppressor activity relates to this enzymatic function. Unlike most tumor suppressors except for APC, the FHIT protein does not bind DNA. Rather, it (like APC) enhances microtubule assembly. It is also felt to promote apoptosis via caspase-8 activation (see Chapter 1). Lack of *FHIT* expression is associated with enhanced resistance to apoptosis. FRA3B alterations are particularly common in human cancers associated with environmental carcinogens.

Other important tumor suppressors that are commonly inactivated during genomic alterations involving CFSs include Wwox, Parkin and caveolin-1. The gene encoding Wwox spans the FRA16D common fragile site. Wwox is important in growth regulation and some forms of apoptosis. Levels of this protein are decreased in most human malignancies. Parkin, an E3 ubiquitin ligase, plays an important role in autophagy (see later and Chapter 1) and is often lost in certain solid tumors. **Caveolin-1** is one of two tumor suppressors (the other being testin) located at FRA7G. Caveolins regulate several cellular functions, including signal transduction. This site is often lost in diverse human cancers, both solid and hematopoietic tumors.

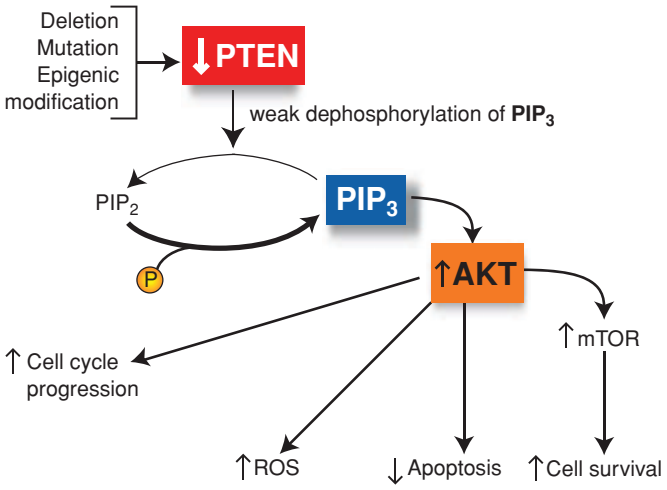


FIGURE 5-42. The consequences of decreased PTEN activity. If activity of PTEN is decreased by mutation or by epigenetic means, phosphatidylinositol-3,4,5-trisphosphate (PIP3) accumulates, activating Akt, a central signaling intermediate. As a result, certain regulators—p27, Bad and FOXO—are not activated, thus promoting cell cycle progression and decreasing apoptosis. At the same time, activation of mTOR (mammalian target of rapamycin) stimulates cell survival. Loss of PTEN activity therefore facilitates the development of uncontrolled cell proliferation and cancer.

Altered Levels of Tumor Suppressor Proteins and/or Activity

It would be tempting to understand loss of effective tumor suppression as basically a matter of altered TSG structure, or LOH, as above. It would also be wrong. For some important tumor suppressors, the critical parameter is the level of tumor suppressor activity, the presence or absence of mutations being important mainly insofar as it determines protein level. Such a tumor suppressor is PTEN (phosphatase and tensin homolog detected on chromosome 10).

PTEN Function

PTEN is a phosphatase that dephosphorylates both proteins and lipids. It regulates the many signals that connect growth factor–triggered signals from receptors at the cell surface to nuclear transcription factors that mediate many cellular functions. PTEN dephosphorylates the highly active signaling intermediate, PIP3, to its inactive 3,4-bisphosphate (PIP2) form. In so doing, PTEN inhibits the AKT-mTOR pathway (Figs. 5-38 and 5-42). As well, PTEN and p53 physically interact and regulate each other. PTEN may be necessary for p53 to be functional and protects p53 from MDM2-mediated degradation. Overall, by virtue of both its lipid phosphatase and other activities, PTEN protein is critical for the DNA damage repair response, apoptosis, regulation of cell cycle progression, maintenance of epithelial polarity and inhibition of EMT. It also regulates cell metabolism to limit glycolysis, as opposed to oxidative phosphorylation (see later).

PTEN protein is normally maintained at a steady, high concentration. Thus, anything that changes PTEN protein levels even slightly, whether inactivation of one or both alleles, altered promoter activity or other epigenetic or post-translational change, may lower the concentration of the protein to a point where it is unable to modulate levels of PIP3 effectively. Decreased PTEN activity permits PIP3 to accumulate and constitutively activate a variety of signaling pathways involved in cell proliferation and survival, which are key in cancer development.

Alterations in PTEN Levels and Function

PTEN is the second most frequently mutated gene in human cancers, after p53. However, it is the level of PTEN activity that is the key to its tumor suppressor activity, and even small decreases in PTEN activity may allow some tumors to develop.

There are many mechanisms, and many points in the pathway from gene to functional PTEN activity, at which the levels of that activity are subject to upregulation or, mostly, downregulation (Fig. 5-43):

- **Regulation of transcription:** Several epigenetic mechanisms (see below) are known to decrease levels of

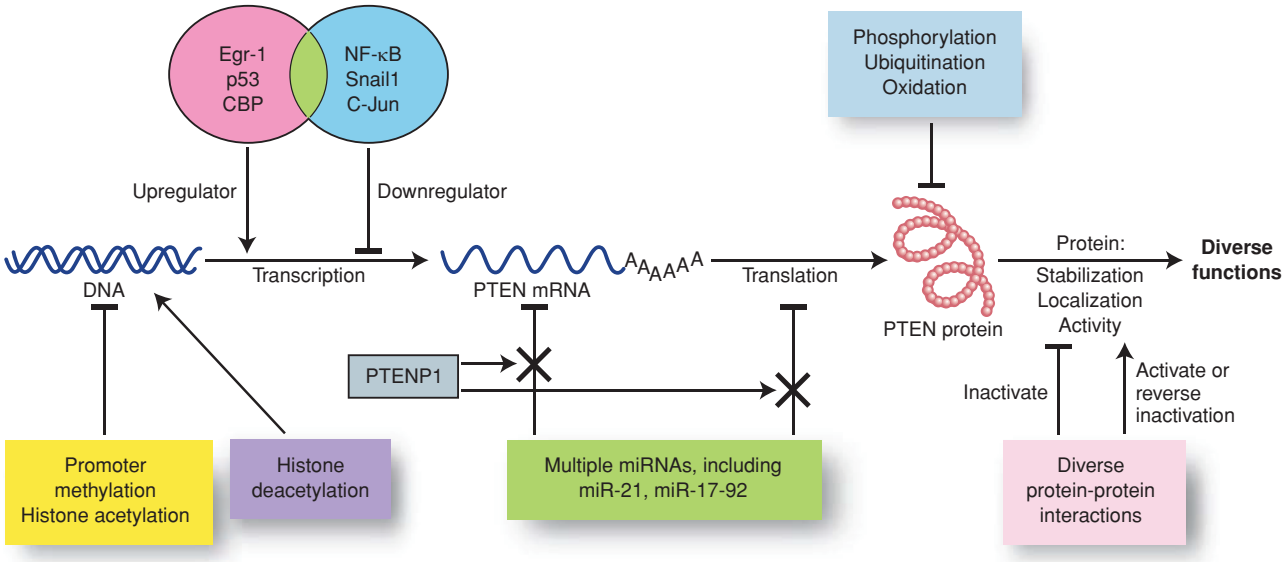


FIGURE 5-43. Regulation of levels of PTEN expression and activity. PTEN is a fundamental regulator of many cellular activities involved in oncogenesis (Fig. 5-42). The levels of PTEN expression and activity are crucial to cellular homeostasis. PTEN can be regulated by multiple mechanisms, as illustrated here, from altered transcription to messenger RNA (mRNA) stability to protein modifications.

transcription of the *PTEN* gene. These include altered histone structure and promoter DNA methylation. Concentrations of proteins that increase or decrease promoter activity are also important.

- **miRNAs:** MicroRNAs (see below) bind mainly the 3′ untranslated region of the PTEN transcript and may cause that mRNA to be degraded, or may prevent its translation.
- **Pseudogene transcripts as decoys:** A gene, called *PTENP1,* which does not code for a protein, produces an untranslated RNA that closely resembles PTEN mRNA. This transcript is representative of a class of transcripts called **competing endogenous RNAs (ceRNAs)**. Sequence homology between the PTENP1 ceRNA and the PTEN mRNA allows the former to bind to miRNAs that would otherwise target and inhibit PTEN transcripts. The likely significance of this decoy to tumor suppression is illustrated by the fact that the *PTENP1* gene is commonly lost in some human cancers, leading to lower PTEN protein levels in such tumors.
- **Protein modifications:** Known posttranslational modifications of PTEN protein can inactivate it (acetylation, oxidation), target it for degradation (polyubiquitination), direct it to subcellular sites where its activity is particularly needed (monoubiquitination) or reverse inactivating modifications (e.g., SIRT1 deacetylates PTEN).

This intricacy and diversity of systems that control amounts of active PTEN protein maintain its functionality in normal tissues within very narrow tolerances. Data suggest that a decrease of only 20% in PTEN levels contributes to oncogenesis. In this context, some tissues (e.g., endometrium, hematopoietic) are more susceptible than others (e.g., prostate) to tumorigenesis when PTEN activity is slightly reduced. These observations underscore two important facts: (1) loss of protective tumor suppressor function may occur when levels of a tumor suppressor protein are reduced by as little as 20%, in the absence of an inactivating mutation in the TSG itself; and (2) different tissues vary greatly in their susceptibility (see below) to oncogenic stimuli—be they decreased tumor suppression or increased tumor promotion.

PTEN is not the only such protein to which these conclusions apply. Protection afforded by several tumor suppressors, like the breast cancer susceptibility proteins BRCA1 and BRCA2, is impaired when one allele is mutant while the other is still active, and so does not necessarily require LOH.

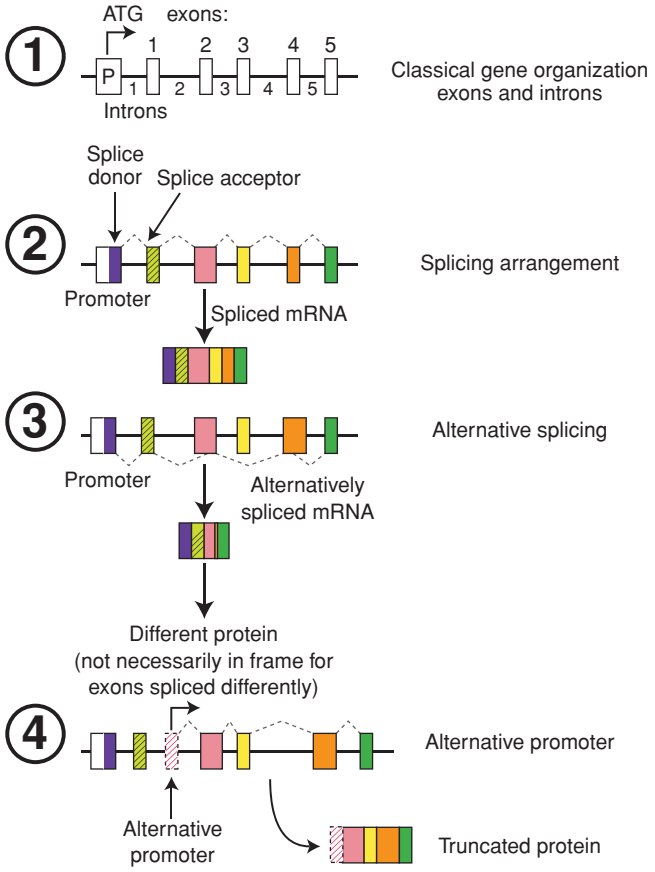


FIGURE 5-44. How alternate splicing affects a gene's products and their activities. RNA splicing is an important mechanism by which gene activity is controlled. It is illustrated here. **1.** The organization of exons and introns in a hypothetical gene is shown. **2.** Transcribed RNAs are spliced; that is, a splice "donor" site at the 3′ end of one exon is linked to a splice "acceptor" site at the 5′ end of the following exon, with the RNA corresponding to the intervening intron removed. The result is a messenger RNA (mRNA) that is exported from the nucleus for translation by ribosomes. **3.** However, there may be alternative splice donor and acceptor sites at different points within the several exons, so that an entirely different mRNA may be generated. The resulting protein may have variable sequence homology to the protein produced in **2,** depending on whether the alternate splice sites result in an mRNA that is in frame with the original or not. **4.** Another strategy for generating a different protein from the same gene is alternate promoters. In this situation, a promoter different from the promoter in **2** mediates transcriptional activation, and transcription begins from a completely different site in the DNA sequence. The result may be a partial protein, a protein that is spliced differently from the original (in **2**) and thus potentially completely different or some variation thereof.

Effects of Alternate or Aberrant Forms of Tumor Suppressors

Mechanisms

A single gene may encode multiple proteins, independent of alterations in DNA structure. Among the most important of these are alternate splicing and the activities of multiple promoters (Fig. 5-44 frames 1,2). About 95% of human genes with multiple exons are known to be spliced in multiple ways, generating proteins of different sizes, often containing different amino acid sequences and having diverse functions.

Two characteristically important aspects of alternative splicing are shown in Fig. 5-44 frames 3,4. What is important here is that the same genomic DNA sequence is responsible for generating multiple variants of an individual protein. Alternative splice donor and acceptor sites may cause an entirely, or partially, different protein to be produced, depending on whether the resulting splicing is in frame or not, compared to the original, "classical," transcript and its derivative protein.

Alternatively, transcription may be initiated from a second promoter (often designated P2). This may yield a protein homologous to the "classical protein," yet truncated at its amino terminus. Such amino terminal variants are often designated ΔN. Of course, these two mechanisms may operate in tandem to produce an array of variant proteins of different sizes, compositions and degrees of homology.

The factors that determine how splicing occurs, what sites are suitable donors and acceptors and so forth are quite complex. They involve many participant molecules. Some of

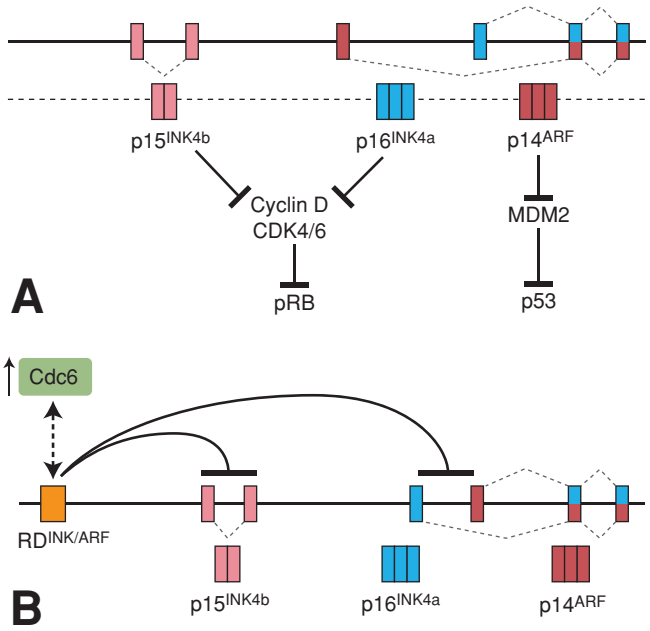


FIGURE 5-45. Multiple tumor suppressors from one locus. A. The organization of the INK/ARF locus. This gene encodes three major and different tumor suppressors, as shown. $p15^{INK4b}$ and $p16^{INK4a}$ are both critical regulators of cyclin D and cyclin-dependent kinases (CDKs) 4 and 6. p15 is transcribed separately from p16. However, p16 and $p14^{ARF}$ (ARF stands for alternate reading frame) coding sequences overlap. Owing to alternate splicing (Fig. 5-44), their coding sequences are totally different. The functions of p16 and p14 are also distinct, as shown. **B.** The area $RD^{INK/ARF}$ represents a region 5′ to the transcriptional start sites that is the target for Cdc6-mediated transcriptional silencing of all of these proteins. All three tumor suppressors are silenced by Cdc6, however, as Cdc6 binds the promoter for p15 and recruits histone deacetylases to the entire gene complex, inhibiting transcription of all three tumor suppressors.

these are tissue specific, so that different mRNA alternatives and resulting variant proteins may be dissimilar in different cell or tissue types.

Alternative Splicing, Tumor Suppression and Interference with Tumor Suppression

Alternative splicing, etc., is important in generating specific tumor suppressor proteins, in determining their ability to protect from cancer and the spread of cancer, in regulating tumor suppression and in escape from tumor suppression.

The ARF-INK4 Locus. One of nature's more impressive mistakes was to have concentrated three major (and several minor) tumor suppressors at the same locus (Fig. 5-45A). This renders them all simultaneously susceptible to elimination with a single deletion event. Even worse, these tumor suppressors can all be turned off by a single interaction between a repressor protein and one specific area in the gene complex. Thus, $p14^{ARF}$, $p15^{INK4b}$ and $p16^{INK4a}$ (see above) are all encoded on chromosome 9p21. Loss of this locus is very common in human cancers and leads to deregulation of Rb-related cell cycle control and excessive inhibition of p53 (Fig. 5-45A).

The tumor suppressor transcripts are all driven by different promoters, $p15^{INK4b}$ being upstream of the others. However, some of the exons for $p14^{ARF}$ and $p16^{INK4a}$ are shared (Fig. 5-45A). The shared coding sequences are in different reading frames, though, so that the two proteins have no sequence homology.

Despite having different promoters, these three open reading frames all share a common repressor site (Fig. 5-45B). The protein, Cdc6, binds a common site prepressor and can simultaneously extinguish expression of the three critical tumor suppressors.

An additional mechanism of tumor suppression is exemplified at this locus, that is, **long, noncoding RNAs** (**lncRNA**s; see below). An important lncRNA, called *ANRIL*, suppresses expression of $p15^{INK4b}$ and is particularly important in some leukemias and prostate cancers.

p53 and Its Cousins. As mentioned above, p53 is the most prominent member of a family of tumor suppressors with diverse functions. All of them are transcriptional activators and repressors, and all inhibit one or more cancer attributes (see above). Like p53, p63 and p73 are active in this mode as homotetramers. The p53/p63/p73 story is more complex than that, however. Each of these genes gives rise to multiple transcripts, for which diverse, sometimes antagonistic, functions have been identified.

The best understood of these variants are shortened transcripts, generated by alternative splicing and/or different internal promoters. These variant mRNAs encode proteins lacking variable amounts of the full-length proteins. Transcriptionally deficient forms of each family are known: ΔNp53, ΔNp63, ΔNp73. Many splice variants, designated with α, β and so forth, are also known. These ΔN and splice variants may oligomerize with the full-length proteins to form transcriptionally inactive tetramers. Furthermore, these variants, especially the ΔN variants, can bind to promoters normally activated by the full-length proteins (e.g., proapoptotic proteins such as Bax, Puma) and block access to these promoters (Fig. 5-46). The presence of multiple additional promoters and many splice variants for each protein further complicates matters.

Nonetheless, ratios of full-length:ΔN variants are tightly regulated in normal tissues. Although the genes for p63

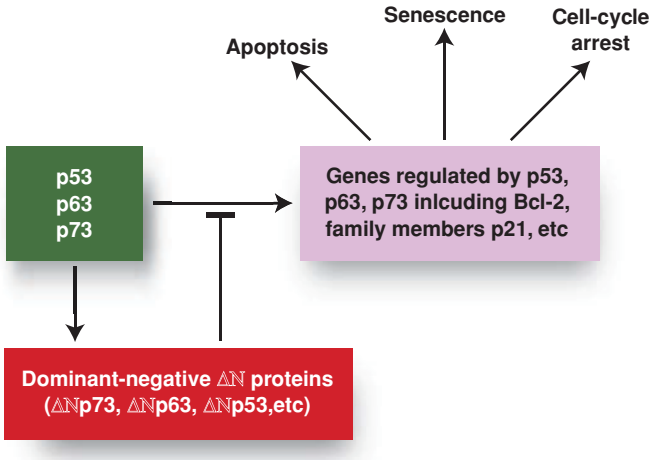


FIGURE 5-46. The isoforms of the members of the p53 family and their interactions. All members of the p53 tumor suppressor protein family have alternate isoforms deleted at their amino termini (ΔN), which act as dominant negative proteins, and impede the transcriptional and other activities of the full-length p53, p63 and p73.

and p73 are not often mutated in cancers, the ΔN forms of p53 family member proteins are upregulated—or ΔN:full-length ratios altered—in many tumors. For example, ΔNp63 predominance is associated with poor response to certain chemotherapies that target DNA and may portend poor prognosis.

Thus, complex as it is, the availability of alternate transcripts, and resulting variants or different proteins, encoded by the same locus represents an important means by which cancers can evade tumor suppression mechanisms.

INHERITED CANCER SYNDROMES

Cancer syndromes attributed to inherited mutations make up only 1% of cancers. These mutations principally involve tumor suppressor and DNA repair genes. As previously discussed for *Rb*, inheritance of a single mutated allele of a tumor suppressor gene results in a heterozygous state and high risk for LOH (i.e., inactivation of the normal allele). What is inherited in this setting is a high degree of susceptibility to developing cancer. Although the germline genotype of such people is heterozygous, both tumor suppressor alleles are inactivated in the tumors that develop in these individuals.

Hereditary tumor syndromes can be arbitrarily divided into three categories:

1. Inherited malignant tumors (e.g., Rb, WT, many endocrine tumors)
2. Inherited tumors that remain benign or have a malignant potential (e.g., APC)
3. Inherited syndromes associated with a high risk of malignant tumors (e.g., Bloom syndrome, ataxia telangiectasia)

These syndromes highlight tumor suppressor activities and the genes that cause them. However, many inherited syndromes entail a different spectrum of tumors than the significance of the mutated gene(s) would suggest. For example, decreased PTEN is very common in many malignancies (see above), but germline loss of PTEN (Cowden syndrome) is mainly associated with benign hamartomas.

Most of these are discussed in chapters dealing with specific organs, and selected examples are given in Table 5-6.

Some disorders, called **phacomatoses** (e.g., tuberous sclerosis, neurofibromatosis), are difficult to classify. These have both developmental and neoplastic features. Tumors associated with these syndromes mostly involve the nervous system.

Only a small proportion of all cancers show Mendelian inheritance, but certain malignancies undeniably tend to run in families. For many tumors, other family members of an affected person have a twofold to threefold increased risk of developing that type of cancer. This predisposition is particularly marked for cancers of the breast and colon, but is also exemplified in the interplay of heredity and environment. Thus, smokers who are closely related to someone with lung cancer have a higher risk of developing lung cancer than do smokers without this familial background.

Organ Specificity in Inherited Cancer Syndromes

Many of the inherited germline mutations cited above (e.g., *BRCA1* or *VHL* genes) lead to specific tumor syndromes. However, it remains unclear why alterations in certain genes tend to affect some organs but not others. Thus, the importance of BRCA1 in repair of DNA double-strand breaks is well established, but it is obscure why germline *BRCA1* mutations lead mainly to breast and ovarian cancers and not other types of cancer, and why women are so profoundly affected, as compared to men.

EPIGENETIC MECHANISMS AND CANCER

Structural changes in the coding sequences of genes, the study of which is called **genomics,** are important determinants of cancer development. It has, however, become clear that tumorigenesis and tumor suppression cannot be understood solely in terms of changes in DNA sequence leading either to dysfunctional proteins or loss of proteins entirely. Rather, regulation of the amounts of proteins in cells influences cell behavior at least as profoundly as the DNA sequences that encode those proteins. **Epigenetics** is the umbrella term for the mechanisms that control gene expression, independently of DNA base sequences.

The most important epigenetic mechanisms that are involved in neoplasia, to suppress tumor development, to facilitate it or both, are listed in Table 5-7. Some were mentioned above. These are physiologic processes that are obligatory parts of normal cellular equilibrium. *It is when these processes go awry that problems, including cancer, develop. Thus, no individual modality is necessarily cancer promoting or cancer suppressing, but may act both to inhibit tumor development and to promote it, the net result depending on the specific genes involved and how they are affected.* These mechanisms interact with each other, so that effects of one may require others to participate.

DNA Methylation at CpGs Regulates Promoter Activity

The amounts of a protein in a cell can be at least as important as the structure of that protein in determining how effectively it, for example, repairs DNA or restrains cell proliferation. The level of transcription of a gene is among the most basic determinants of how much protein is made. Transcription, in turn, depends on promoter activity. The promoters of many genes contain disproportionate concentrations of CpG dinucleotides, called "**CpG islands**." (The *p* represents the interbase phosphodiester bond.) These islands are inhomogeneously distributed in the genome. They predominate in

TABLE 5-7
MAJOR MECHANISMS OF EPIGENETIC REGULATION THAT AFFECT ONCOGENESIS

Mechanism	Example
Covalent modifications of DNA	CpG methylation
Covalent modifications of histones	Acetylation
Remodeling/repositioning of nucleosomes	Incorporation of histone variants
Small noncoding RNAs	MicroRNAs
Long noncoding RNAs	Pseudogene transcripts

the promoter regions of many genes and in repetitive DNA sequences, particularly transposable elements (see below).

DNA Hypermethylation. DNA methylation is the work of a family of enzymes called DNA methyl transferases (DNMTs). DNMTs, especially DNMT3A, are recruited in part by modified histones (see below) to the specific CpG islands to be methylated. Methylation of cytosines at promoter CpGs generally silences the gene immediately downstream. This occurs both because CpG methylation prevents transcription factors from binding the promoter and because methylation may recruit transcriptional suppressors to the site. In many cancers, methylation inactivates TSG activity and is generally more common as a means by which tumors evade suppression than TSG mutations. Some genes, especially $p15^{INK4b}$, FHIT and BRCA1 (see above), are highly susceptible to downregulation by promoter methylation. CpG methylation may also complement mutation, to complete the inactivation of both of a pair of TSG alleles. Thus, if one allele of the DNA mismatch-repair gene *MLH1* is mutated in a colon cancer, the other is likely to be inactivated by promoter methylation.

It should be noted that DNA methylation patterns are not automatically transmitted to both daughter cells after mitosis. A special enzyme, DNMT1, is responsible for perpetuating parental cellular DNA methylation patterns to mitotic progeny. This enzyme is lost or impaired in some tumors.

Hypomethylation: On average, the DNA of most cancer cells is hypomethylated, compared to their normal tissue counterparts. This occurs in repetitive DNA sequences, as well as in exons and introns of protein-encoding genes. The extent of DNA hypomethylation may increase as oncogenesis advances from a benign proliferation to a malignant tumor. Undermethylation destabilizes DNA structure and favors recombination during mitosis, leading to increased deletions, translocations, chromosomal rearrangements and aneuploidy, all of which contribute to malignant progression. Transposable DNA sequences are particularly prone to undergo translocation when hypomethylated. Decreased methylation of genes associated with cell proliferation may increase transcription of such genes. The same principle applies to latent human tumor viruses (e.g., HPV, Epstein-Barr virus), hypomethylation of which may lead to tumor development.

Mechanisms of Methylation and Demethylation

Lest the above appear too straightforward, reality is always more complex. In fact, even though tumors may benefit from overall hypomethylation, in fact, levels of CpG methylation vary considerably, from one gene to the next. Some genes' promoters are hypermethylated (often TSGs), while others, such as oncogenes, transposable sequences and other repetitive DNA stretches, are likely to be hypomethylated.

Demethylation may also occur, although it tends to be more complex and may involve nonenzymatic reactions (see below), increased oxidation and removal of affected cytosines by DNA damage repair pathways.

DNMTs are also not immune to tumor-related genomic instability. Mutations in DNMT3A are important in allowing leukemic hematopoietic stem cells to proliferate, and so are also associated with poor prognosis. Thus, *the basic alteration is* ***aberrant methylation*** *and is site specific. CpG methylation patterns in every tumor and every gene are different.*

MicroRNAs May Act as Oncogenes and as Tumor Suppressors

Not too long ago, researchers noticed that a specific area in a B-lymphocytic leukemia (see Chapter 26) tended to be disrupted, but the affected region did not code for any known protein. On further analysis, they determined that the disrupted gene encoded not a protein, but a tiny RNA species that acted as a tumor suppressor. Loss of that miRNA tumor suppressor was linked to development of that type of leukemia. Since then, over 1000 miRNAs have been discovered. Functions relating to neoplasia have been ascribed to many of these (Table 5-8).

TABLE 5-8
EXAMPLES OF MICRORNAS (miRNAs) THAT ACT AS ONCOGENES, TUMOR SUPPRESSORS OR BOTH, AND THE TUMOR TYPES FOR WHICH THEY DISPLAY THOSE ACTIVITIES

Oncogenic miRNAs		Tumor Suppressor miRNAs		miRNAs That Act as Both Suppressors and Oncogenes	
miRNA	**Organ**	**miRNA**	**Organ**	**miRNA**	**Organ**
miR-21	Breast, CLL, colorectal, esophagus, glioblastoma, liver, lung, pancreas, prostate	miR-143	Breast, CLL, colorectal, lung, prostate	miR-23 group	Bladder (o), breast (o), CLL (o), prostate (s)
miR-23	Bladder, breast, CLL	let-7group[a]	ALL, breast, CLL colorectal, lung, pancreas	miR-23 group	Bladder (o), breast (o), CLL (o), prostate (s)
miR-221	AML, bladder, CLL, glioblastoma, liver, pancreas, prostate, thyroid	miR-145	Bladder, breast, colorectal, lung, ovary, prostate	miR-181 group	ALL (o), AML (s), breast (o), CLL (s), glioblastoma (s), pancreas (o), prostate (o), thyroid (o)
miR-17–92 cluster[b]	ALL, CML, colorectal, lung, ovary			miR-125 group	ALL (o, s), AML (o), breast (s), glioblastoma (s), liver (s), ovary (s), pancreas (o), prostate (s), thyroid (s)

[a]The let-7 group includes let-7-a-a1 through -a3, -b through -g, -i and miR-98.
[b]The miR17-92 cluster includes structurally homologous miRs 17-3p, 17-5p, 18a, 19a, 19b-1, 20a and 92a-1.
ALL = acute lymphoblastic leukemia; AML = acute myeloblastic leukemia; CLL = chronic lymphocytic leukemia; CML = chronic myeloid leukemia.

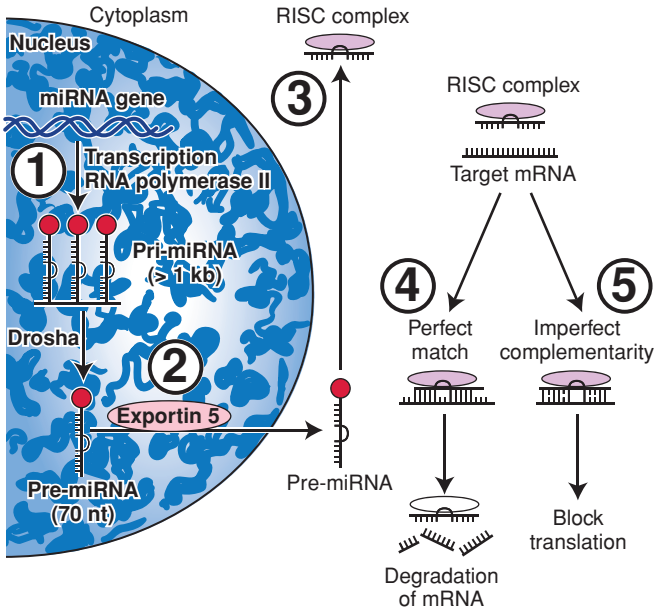


FIGURE 5-47. Production, modification and activities of microRNAs (miRNAs). 1. Most miRNAs are transcribed by RNA polymerase II, the same enzyme that transcribes messenger RNAs (mRNAs) for protein production. **2.** However, the original transcript, which is often more than 1 kb in length, is processed by an enzyme, Drosha, to a shorter form, which is called a pre-miRNA. **3.** This form is exported from the nucleus. In the cytosol, it joins an RNA-induced silencing complex (RISC), where the pre-miRNA is tailored further to the final miRNA by an enzyme called Dicer. A member of this complex, a protein called Argonaute, or Ago, can cleave targeted mRNAs. The nature of the effect of miRNAs depends on the extent of complementarity with a particular mRNA. **4.** If the nucleotides 2–8 of the miRNA align with the 3′-untranslated region of a target perfectly, the target is digested and degraded. **5.** If, on the other hand, the complementarity is imperfect, the miRNA inhibits translation of the target mRNA.

Generation and Actions of miRNAs

MicroRNAs may be encoded anywhere in the genome: intergenic DNA, introns, exons, 3′ untranslated regions (UTRs) and so forth. They are usually transcribed by RNA polymerase II (pol II), the same enzyme that transcribes protein-encoding genes. The initial transcripts that will eventually become miRNAs are often long (>1 kb). These are processed to precursor miRNAs about 70 bases long, which are exported (Fig. 5-47) to the cytosol. There, they are processed further and incorporated as single strands about 22 bases long into an RNA-induced silencing complex **(RISC)**. RISC includes an enzyme (Argonaute, or Ago) that can cleave target mRNAs.

If the recognition sequence (bases 2–8) of an miRNA matches an mRNA—usually the 3′ UTR—perfectly or nearly perfectly, Ago may degrade the targeted transcript. If miRNA complementarity for an mRNA is imperfect, translation of the latter is blocked without degrading the target. miRNAs are thus promiscuous, and any individual miRNA may regulate many different transcripts.

miRNAs and Cancer

miRNAs are critical controllers of many activities, such as embryogenesis and development, cell cycling, differentiation, apoptosis and maintaining stem cell pluripotency ("stemness"). They also regulate many steps in oncogenesis.

miRNAs may inhibit tumor suppressor proteins or may themselves act as tumor suppressors. In the latter case, they may perform several functions, including directly targeting oncogene transcripts. They may also upregulate cell proliferation, and so act as oncogenes. In some cases, one miRNA species, or clusters of related species, may promote tumor development in some tissues but suppress it in others. This context dependence recalls the ambidexterity of some proteins that may be tumor suppressors sometimes and tumor activators at other times (see above). Examples of cancer-related activities for a small number of the more than 1000 known miRNAs are shown in Table 5-8.

miRNAs That Promote Oncogenesis

The cluster of homologous miRNAs designated miR-17–92 is commonly increased in certain hematologic cancers. Expression of these miRNAs is induced by c-Myc. The miR-17–92 cluster protects cells from oncogene-induced apoptosis (see above) in several ways, including by tightly regulating Myc-induced proliferation and downregulating the proapoptotic protein Bim (see Chapter 1). These miRNAs also inhibit the tumor suppressor PTEN and the cell cycle regulator $p21^{WAF1/CIF1}$ (see above).

MiR-21 also restricts apoptosis and other tumor suppressor functions. It downregulates p53 and proteins important in mitochondrial apoptosis. MiR-21 also targets key regulators of cell proliferation, such as TGF-β signaling and PTEN pathways (see above). In various tumor types, miR-21 reduces other tumor suppressor and proapoptotic activities, underscoring both the context dependence and promiscuity of miRNA actions. This miRNA is overexpressed in many human tumors, including those in lung, pancreas and colon cancers.

Tumor Suppression by miRNAs

The let-7 family of miRNAs contains 12 highly conserved members. They have overlapping specificities and target ranges, especially downregulating proteins that activate cell proliferation, such as K-Ras, N-Ras and Myc. miRNAs of this group also target CDK6 and CDC25A, thus blocking cell cycle transit through $G_1 \rightarrow S$ transition. Levels of let-7 members are reduced in many human tumors, especially lung cancers.

Important miRNAs that target the prosurvival (anti-apoptotic) branch of the Bcl-2 family include the cluster of miR-15/16 species. These directly inhibit Bcl-2, the main mitochondrial antiapoptotic protein (see above, Chapter 1). They also block production of important cell cycle drivers, including cyclins D and E. MiR-15/16 are often decreased or absent in solid tumors and certain lymphomas.

Histone Modifications Alter the Ability of Nonhistone Regulators to Reach the DNA

Chromatin is a complex of DNA and proteins that promotes DNA stability and allows it to fit in a small space (the nucleus). It contains repeating units, **nucleosomes,** periodically spaced structures that consist of a combination of 4 histone proteins (H2A, H2B, H3, H4), wrapped in DNA. Covalent alterations to histones include methylation, acetylation, ubiquitination, phosphorylation and others. These occur via specific histone-modifying enzymes,

and are reversible. The operation of histone methylases can be undone by histone demethylases. The work of histone acetylases (HACs) can be reversed by histone deacetylases (HDACs). Covalent changes to histone structure control such gene activities as transcription, DNA repair and DNA replication. Not surprisingly, then, the enzymes that acetylate or deacetylate and methylate or demethylate histones are key regulators of many activities, including oncogenesis.

Histone methylation. Lysines are the principal targets of histone modifications, including acetylation and methylation. However, there are many lysines on several histone species, so the consequences of histone methylation (etc.) depend on where (on which histone, on what amino acids and near what gene) and how much the histone is methylated. In some cases, a specific transcriptional repressor complex, the **polycomb repressor complex-2** (PRC2), is recruited to promoters that are to be inactivated. This complex methylates a specific residue on H3 and silences transcription. If, by different means, a different H3 lysine is methylated, the opposite effect (transcriptional activation) occurs. The histone methylating enzyme, EZH2, is part of PRC2. Altered expression of EZH2 occurs in many cancers and has been associated with a poor prognosis. Cells may gain or lose EZH2 function by mutation or by altered levels of a miRNA that inhibits it (miR-101).

Histone acetylation. Histone acetylation tends to open chromatin and is generally associated with increased transcriptional activity. Histone deacetylation causes chromatin condensation, making it inaccessible for transcription, and so is associated with transcriptional silencing. HDACs are often dysregulated in cancers, causing both silencing of tumor suppressor genes and derepression of oncogenes. The combination of histone modifications and DNA methylation constitutes an intricate regulatory network whose disruption plays an important role in oncogenesis.

The role of histone acetylation status in regulation cannot, apparently, be overestimated. A recent report indicates that histone acetylation is a key determinant of monogamous mating behaviors in prairie voles. Apparently, monogamy in these animals occurs (at least in part) because of histone acetylation near oxytocin and vasopressin receptor genes in the nucleus accumbens in their brains.

Modified Histones and DNA Methylation

As indicated above, the acetylation state of histones affects the transcriptional activity of the gene in question. Histone methylation and DNA methylation also regulate transcription. Histone methylases may directly recruit DNMTs to a gene to be silenced. DNMTs, in turn, can bring HDACs to these sites to deacetylate histones and silence expression. These relationships are complex, however. In some cases, DNA methylation appears to precede histone methylation and deacetylation, and vice versa. Thus, the three processes are linked, but the sequence of events and the final status of the DNA and histones at a site are probably all specific for individual genes.

Nucleosome Positioning and Histone Composition Influence Gene Activity

Chromatin structure is dynamic and varies with a cell's needs at the moment. Nucleosomes tend to leave open those parts of genes where the transcriptional apparatus binds to start gene expression, and again where that apparatus releases the DNA at the end of transcription. Remodeling complexes busily modify nucleosome position and composition, causing nucleosomes to slide or be removed as needed to tailor gene expression to changing cellular circumstances.

The process of synchronizing nucleosome positioning entails incorporation of modified or variant histones into chromatin. These also substitute for their more conventional cousins on an ongoing basis and strongly influence the susceptibility or resistance of associated DNA to silencing by methylation. Such histones help to determine nucleosome positioning itself. Continued modification of histone proteins, as well, is part of the dynamic of chromatin remodeling. For every histone acetylating, methylating, ubiquitinating or phosphorylating an enzyme, there is another that undoes these modifications. As we will see below, these modifiers of histone structure, as well as the mechanisms that sense them, are often central to tumor development.

Long Noncoding RNAs Play Major Regulatory Roles

About 3% of the human genome encodes proteins. Not long ago, it was thought that most of the rest was inactive, or "junk" DNA. Nothing could be further from the truth. It is now clear that over 90% of the human genome is actively transcribed, almost all of it as transcripts that do not make proteins. Furthermore, many DNA sequence changes associated with cancer and other diseases occur within the regions that encode these untranslated RNAs.

These RNAs are called ***long noncoding RNAs,*** *which are defined as RNAs, either primary or spliced transcripts, that do not fit into recognized classes such as structural, protein-coding or small RNAs.* DNA sequences almost anywhere can encode lncRNAs, including intergenic regions, introns, exons and even antisense to coding regions. LncRNAs may be quite large, often 1000s or 10,000s of bases. About 5000 are currently cataloged, and over 20,000 different species may exist. However many there are, they are very low in abundance, poorly understood and almost completely uncharacterized. They do, though, play many important regulatory epigenetic roles, including processing of small RNAs, controlling transcription and acting as organizers, decoys, signal transducers and scaffolds that bind to proteins, DNA or other RNAs. For example, inactivation of one of the pair of X chromosomes in females is the work of an lncRNA called Xist. LncRNAs also help direct chromatin remodeling and DNA methylation and determine the stability and fate of protein-coding RNAs.

We have mentioned lncRNAs in several contexts (above), including as the products of pseudogenes (e.g., PTENP1). These pseudogene lncRNAs may act as decoys for regulatory miRNAs (i.e., as alternative targets for degradative miRNAs), allowing their protein-coding tumor suppressor cousin (here, PTEN mRNA) to survive unmolested. Examples of lncRNAs involved in cancer are shown in Table 5-9.

Epigenetic Regulators Are Distorted in Cancers

The intricacy of epigenetic control over normal cellular processes should be abundantly evident, as should the limitations of our understanding of it. Tumor development, progression and dissemination all entail extensive disequilibrium at every level of epigenetic activity. The explanations above touch on many of these, both in principle and specifically. *It should be emphasized, in summarizing this topic,*

TABLE 5-9
REPRESENTATIVE LONG NONCODING RNAs (lncRNAs) THAT ARE ALTERED IN CANCERS

lncRNA	Tumor Type	Alteration	Function/Consequence
MALAT1	NSCLC, colorectal	↑	Tumor progression and dissemination
ANRIL	Prostate, leukemias	↑	Silences TSG $p15^{INK5b}$ by recruiting PRC2 to methylate promoter
PTENP1	Lung, prostate, endometrium	↓	Pseudogene decoy protecting PTEN mRNA from miRNA-mediated destruction
HOTAIR	Pancreas, colorectal, breast, liver	↑	Recurrence; metastasis; recruits PRC2 to silence tumor suppressors
HULUC	Liver	↑	Represses expression of tumor suppressor miR-372

miRNA = microRNA; mRNA = messenger RNA' NSCLC = non–small cell lung cancer; PRC2 = Polycomb repressor complex-2; TSG = tumor suppressor gene.

that the devil is always in the details, and that overarching generalizations about how tumors develop may be conceptually useful but often break down when one attempts to apply them to specific situations. Thus, a transcriptional activating function may be overactive in a tumor and so upregulate an oncogene, but the same function may be blocked with respect to a tumor suppressor in the same cancer. With that caveat, it is reasonable to summarize the impact of epigenetics on cancer as follows:

- **DNA methylation:** Generally, cancer cell genomes are hypomethylated. This causes general genomic instability and extensive derepression of transcription affecting many genes, especially oncogenes. At the same time, site-specific hypermethylation (e.g., of tumor suppressor genes) also characterizes cancers. The role of PRC2 (see above) is emerging as fundamental to these changes.
- **Histone modifications:** Many tumors show general loss of histone acetylation, especially associated with silencing of TSGs. HDAC overexpression is common in cancers. This has stimulated development of therapeutic HDAC inhibitors. However, HACs are also often abnormal in cancers, and it is the specific interplay of both HACs and HDACs relative to tumor suppressor genes and oncogenes that determines the end result: which genes are activated and which repressed. Other types of histone-modifying enzymes, such as the methylating enzyme EZH2, are also often involved in silencing tumor suppressor genes.
- **Nucleosome positioning:** Altered chromatin structure in cancer accompanies changes in DNA methylation and histone derivatives. Thus, nucleosome localization in tumor cells differs from that in their nonmalignant cellular counterparts.
- **Noncoding RNAs:** Levels of specific noncoding RNAs, both short and long noncoding RNA species, in cancer differ greatly from those in normal cells. These disturbances of normal equilibrium play central roles in regulating almost all facets of cancer cell behavior.

Environmental Stimuli Shape Epigenetic Regulators

The epigenome is highly dynamic and responds to modulation by nutrition, stress, pharmacologic and toxic agents and other influences. For example, identical twins diverge increasingly over time in patterns of DNA methylation. In fact, patterns of CpG methylation of specific genes change over the course of years in any given individual. The ways in which the cellular milieu influences epigenetic regulation are largely obscure. But what is known suggests that this impact may be fundamental to the processes by which tumors originate and spread.

Epigenetic Remodeling as a Function of Metabolism

Tumor cell metabolism is fundamentally different from that of normal cells (see below). The key differences known to affect epigenetic regulation are (1) reliance on glycolysis for energy (as opposed to oxidative phosphorylation), (2) increased levels of HIF-1α, (3) a highly oxidant-rich environment, (4) mutations in key enzymes in the Krebs cycle and (5) abnormally high levels of fatty acid biosynthesis. These factors influence epigenetic regulators as follows:

- **Histone acetylation:** The net result of HACs' and HDACs' actions on histones largely determines gene expression (see above). The acetyl donor for acetylation is acetyl-coenzyme A (CoA). A combination of decreased Krebs cycle effectiveness, via mutations and other means, and high need for acetyl-CoA for fatty acid synthesis reduces the pool of acetyl-CoA available for histone acetylation. This may increase transcriptional repression, especially of TSGs.
- **Histone deacetylation:** A key class of HDACs (class III) are sirtuins, which use NAD^+ to deacetylate histones. Cancer cells' excessive reliance on glycolysis decreases the pool of available NAD^+, restraining this group of HDACs and causing further disequilibrium in histone acetylation. This situation is exacerbated further by metabolic consequences of HIF-1α upregulation, which occurs often in tumors and further limits NAD^+ availability.
- **Methylation:** DNMTs and histone methyl transferases (HMTs) require S-adenosylmetionine (SAM) as a methyl group donor. High levels of oxidative stress in transformed cells lower SAM levels in several ways and accelerate its conversion to other species (especially the antioxidant glutathione). Reduced SAM availability unbalances both histone and DNA methylation.
- **Base changes and oxidation:** Several changes associated with DNA oxidation and modification may result in base changes, particularly affecting CpGs. Thus, methylated cytosine (5-methylcytosine) can undergo two changes that alter DNA structure. It may be spontaneously deaminated to thymine, thus creating a C→T base change transition. In an oxidant-rich environment, it may also be oxidized to 5-(hydroxymethyl) cytosine. This reaction, catalyzed by an enzyme called **Tet methylcytosine dioxygenase (TET2)**, is of note because the *TET2* gene is specifically mutated in certain hematologic malignancies and premalignant conditions. TET2 activity is also affected by certain metabolic aberrations (see below). The added hydroxyl group may then be further oxidized to a carboxyl group, which is enzymatically removed to regenerate the original, unmethylated, cytosine.

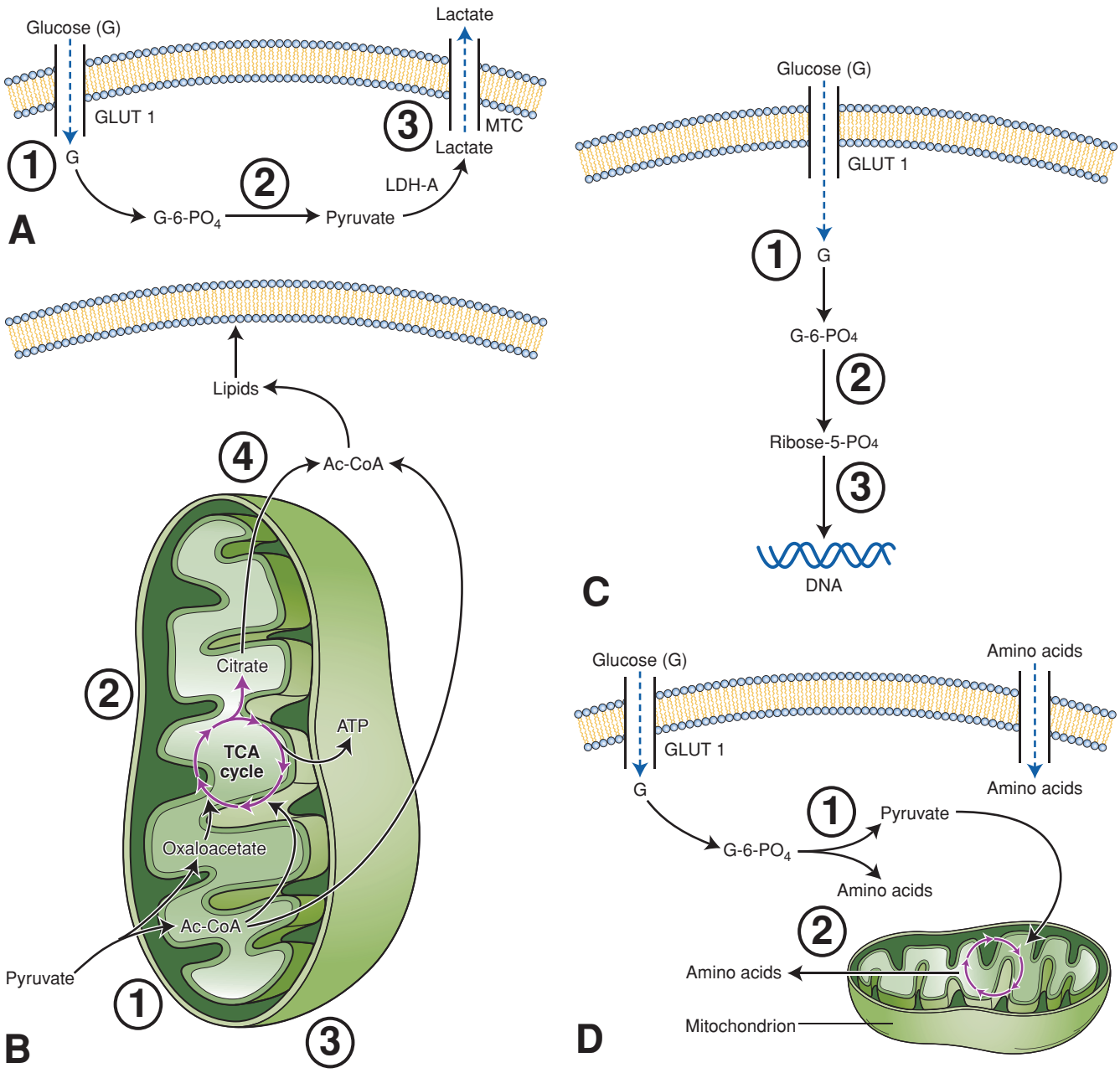


FIGURE 5-48. Cell metabolism. A. Glucose entry. 1. Entry of glucose (G) into the cell is mediated by the glucose transporter, GLUT1. Upon entry, it is converted to glucose-6-phosphate. **2.** Most of the glucose-6-phosphate is metabolized by glycolysis, which leads to production of pyruvate. Pyruvate, in turn, is converted by lactic dehydrogenase-A (LDH-A) to lactate. **3.** Lactate is exported from the cell by a transporter called MTC. **B. Pyruvate utilization in mitochondria. 1.** Some of the pyruvate generated from glucose metabolism enters mitochondria, to become oxaloacetate and to join the tricarboxylic acid (TCA) cycle, which drives oxidative phosphorylation to produce adenosine triphosphate (ATP). **3.** Pyruvate may be converted as well to acetyl-coenzyme A (acetyl-CoA). **4.** Either this acetyl-CoA or citrate from the TCA cycle is exported to the cytosol, where it is incorporated into lipids. **C. Incorporation into DNA. 1.** Glucose-6-phosphate undergoes a number of enzymatic alterations. **2.** It is a precursor for ribose-5-phosphate (ribose-5-PO_4). **3.** Ribose-5-PO_4 enters the nucleus and is an important building block in nucleic acid synthesis. **D. Incorporation into amino acids. 1.** Glucose-6-phosphate metabolism products may be directly converted into certain amino acids. **2.** Alternatively, after pyruvate enters mitochondria, products of the TCA cycle may be converted into amino acids.

As well, oxidation of the G in CpGs may produce 8-oxoguanine. Not only does this disrupt CpG methylation, but also it is highly mutagenic, because 8-oxo-G may be read by polymerases as either G or A.

Therefore, epigenetic regulators are central to normal cellular equilibrium. They become unbalanced during carcinogenesis, but unevenly so: opposite changes in these regulators affect tumor suppressors as compared to oncogenes. Thus, epigenetics is both important and complex: as central as it is to tumor development and spread, there is as yet no single general principle that applies to all aspects of epigenetic regulation.

CANCER CELL METABOLISM

All cells must engage in several critical activities, including to generate energy; produce and repair DNA, RNA, membrane and other lipids; make proteins; and so forth. The proportions of the substrates that cells import or synthesize and that the cell devotes to these different pursuits depends on what the cell does. Most of these functions begin with a source of carbon, which is used to generate energy and build cellular constituents.

Normal Cell Metabolism Favors ATP Generation

Normal cells utilize glucose as their main (but not only; see below) carbon source, both to produce ATP and to synthesize macromolecules. Energy production from glucose includes the following (Fig. 5-48):

- **Glucose entry:** Glucose enters cells via transporters, the best understood being GLUT1 (Fig. 5-48A), although GLUT2, GLUT3 and GLUT4 may also participate.
- **Aerobic glycolysis:** These enzymatic reactions transform glucose to pyruvate and generate 2 net ATPs.
- **Pyruvate:** This product of aerobic glycolysis is the lynchpin of metabolism in normal and malignant cells. Pyruvate enters mitochondria, where it may become acetyl-CoA, in a reaction catalyzed by pyruvate dehydrogenase (PDH; see below). Pyruvate may enter the tricarboxylic acid (TCA) cycle in two ways—after conversion to oxaloacetate or after conversion to acetyl-CoA (Fig. 5-48B).
- **Ribose-5-phosphate:** This sugar is produced from a product of the first reaction that is performed upon glucose after it enters the cell (i.e., its conversion to glucose-6-phosphate). Ribose-5-phosphate is then incorporated into nucleic acids (Fig. 5-48C).
- **Acetyl-CoA:** Pyruvate enters mitochondria and is converted to acetyl-CoA by PDH. This step allows entry into the TCA cycle, which eventually produces 36 ATPs (Fig. 5-48D). Acetyl-CoA can also exit mitochondria to participate in lipid biosynthesis.
- **Amino acid synthesis:** Many amino acids enter the cell via cell membrane transporters. Some are so-called essential amino acids, which humans cannot synthesize and need to derive from foodstuffs. Other amino acids, however, can be synthesized by cells from pyruvate or its metabolites that are part of, for example, the TCA cycle.

Therefore, glucose metabolism provides the cell with much more than just energy. It furnishes key building blocks required for virtually all types of cellular structural and functional constituents.

Glucose Uptake Helps Determine Cellular Metabolism

Cells import glucose (and other carbon sources; see below) in response to both intracellular and extracellular signals. Many of these signals are significantly altered in cancers, contributing to tumor cells' deviant metabolism.

Exogenous signals. The key outside regulators of cellular metabolism are insulin and IGF-I. Upon binding their receptors, these hormones activate intracellular signals that drive many of the processes and mediators that participate in oncogenesis.

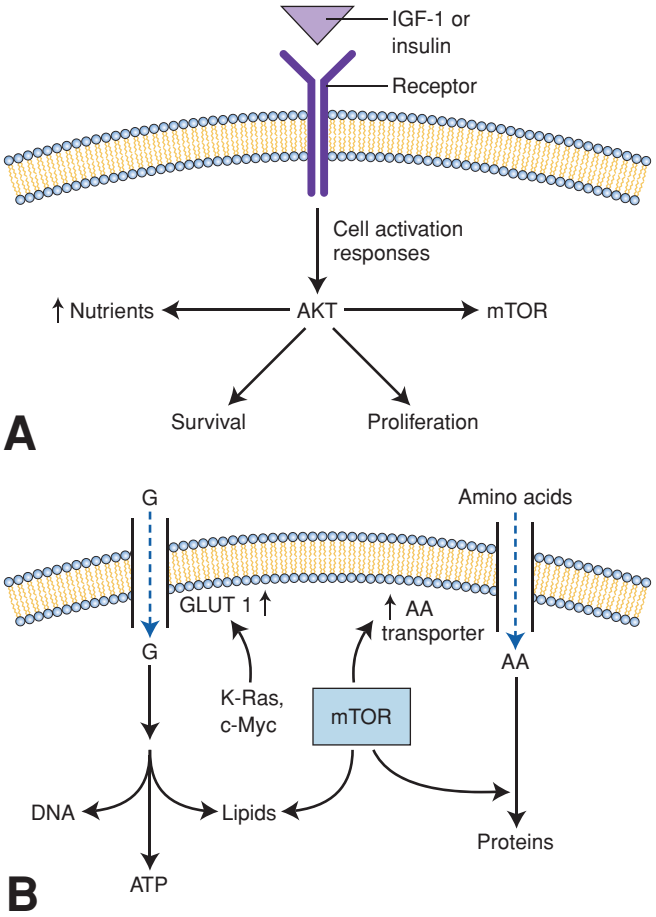


FIGURE 5-49. Effects of metabolic activation on cancer cell metabolism. A. Insulin-like growth factor-I (IGF-I) activation. When IGF-I or insulin binds its receptor, it activates Akt, which in turn elicits many downstream responses. Among the key mediators of Akt effects on cancer cell metabolism is mTOR. **B. Consequences of mTOR activation for cancer cell metabolism.** Operating in tandem with K-Ras– and c-Myc– activated increases in the GLUT1 glucose transporter, mTOR increases synthesis of lipids. It also increases the activity of cell membrane transporters so that increased amino acids are available to support the increased proteosynthetic needs of cancer cells.

Endogenous mediators. At the center of the intracellular response is Akt (Fig. 5-49A). By virtue of the many pathways downstream from Akt (see above and Chapter 1), the cell is protected from apoptosis, stimulated to proliferate and so forth. Akt function is antagonized by the PTEN tumor suppressor. In terms of metabolism, the key downstream effector of Akt is mTOR. This protein stimulates production of amino acid transporters and uptake of amino acids (Fig. 5-49B). It also causes increased lipid and protein synthesis. C-Myc, also upregulated by IGF-I, increases production of GLUT1 and importation of glucose.

Metabolism in Cancer Cells

Cancer cells have different needs from normal cells. As their proliferative rate generally far exceeds that of their normal cousins, they must produce the structural components of their soon-to-be daughter cells at a rate that sustains their mitotic activity. Thus, synthesis of protein, lipid and so forth must march to a much faster drummer than normal.

In 1930, Otto Warburg observed that tumor cells generated energy mainly by aerobic glycolysis in the cytosol, producing pyruvate and 2 ATPs, rather than by mitochondrial oxidative phosphorylation, which generates 36 ATPs, CO_2 and H_2O. The seeming paradox between tumor cells' greater metabolic needs and their preference for a pathway that produces much less energy may be resolved, at least in part, by noting that pyruvate contributes to protein, lipid and other macromolecular synthesis. Furthermore, lactate generated by LDH from pyruvate (Fig. 5-48) may be exported via a special cell membrane channel (monocarbohydrate transporter [MCT]).

Like an athlete who uses an array of different power bars, tumor cells can also generate energy from multiple carbon sources. Lactate, excreted via the MCT by some tumor cells after aerobic glycolysis, may be imported via the same channel. LDH converts this lactate back into pyruvate, for use in any of the several ways described above. Acetate may also be taken up by tumor cells, where it can be made into acetyl-CoA, to be used mostly for lipid synthesis. Another important energy source for cancer cells is glutamine, which is converted to α-ketoglutarate, a TCA intermediate. Although normal cells, depending on their functions, may also exploit these other molecules similarly, cancer cells have turned this multiplicity of carbon sources into an art form.

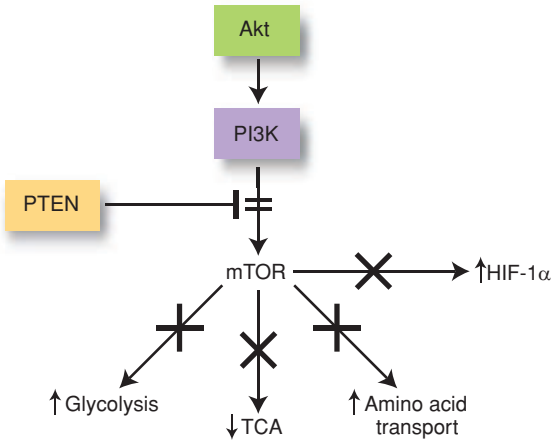


FIGURE 5-50. PTEN controls cellular metabolism and is a suppressor of the metabolic changes that power cancer cell activity. PTEN downregulates mTOR by dephosphorylating phosphatidylinositol-3,4,5-trisphosphate (PIP3) (Fig. 5-38). All downstream effects of mTOR are thus restricted: upregulation of hypoxia-induced factor-1α (HIF-1α), increased glycolysis, increased amino acid transport and decreased tricarboxylic acid (TCA) activity. Thus, PTEN's regulation of mTOR makes it impossible for cancer cells to generate the biosynthetic building blocks they need to sustain proliferation.

Cancer Cells Use Increased Amounts of Glucose

As noted above, cells import more glucose in response to stimulation by exogenous insulin. This phenomenon may explain in part the increased cancer incidence in obese patients with high levels of circulating insulin. Many studies relate both the hyperglycemia and the hyperinsulinemia of type 2 diabetes mellitus (Chapter 13) to worse prognosis for diverse tumors. However, GLUT1, the main glucose importer, is upregulated by several oncoproteins, especially c-Myc, B-Raf and K-Ras. Interestingly, c-Myc also increases glutamine transport into cancer cells and upregulates LDH, which catalyzes the (bidirectional) interconversion of lactate and pyruvate. Myc-dependent tumors are often inhibited if LDH or glutamine is decreased.

HIF-1α helps mediate increased metabolism in cancer cells. It upregulates GLUT1, increases cellular importation of glutamine and stimulates glycolysis. It also inhibits movement of pyruvate into mitochondria by blocking PDH, thus favoring glycolysis over the TCA cycle in energy production. HIF-1α is increased when oxygen tension is low or when its inhibitor, the tumor suppressor VHL, is lost. Activation of mTOR, even in the absence of hypoxia, also augments HIF-1α levels.

Tumor Suppressors Regulate Metabolism

The ability of oncoproteins to accelerate anabolism is normally balanced by the effectiveness of tumor suppressors in preventing runaway metabolism:

- **VHL:** This E3 ubiquitin ligase component is responsible for directing polyubiquitination—and thence degradation—of HIF-1α. VHL thus prevents HIF-1α from redirecting the cell's energy production toward glycolysis.
- **PTEN:** Activation of mTOR, leading to enhanced glycolysis, among other things, is a direct consequence of increased Akt triggering of PI3K (see above). PTEN strongly inhibits PI3K. It decreases mTOR activity, reduces HIF-1α and limits GLUT1 production (Fig. 5-50). Because it inhibits HIF-1α, PTEN also prevents HIF-1α–induced blockage of mitochondrial use of pyruvate to drive the TCA cycle forward.
- **p53:** In addition to its many other regulatory functions, p53 controls and directs cellular metabolism. It has been suggested, in fact, that the main reason for the Warburg phenomenon is that many tumors inactivate p53. The actions of p53 in orchestrating cellular metabolism are summarized in Fig. 5-51 and described below. *It is instructive not only to view p53 activities as directing certain energy-producing functions but also to appreciate how loss of p53 activity (e.g., by mutation) affects all of these activities.* p53 is activated by AMPK in response to metabolic stress. As a result, p53:
  - upregulates a glycolysis regulator, **TIGAR** (TP53-induced glycolysis regulator) that blocks aerobic glycolysis and shunts its intermediates to other pathways;
  - decreases glucose transporter (mainly GLUT1) synthesis and so impedes glucose entry into cells;
  - blocks nuclear factor-κB (NFκB), thus hindering its direct and indirect activation of glycolysis (NFκB upregulates HIF-1α);
  - increases the synthesis of a stimulator of cytochrome oxidase, **SCO_2,** which then increases mitochondrial electron transport and thus
  - increases pyruvate and glutamine importation into mitochondria and incorporation into the TCA cycle by upregulating PDH;
  - lowers c-Myc levels by activating miR-145, a direct inhibitor of c-Myc; this, in turn, decreases c-Myc-stimulated HIF-1α production and increases oxidative phosphorylation;
  - indirectly impedes fatty acid biosynthesis; and
  - upregulates intermediate molecules that can trigger autophagy (see below).

  The net metabolic effect of p53, acting in all of these ways, is to shunt energy production away from glycolysis and toward oxidative phosphorylation. Thus, loss of p53 leads directly to the Warburg phenomenon.

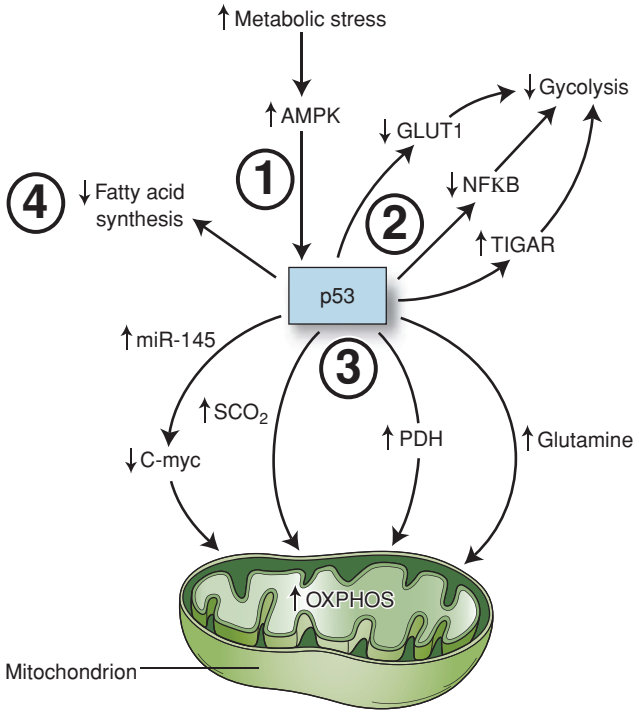


FIGURE 5-51. p53 regulation of cellular metabolism. In addition to its other functions, p53 is a critical metabolic regulator. It prevents cancer cells from achieving their malignant potential by multiple pathways. **1.** p53 is activated by the increased metabolic stress attendant to increased cellular proliferation. This activates adenosine monophosphate (AMP)-protein kinase (AMPK), which in turn activates p53. **2.** p53 directly downregulates transcription of GLUT1 and nuclear factor-κB (NFκB). It also upregulates TIGAR (TP53-induced glycolysis regulator), which impedes glycolysis and directs glycolytic intermediates into other pathways. **3.** It increases TCA cycle activity in several ways. p53 upregulates SCO_2, a stimulator of cytochrome oxidase that directly increases mitochondrial electron transport. It also increases pyruvate and glutamine incorporation into the tricarboxylic acid (TCA) by upregulating pyruvate dehydrogenase (PDH). As well, it upregulates miR-145, which directly downregulates c-Myc and so prevents Myc-mediated metabolic effects (Fig. 5-49). **4.** As well, p53 downregulates a key enzyme that mediates fatty acid synthesis.

It is worth noting in this context that p53-related metabolic protection is triggered by a sequence of events in which AMPK is activated. AMPK directly inhibits mTOR (see above), and pharmacologic stimulation of AMPK (e.g., with metformin) has been used in tumor therapy.

- **Isocitrate dehydrogenase (IDH):** This TCA enzyme has turned out to be a potent tumor suppressor. One allele of IDH is mutated in a high percentage of malignant gliomas (see Chapter 32) and in myelodysplastic syndromes (MDSs; see Chapter 26). The oncogenic mutation results in a gain-of-function alteration, which leads to generation of large amounts of a new product (called **R-2-hydroxyglutarate, or R2HG)**. R2HG directly inhibits the TET family of DNA hydroxylases and also a family of histone demethylases (see above). The result is that TET2-related histone demethylation and 5-methylcytosine hydroxylation (see above) are not available to undo the downregulation of tumor suppressor gene promoters by CpG and histone methylation, such as occurs during oncogenesis. This inactivation of TET tumor suppressors facilitates tumor development.

Tumor-Associated Stroma Supports Cancer Cell Metabolism

The Warburg effect, which is based on observations of cultured cancer cells, is generally accepted as reflecting cancer cell metabolism. It may not, however, be the whole story. In vivo, cancers contain tumor cells and stromal cells. These latter both strongly influence, and are strongly influenced by, the metabolism of their malignant neighbors.

Stromal Cell Responses to Tumor Cell–Derived Triggers

Tumor cells create a milieu of oxidative stress. Stromal fibroblasts respond to this in a sequence of events that involves impaired mitochondrial function. This, in turn, increases their ROS levels. Increased ROS further damages stromal cells' mitochondria, which leads to increased ROS and even greater impairment of mitochondrial function, in a vicious cycle (Fig. 5-52A). Resulting ROS generated by the stromal cells affect adjacent tumor cells by promoting even greater destabilizing changes in their already destabilized cancer cell DNA.

The mitochondrial injury that stromal cells sustain in this process eventually leads to autophagic destruction of the damaged organelles (mitophagy; see Chapter 1). Deprived of much of the machinery of oxidative phosphorylation, stromal cells engage in more aerobic glycolysis. They therefore produce and export more lactate (see above), which is used as a source of energy and a biosynthetic substrate by nearby cancer cells, as detailed above (Fig. 5-52B). Therefore, tumor cells induce metabolic alterations in their nonmalignant stromal neighbors that cause them to undergo oxidant injury and autophagy, and to supply tumor cells with abundant lactate for use in sustaining multiple nefarious cancer cell activities.

Autophagy Is Closely Regulated in Cancer Cells

Autophagy (see Chapter 1) is a process of recycling and removing, mostly of cellular constituents. It was first noted to be a cellular response to supplying metabolic needs in times of stress (e.g., starvation). As such, it might be of considerable utility to tumors that experience episodic depletion of energy and metabolic substrates. However, perhaps counterintuitively, the key activator of autophagy, Beclin-1, is among the genes most commonly mutated in human cancers. Mice with one or both Beclin-1 genes deleted develop far more cancers than do animals with both genes intact.

Why? To date, the best understanding of the explanation focuses on functions of autophagy not directly related to cellular nutrition. Autophagy is a key means by which oxidant-damaged cell proteins and organelles are recognized and removed. If autophagy is impaired, oxidant injury accumulates, with two important consequences. First, oxidatively damaged cellular molecules can accumulate, aggregate and mediate further oxidant injury (see Chapter 1), eventually increasing the DNA mutation rate and leading to genomic instability (see below). Accumulation of damaged cell constituents relies on the autophagy-related protein, p62 (see Chapter 1). p62 is in fact central to tumorigenesis and is overexpressed in many human tumors. Without it, the oxidized aggregates do not form and resultant oxidant genetic damage does not occur.

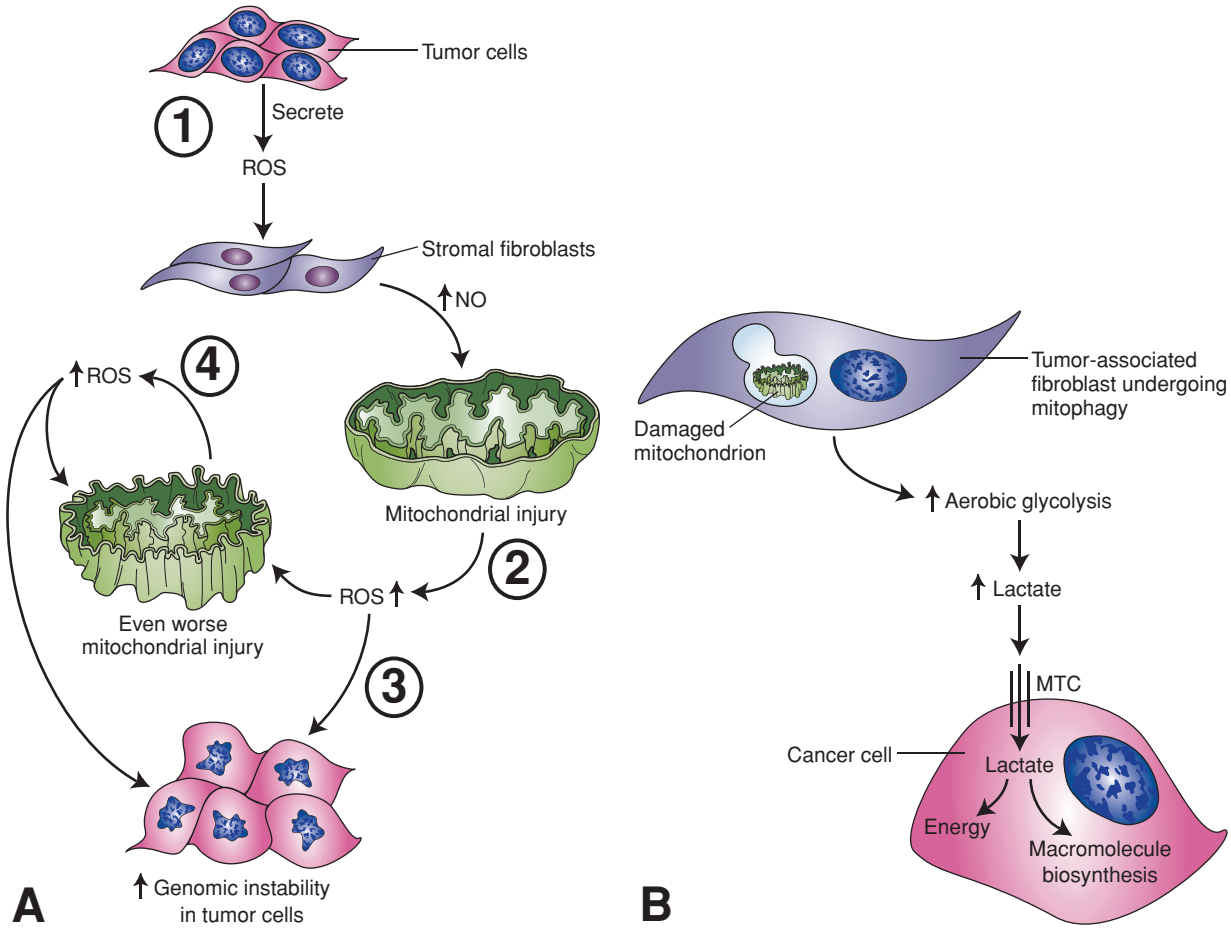


FIGURE 5-52. Stromal cell responses to tumor cell–derived signals. A. Role of reactive oxygen species (ROS). Tumor cells manipulate stromal cells so as to augment tumor cell metabolic activity. **1.** ROS elaborated by tumor cells stimulate stromal cells to increase their production of nitric oxide (NO). This NO causes mitochondrial injury in the stromal cells. **2.** As a result, stromal cells generate excessive ROS. **3.** Increased stromal cell ROS generates more oxidative injury in neighboring cancer cells, leading to increased genomic instability in the tumor. **4.** Increased stromal cell ROS also increases stromal cell mitochondrial injury, creating a vicious circle and magnifying tumor cell genomic instability. **B. Altered metabolism in tumor-associated fibroblasts.** Mitochondrial damage in tumor-associated fibroblasts leads to autophagy of damaged mitochondria (mitophagy). Resulting loss of mitochondria directs more fibroblast metabolism toward glycolysis, producing lactate, which is secreted by the stromal cells. Lactate is taken up by tumor cells, via MTC, and used for macromolecule biosynthesis and other tumor cell metabolic activities.

Second, if autophagy is impaired, accumulation of damaged and damaging cell components will lead to cell death. But p53-dependent apoptosis in such settings requires that the process of autophagy is intact. As a consequence, the cell dies, not by apoptosis, but by necrosis. Unlike apoptosis, necrotic cell death elicits inflammatory responses, including such shady characters as tumor-associated macrophages that facilitate and further tumorigenesis (see above).

Many tumor suppressors, such as PTEN and the tuberous sclerosis proteins (TSC1, TSC2), constitutively facilitate autophagy. The supreme tumor suppressor, p53, has an ambiguous relationship with autophagy, stimulating it in some ways and inhibiting it in others. Several oncogenic proteins (Akt, Bcl-2, mTOR) impair autophagy, underscoring its importance as an antioncogenic process. Important genes involved in autophagy, such as Beclin-1, are commonly mutated in many human cancers. Interestingly, several antineoplastic medications strongly promote autophagy. Although the connection between autophagy and cancer is not fully understood, impairment of the tumor suppressor function of autophagy may result in accumulation of materials within the cell that cause chromosomal instability, which ultimately may lead to cancer development.

GENETIC INSTABILITY IN CANCER

The pathogenesis of cancer involves multiple genetic—and, undoubtedly, epigenetic—changes, and genomic instability is a key contributor to these processes. Although not universal in tumors, **chromosomal instability (CI)** entails additions or deletions of entire chromosomes, or portions thereof, to yield variable cellular karyotypes. CI may result in **aneuploidy** (abnormal chromosome number), **gene amplification** (increased copy number of a gene) and **loss of heterozygosity** (loss of one allele out of a pair). LOH may reflect loss of a whole chromosome, deletion of a bit of DNA bearing the gene in question or inactivation of that gene. As a result, the remaining allele is the only one for that locus and controls the phenotype. If that remaining allele is rendered abnormal, the lack of a second allele to counterbalance it means that its abnormal phenotype is unopposed. Moreover, the phenotype of

the remaining allele may promote the development of cancer. Typically, about one fourth of alleles are lost in malignancies.

Mechanisms of Altered Activation of Cellular Genes

There are three general mechanisms by which proto-oncogenes become activated:

- A mutation in a proto-oncogene leads to **constitutive production of an abnormal protein.**
- Increased expression of a proto-oncogene causes **overproduction of a normal gene product**.
- Activation or expression of proto-oncogenes is regulated by numerous auto-inhibitory mechanisms that safeguard against inappropriate activity. Many mutations in proto-oncogenes render them **insensitive to normal auto-inhibitory and regulatory constraints** and lead to constitutive activation.

The converse processes apply to inactivation of tumor suppressors (see above). That is, (1) they may suffer mutations that increase production of an abnormal protein that either lacks or interferes with tumor suppression; (2) their effectiveness is rendered useless when a regulatory target is overexpressed, overwhelming a normally expressed suppressor; or (3) their expression is impaired, whether by an inactivating mutation or epigenetic inactivation.

Multiple Mechanisms Generate Genetic Instability

Several mechanisms of genetic instability contribute to tumorigenesis. These include (1) point mutations, (2) translocations, (3) amplifications and deletions, (4) loss or gain of whole chromosomes and (5) epigenetic changes. These types of instability occur in many ways. Among the most important is the loss—whether by inheritance, mutation or epigenetic inactivation—of proteins that protect the cell from mutations. These include cell cycle regulatory proteins (checkpoints, proofreaders, mitosis-related chromosomal sorting proteins, etc.) and proteins that mediate DNA repair functions.

Role of Defects in DNA Repair Systems

An understanding of how defects in DNA repair contribute to oncogenesis was derived in part from observations made in familial cancer syndromes. For example, a type of colon cancer syndrome, hereditary nonpolyposis colon cancer (HNPCC, Lynch syndrome), entails a 75% lifetime risk for colon cancer. The large majority of HNPCC patients have mutations in MLH1 or MSH2 DNA mismatch repair enzymes (see above).

Xeroderma pigmentosum (XP), a hereditary syndrome characterized by enhanced sensitivity to UV light and development of skin cancer, reflects defects in nucleotide excision repair (NER) enzymes. In some common types of spontaneous lung cancer, a majority of cases exhibit mutant proteins involved in NER.

Double-Strand Break Repair and Cancer

As mentioned above, detection of DSBs and initiation of repair processes involve the ATM protein. Mutations in ATM and other enzymes involved in DSB repair are associated with a high frequency of malignant tumors.

Point Mutations

Although humans have evolved highly efficient mechanisms to recognize and repair DNA base changes, single base changes do occur normally, at the rate of 10^{-9}/base/cell division in somatic cells and 10^{-11} in germ cells. Application of advanced DNA sequencing techniques has allowed detection of many of these single base changes—called single nucleotide polymorphisms, or SNPs—in tumors.

Activation by Point Mutation

Conversion of proto-oncogenes into oncogenes may involve (1) point mutations, (2) deletions or (3) chromosomal translocations. The first oncogene identified in a human tumor was activated *HRAS* in a bladder cancer. This gene had a remarkably subtle alteration—a point mutation in codon 12. This change led to the substitution of valine for glycine in the H-ras protein. Subsequent studies of other cancers have revealed point mutations involving other codons of the *ras* gene, suggesting that these positions are critical for the normal function of the ras protein. Since the discovery of mutations in *HRAS*, alterations in other growth-regulatory genes have been described.

Activating, or gain-of-function, mutations in proto-oncogenes are usually somatic rather than germline alterations. Germline mutations in proto-oncogenes, which are known to be important regulators of growth during development, are ordinarily lethal in utero. There are several exceptions to this rule. For example, c-*ret* is incriminated in the pathogenesis of certain heritable endocrine cancers, and c-*met*, which encodes the receptor for hepatocyte growth factor, is associated with a hereditary form of renal cancer.

Chromosomal Translocation

Chromosomal translocations involve joining of a piece of one chromosome with a part of another. These rearrangements generally contribute to tumorigenesis in one of two ways. Sometimes they place a normal gene, like a proto-oncogene, under the control of a promoter that is regulated less effectively than the native proto-oncogene promoter.

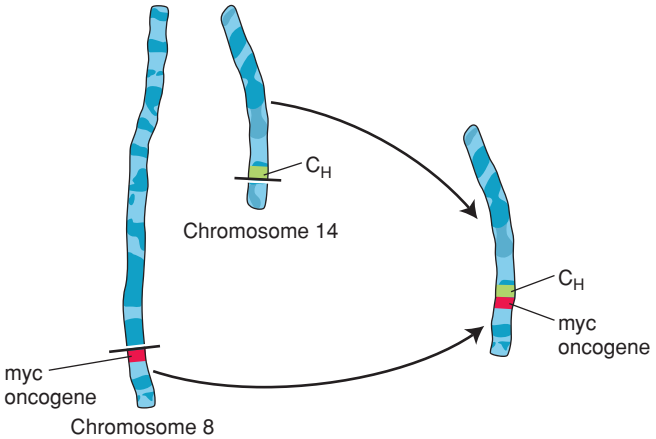


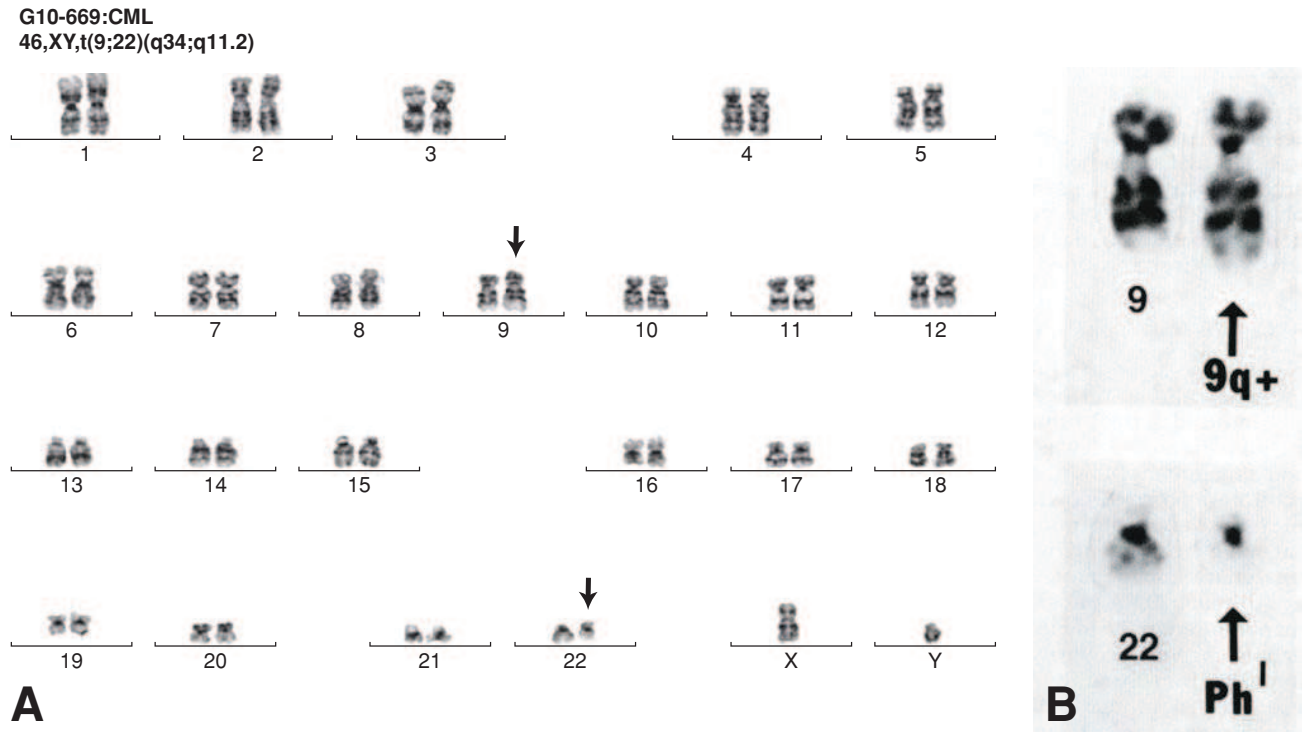
FIGURE 5-53. Schematic representation of the t(8;14) translocation in Burkitt lymphoma. In this disorder, chromosomal breaks involve the long arms of chromosomes 8 and 14. The c-*myc* gene on chromosome 8 is translocated to a region on chromosome 14 adjacent to the gene coding for the constant region of an immunoglobulin heavy chain (C_H). The expression of c-*myc* is enhanced by its association with the promoter/enhancer regions of the actively transcribed immunoglobulin genes.

In 75% of patients with Burkitt lymphoma (see below and Chapter 26), there is a translocation of c-*myc*, a proto-oncogene involved in cell cycle progression, from its site on chromosome 8 to a position on chromosome 14 (Fig. 5-53). This translocation places c-*myc* adjacent to genes that control transcription of the immunoglobulin heavy chains. As a result, the c-*myc* proto-oncogene is activated by the promoter/enhancer sequences of these immunoglobulin genes and is consequently expressed constitutively rather than in a regulated manner. In 25% of patients with Burkitt lymphoma, the c-*myc* proto-oncogene remains on chromosome 8 but is activated by translocation of immunoglobulin light-chain genes from chromosome 2 or 22 to the 3′ end of the c-*myc* gene. In either case, a chromosomal translocation does not create a novel chimeric protein but stimulates the overproduction of a normal gene product. In Burkitt lymphoma, the excessive amount of the normal c-*myc* product, probably in association with other genetic alterations, leads to the emergence of a dominant clone of B cells, driven relentlessly to proliferate as a monoclonal neoplasm. Many other hematopoietic malignancies, lymphomas and solid tumors reflect activation of oncogenes by chromosomal translocation. Although some malignant conditions are **initiated** by chromosomal translocations, during the **progression** of many cancers, myriad chromosomal abnormalities take place (translocations, breaks, aneuploidy, etc.).

Activation by Chromosomal Translocation

In addition, chromosomal translocation may lead to production of a new, abnormal, protein. Thus, a part of one chromosome including part or all of the coding region from a protein (e.g., a proto-oncogene) moves to another chromosome, into the coding region of another gene. The result is a new protein, sharing sequence homology with the original ones, but active in driving oncogenesis in a way that the originals are not.

This process has been implicated in the pathogenesis of several human leukemias and lymphomas. The first and still the best-known example of an acquired chromosomal translocation in a human cancer is the **Philadelphia chromosome,** which is found in 95% of patients with chronic myelogenous leukemia (CML; Fig. 5-54). The c-*abl* proto-oncogene



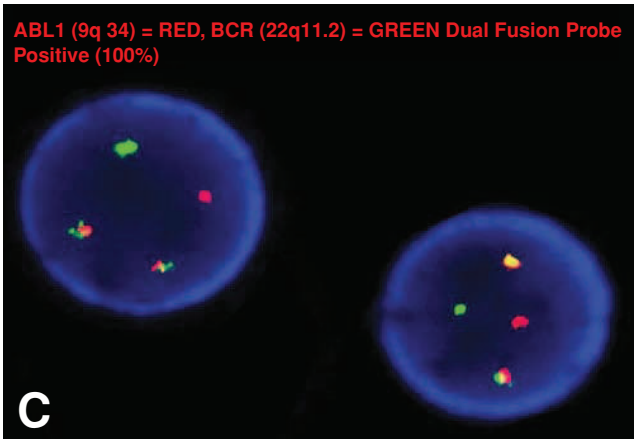


FIGURE 5-54. The t(9;22) translocation in chronic myelogenous leukemia. A. Abnormal karyotype with the shortened chromosome 22 and the longer chromosome 9 highlighted. B. Higher magnification of the translocated chromosomes. C. Fluorescence in situ hybridization (FISH). This assay demonstrates the fusion chromosome using a red ABL chromosome 9 probe and a green BCR chromosome 22 probe, the joining of which yields a yellow signal. Two tumor cells are shown. Each has one normal chromosome 9 and one normal chromosome 22.

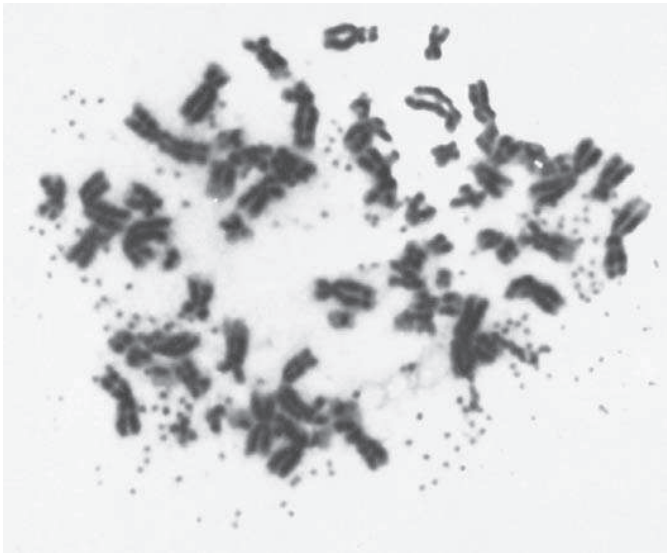


FIGURE 5-55. Double minutes in human cancers. Double minutes in a karyotype of a soft tissue sarcoma appear as multiple small bodies.

on chromosome 9 is translocated to chromosome 22, where it is placed in juxtaposition to a site known as the breakpoint cluster region (*bcr*). The c-*abl* gene and *bcr* region unite to produce a hybrid oncogene that codes for an aberrant protein with very high tyrosine kinase activity, which generates mitogenic and antiapoptotic signals. The chromosomal translocation that produces the Philadelphia chromosome is an example of oncogene activation by formation of a chimeric (fusion) protein. Inhibition of the resulting abnormal kinase by imatinib causes long-term remissions in CML.

Amplifications and Deletions

Genetic amplifications are duplications of variable-sized regions of chromosomes. Cytogenetically, such modifications appear as small DNA fragments that are not part of any chromosome, called "double minutes" (Fig. 5-55), or as increased signal intensity when fluorescent probes for specific regions hybridize with chromosomes. These changes not infrequently affect oncogenes, drug resistance genes or related nefarious characters along with adjacent genomic fragments.

Activation by Gene Amplification

The *ERBB2* proto-oncogene is amplified in up to a third of breast and ovarian cancers. The *ERBB2* gene (also called *HER2/neu*) encodes a receptor-type tyrosine kinase that structurally resembles the EGF receptor. Amplification of *ERBB2* in breast and ovarian cancer (Fig. 5-56) may be associated with poor overall survival and decreased time to relapse. In this context, an antibody targeted against *HER2/neu* (trastuzumab) is now used as adjunctive therapy for breast cancers that overexpress this protein.

Inactivation by Deletion

Deletions, naturally, are lost chromatin. These can vary from tiny pieces to whole arms of chromosomes. Just as amplifications tend to occur at sites of oncogenes, deletions that come to our attention in cancer cells tend to affect tumor suppressor genes.

Alterations in Chromosome Number

Addition or loss of whole chromosomes generally occurs during mitosis and is thought to reflect defects in binding of the mitotic spindle to chromosomal kinetochores (see above), possibly due to malfunctioning of the Aurora B kinase apparatus (see above). As a consequence, chromosomes attach too avidly to mitotic spindles and fail to separate and segregate appropriately.

Almost all solid tumors have abnormal karyotypes. Commonly, tumors lose one copy of a chromosome 10, where the gene for PTEN (see above) resides, or possess extra copies of chromosomes that carry particular oncogenes.

A tumor with a normal karyotype may still have experienced chromosomal loss, however. One parental chromosome of any particular pair may be lost, only to be replaced by a reduplicated copy of the copy of that chromosome derived from the other parent. That is, one parent's copy of a chromosome may be replaced by a duplicated copy of the other parent's chromosome of the same number. The resulting so-called copy-neutral loss of heterozygosity (CN-LOH) is called **uniparental disomy**, and is common in many malignancies. CN-LOH has prognostic significance in several cancer types, such as acute myeloid leukemias.

Epigenetic Modifiers in Cancer Genomic Instability

There are, as discussed above, many heritable factors that can affect gene expression without necessarily changing DNA base sequence. The scope of epigenetic alterations in human cancers is barely understood, but all evidence to date indicates that such modifications impact cancer development and progression profoundly.

A mutation in a gene that participates in chromatin remodeling is seen in most malignant rhabdoid tumors. This mutation occurs in diploid cells without obvious genomic

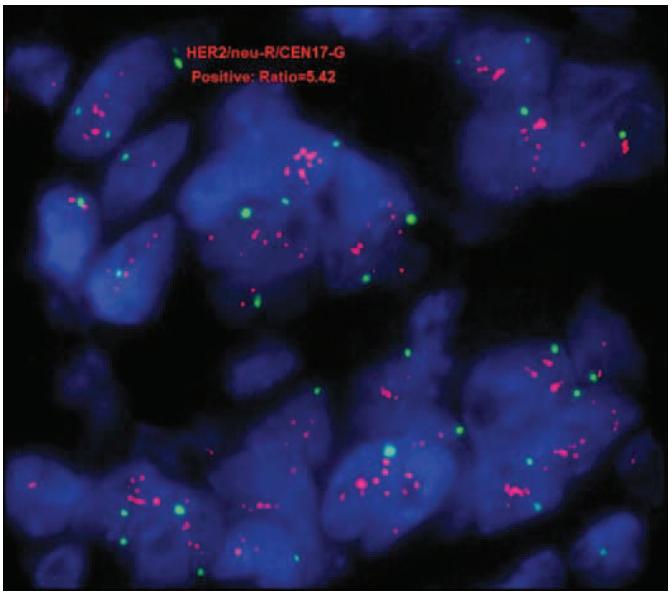


FIGURE 5-56. *ERBB2* amplification in human cancers. ERBB2 also called HER2/neu amplification in a human breast cancer (fluorescence in situ hybridization [FISH]), showing the multiple copies (red fluorescence) as minute bodies. As a chromosome control, a green probe for chromosome 17 is shown.

alterations or gene amplifications/deletions. It is, however, associated with profound changes in gene expression.

Epigenetic modifiers need not only involve direct mechanisms of cell proliferation. They may, for example, affect cellular sensitivity to chemotherapeutic agents or provide escape routes if an enabling mutation is targeted by a particular drug.

The Role of the Immune System in Carcinogenesis Is Unclear

The immune system distinguishes self from nonself molecules and is very effective in combating infectious agents. The notion that it plays a role in suppressing tumor development is rooted in the concept of tumors as nonself entities, with unique "tumor-specific antigens" that can elicit protective immunologic responses. This principle has been extensively demonstrated in experimental animals.

Experimental systems in which tumors are induced by powerful chemical carcinogens have shown that such tumors may be highly immunogenic, particularly when transplanted into immunocompetent recipients. Mice with defects, whether in innate or adaptive immunity (see Chapter 4), develop tumors more often than immunocompetent animals. Similarly, people with immune deficiencies, such as patients with AIDS, are also more prone to cancers than are immunocompetent individuals.

The tumors that develop in these settings, however, bear little resemblance to most human cancers. Potent carcinogens are powerful mutagens and can cause new tumor antigens to arise because of substantial genetic alterations. There is little evidence that spontaneous human tumors bear such antigens. As well, the tumors that occur in immunocompromised humans and animals are almost always virus induced and, again, dissimilar to the tumors that normally afflict people (lung, colon, etc.) that are not connected to infectious agents. The immune deficits thus can be seen as defects in virus clearance (or removal of virus-infected cells), rather than as antitumor surveillance or defense mechanisms.

Inflammatory Cells Nearby Tumors: Friends or Foes?

Pathologists have observed for many years that mononuclear inflammatory cells often accompany cancers (Fig. 5-57). Once an understanding of the role of lymphocytes in immune function had developed, it was a short step to conclude that these lymphocytes (and other cells) were part of a host response to the presence of tumor. The conclusion that tumors produced antigens that elicited such a response was seemingly inescapable.

There are reports that, for certain kinds of tumors, abundant mononuclear infiltration near the tumor may correlate with a good prognosis. Such observations led to many attempts at "immunotherapy," either to elicit or to magnify immune responses against tumors. Immunotherapy was particularly attempted for tumors for which such infiltrates were common, if not invariable, concomitants. Melanomas are an example. To date, immunotherapy of any type of tumor remains experimental, with effectiveness as a treatment modality still unproven.

Even the presence of mononuclear infiltrates near tumors does not necessarily mean that those mononuclear cells signify host immunity against the tumor. Tumor-associated mononuclear infiltrates may not, in fact, be inflammatory. Rather, as indicated above, such cells—far from representing host defenses against a foreign invader—often actually serve the purposes of developing tumors. In short, the function of tumor-associated mononuclear infiltrates is not settled.

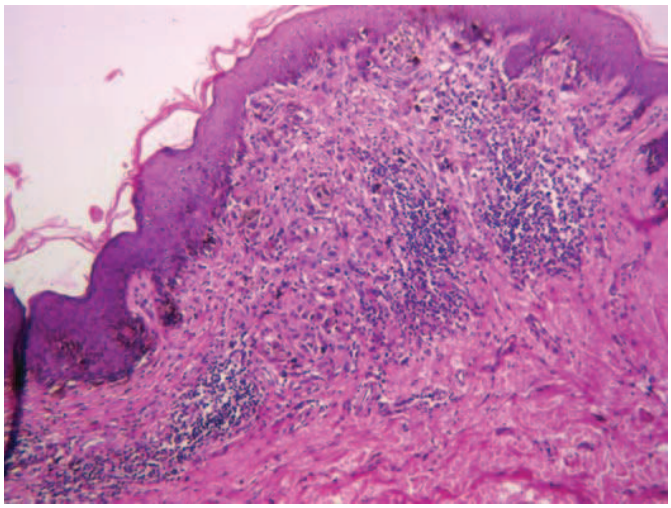


FIGURE 5-57. Mononuclear infiltrate adjacent to a malignant melanoma. Extensive mononuclear cell (mostly lymphocytes) near a primary malignant melanoma in the skin. The significance of the mononuclear cells, traditionally considered to represent host immune responses to the presence of a "foreign tumor antigen", is unclear.

Tumor Antigens

Most human cancers reflect somatic mutations that may theoretically produce mutant proteins, which in turn serve as targets of the immune system. In addition, normal proteins may be overexpressed, and posttranslational modifications of normal proteins may produce altered antigens. Tumor antigens not associated with oncogenic viruses may be categorized as follows:

- **Tumor-specific antigens (TSAs):** These represent somatic mutations or alterations in protein (and other) processing, unique to tumors.
- **Tumor-associated antigens (TAAs):** These reflect the production of normal proteins, either in excess or in a setting different from their normal expression.

Tumor-Specific Antigens

Most tumor-related mutations occur in intracellular proteins, which could theoretically offer immunologic targets. However, most TSAs tend to be specific for individual patients' tumors, and not for tumor types, making immunologic targeting for therapy complicated and highly personalized. Nevertheless, since TSAs are expressed only by the cancer cells and not in normal tissues, there should be no preexisting immune tolerance to them and they are theoretically excellent candidates for tumor immunotherapy. These conclusions hold true for normal proteins that undergo aberrant posttranslational modifications, such as altered glycosylation, lipid association and so forth.

Tumor-Associated Antigens

TAAs are molecules that are shared between cancer cells and normal cells and include:

- **Oncospermatogonial antigens:** These molecules are normally only seen in testicular germ cells but may be produced by malignant cells. Since the testis is an immunologically privileged site, such molecules are not normally exposed to the immune system. However, immune reactivity of both cell- and antibody-mediated limbs to these antigens tends to be weak.
- **Differentiation antigens:** These molecules are seen on normal cells of the same derivation as the cancer cells. As an example, CD20, which is a normal B-cell differentiation antigen, is expressed by some lymphomas, and anti-CD20 antibody (rituximab) is effective treatment for such tumors.
- **Oncofetal antigens:** These antigens are made by normal embryonic and fetal structures and by several cancers (e.g., carcinoembryonic antigen, α-fetoprotein).
- **Overexpressed antigens:** These are normal proteins that are overproduced in certain malignant cells (e.g., prostate-specific antigen, HER2/neu).

Since TAAs represent a class of antigens that is principally recognized as "self" by the immune system, and so have elicited tolerance, they do not lead to effective immunologic responses.

To date, the evidence for natural control of neoplasia by immunologically mediated mechanisms (immune surveillance) in humans is scanty. Most interest in this area is directed toward possible therapeutic applications.

The potential development of effective cancer immunotherapy is complicated by tumor mechanisms to evade immunologically mediated destruction (Table 5-10). Among tumors' escape routes from immune attack are production of immunosuppressive cytokines, resistance to lysis by cytotoxic lymphocytes, inhibition of apoptotic signaling and changes in antigenic profiles. Interestingly, there is substantial evidence implicating mutant p53 as protecting cancer cells from granzyme-mediated apoptosis (see Chapter 1) caused by cytotoxic T lymphocytes (CTLs).

Another effect of cancer immunotherapy that must be overcome is related to tumor heterogeneity. Antibodies or CTLs directed against tumor antigens may lead to selective emergence of malignant clones that have lost these antigens. Nevertheless, major efforts to develop new immune therapies for cancer continue.

TABLE 5-10
POTENTIAL PATHWAYS FOR TUMOR CELL AVOIDANCE OF IMMUNOLOGICALLY MEDIATED DESTRUCTION

Related to CTLs
Development of immune tolerance
Failure of helper T cells
Low numbers of sensitized CTLs
Lack of specificity for malignant cells
Barriers to entry of CTLs into tumor environment
Impairment of signal transduction in T cells
Deficiencies in CTL cytolytic activity
Regulatory T cells block antitumor activity
Related to Tumor Cells
Failure of tumor cells to stimulate latent lymphocyte reactivity
Low levels of tumor antigen production
Weak immunogenicity of tumor antigens
Decreased MHC antigens on tumor cell membranes
Elaboration of immunosuppressive molecules by tumors
Resistance of cancer cells to apoptosis and other cell death mechanisms
Tumor cells cause CTLs to undergo apoptosis

CTL = cytotoxic T lymphocyte; MHC = major histocompatibility complex.

CANCER STEM CELLS AND TUMOR HETEROGENEITY

Most Cancers Are of Monoclonal Origin

Studies of human and experimental tumors indicate that most cancers arise from single transformed cells. This conclusion is best established for proliferative disorders of the lymphoid system, in which clonality is easiest to assess. Neoplastic plasma cells in multiple myeloma produce a single immunoglobulin species, unique to each individual patient and consistent in that patient over time. Monoclonal T-cell receptor and immunoglobulin gene rearrangement, as well as monoclonal cell surface markers, establish a monoclonal origin for many lymphoid malignancies. The cells of B-cell lymphomas exclusively display either κ or λ light chains on their surfaces, while polyclonal lymphoid proliferations—which are almost always benign—contain a mixture of cells, some with κ, and some with λ, light chains.

Monoclonality has also been demonstrated for many solid tumors. One of the best examples of this principle utilized glucose-6-phosphate dehydrogenase in women who were heterozygous for its two isozymes, A and B (Fig. 5-58). These isozymes are encoded by genes located on the X chromosome. Since one X chromosome is randomly inactivated, only one of the two alleles is expressed in any given cell. Thus, although the genotypes of all cells are the same, half of cells express only A; the rest express only B. Examination of benign uterine smooth muscle tumors (leiomyomas, or "fibroids") revealed that in any individual tumor all cells expressed either A or B. No tumor included a mixture of A-expressing cells and B-expressing cells. Thus, each tumor was derived from a single progenitor cell. Oligoclonal tumors have been described, but they are rare and are usually caused by infection with oncogenic viruses (see below).

Cancer Stem Cells Are Primordial Malignant Cells from Which Tumors Arose and Which Can Generate, and Regenerate, the Tumors

Only a minute proportion of the cells in a malignant tumor can produce a new tumor when they are transplanted

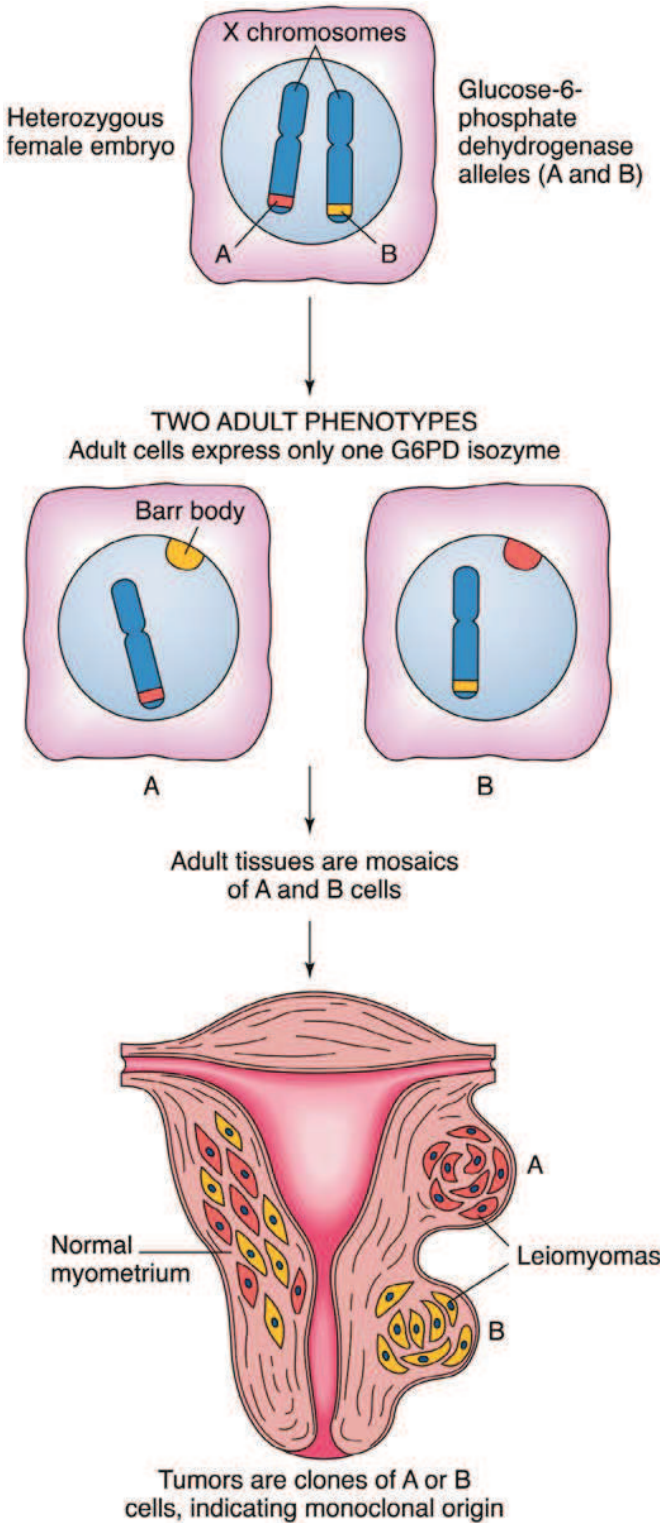


FIGURE 5-58. Monoclonal origin of human tumors. Some females are heterozygous for the two alleles of glucose-6-phosphate dehydrogenase (G6PD) on the long arm of the X chromosome. Early in embryogenesis, one of the X chromosomes is randomly inactivated in every somatic cell and appears cytologically as a Barr body attached to the nuclear membrane. As a result, the tissues are a mosaic of cells that express either the A or the B isozyme of G6PD. Leiomyomas of the uterus have been shown to contain one or the other isozyme (A or B) but not both, a finding that demonstrates the monoclonal origin of the tumors.

into immunologically deficient animals. Normal tissues contain pluripotent somatic stem cells, which can both replenish their own numbers (self-renewal) and differentiate into more mature derivative cells. Cancers also have a small population of malignant cells with such capabilities. These are called **cancer stem cells (CSCs)**. Their existence has been most convincingly demonstrated in hematologic malignancies like acute myeloblastic leukemia (AML), but there is also strong evidence for their existence in an increasing number of solid tumors.

In AML, far less than 1% of leukemic cells express hematopoietic stem cell membrane markers ($CD34^+$, $CD38^-$). Only these cells among the entire leukemic population can reestablish leukemia in an appropriate transplant recipient host. Comparable, but not identical, data have been obtained from studies of cancers of the breast, colon and brain, in which different markers identify CSC-rich cell populations and exclude the vast majority of tumor cells, which cannot recapitulate tumorigenesis. CSCs are defined functionally. The respective markers allow us to identify populations that are enriched for stem cells, but not pure CSCs. Only some of the cells in those populations function as cancer stem cells.

Derivation of CSCs

The origins of CSCs are murky. In some cases, they may derive from the pluripotent somatic stem cells of the affected organ, for example, hematopoietic stem cells in the case of AML (Fig. 5-59A). In other cases, lineage-committed progenitor cells may be the culprits. Such cells are multipotent, but not pluripotent, at the time of transformation. They may reacquire a degree of "stemness," allowing them both to repopulate their own numbers and to differentiate into more committed cells (Fig. 5-59B). Therefore, it is most likely that CSCs can arise both from tissue stem cells and from slightly differentiated immediate progeny of these tissue stem cells. Lurking within the tumor, they function as a reservoir of cells that continue to provide more differentiated tumor cells and that can regenerate the entire tumor, should that become necessary.

Tumor Cells Derived from CSCs

Although almost all tumors begin as single clones of neoplastic cells, as they grow, their cells show considerable variation in appearance (Fig. 5-60) and behavior. Diversity of cells among a tumor population has broad implications for tumor progression and dissemination, as well as for responses to chemotherapy and the development of resistance to these agents. Several theories, which are not necessarily mutually exclusive, and which all may be correct in some cases, have been proposed to account for the development of phenotypic diversity of cells in tumors.

It is critical to understand that the overwhelming majority of tumor bulk is composed of these derivative cells. Treatments that reduce tumor volume mostly target these cells, and consequent reduced tumor volume reflects the susceptibility of these cells—not CSCs—to the therapies employed. However, as will become clear (see below), reduced tumor volume does not equate with elimination of CSCs, nor does it necessarily affect the ability of CSCs to regenerate the tumor after treatment.

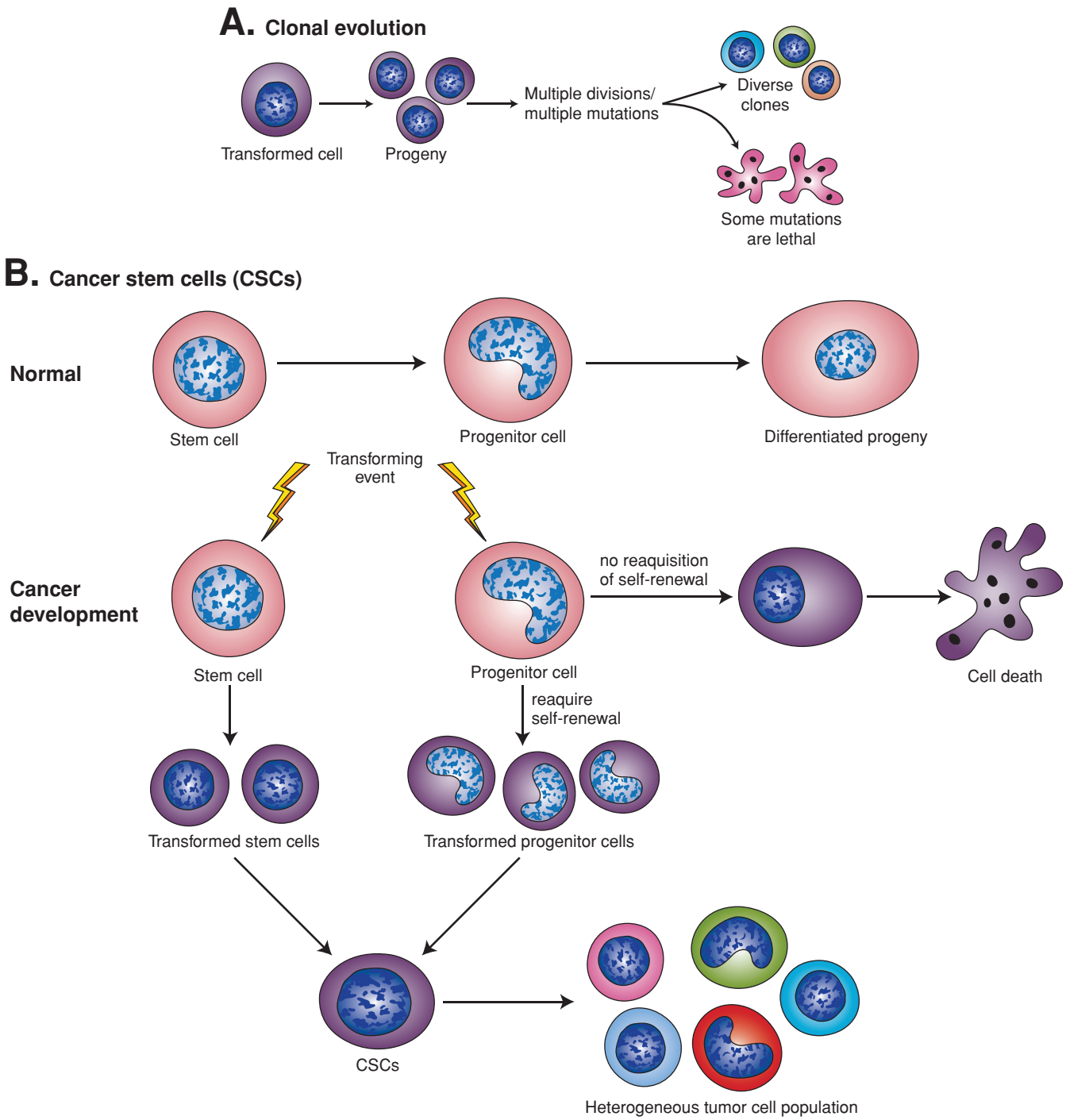


FIGURE 5-59. Tumor stem cells and tumor heterogeneity. A. Linear progression of tumor clonal evolution. Proliferating cancer progenitor cells eventually develop a variety of mutations, with different individual cells acquiring different mutations, leading to heterogeneity in the tumor cell population. Some such mutations are inconsistent with cell survival, while others facilitate cancer progression. This model is most consistent with critical enabling primordial mutations in a stem cell that must be retained throughout subsequent tumor evolution. **B. Cancer stem cells and progenitor cells.** Normally (*above*) stem cells give rise to committed progenitor cells. These then produce terminally differentiated cells. An oncogenic stimulus (*below*) to a stem cell may lead to an expanded pool of transformed stem cells. These become cancer stem cells (CSCs). Alternatively, the oncogenic stimulus may affect a committed progenitor cell. If the latter recapitulates a program of self-renewal, the resulting transformed progenitor may become a CSC. If it does not activate the self-renewal program, resulting differentiated progeny will be produced and eventually die. CSCs generated either via transformation of stem cells or transformation of committed progenitors may then be the antecedents of a heterogeneous malignant cell population.

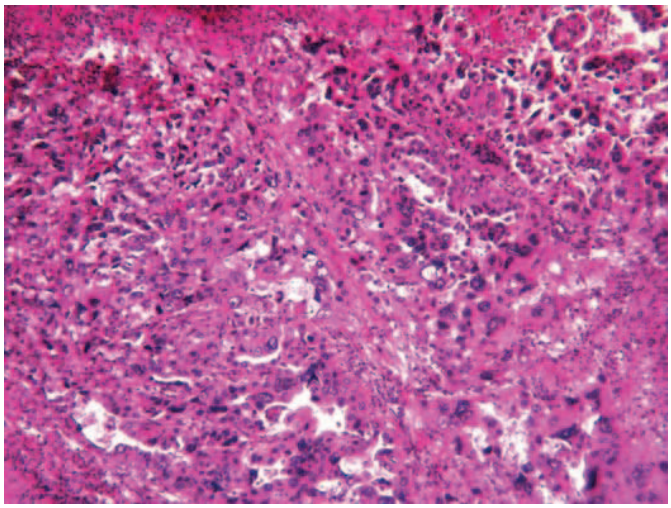


FIGURE 5-60. Phenotypic diversity in human tumors. Human tumor cells show great heterogeneity in their appearance, proliferative activity and so forth. Thus, most human tumors are mixtures of small and large cells, often with diverse shapes, varying nuclear appearances and differences in mitotic activity.

Clonal Evolution

The original explanation of tumor heterogeneity holds that tumor cells progressively accumulate new mutations as they proliferate. A tumor in which many cells are dividing can thus, over time, generate a diverse population of genetically different cells. Some of these cells may be destined for ignominious death, while others may flourish as genetically distinct subclones of the original malignant cells (Fig. 5-59A). Darwinian-style selection—whether due to localized hypoxia, differences in proliferation rates, potential for invasion and metastasis, therapy and so forth—governs which subclones will succeed and which will perish, which will metastasize and which will remain localized.

Epigenetic Cancer Cell Plasticity

Cancer's evil machinations have led to even more devious ways for tumors to maintain themselves and to grow and spread. Thus, for some tumors (e.g., malignant melanomas), tumors adapt to the progressive challenges to survival and dissemination via epigenetic changes (e.g., noncoding RNAs, or expression of proteins that modify histones) (Fig. 5-61). A mass of slowly proliferating tumor cells may alternate among

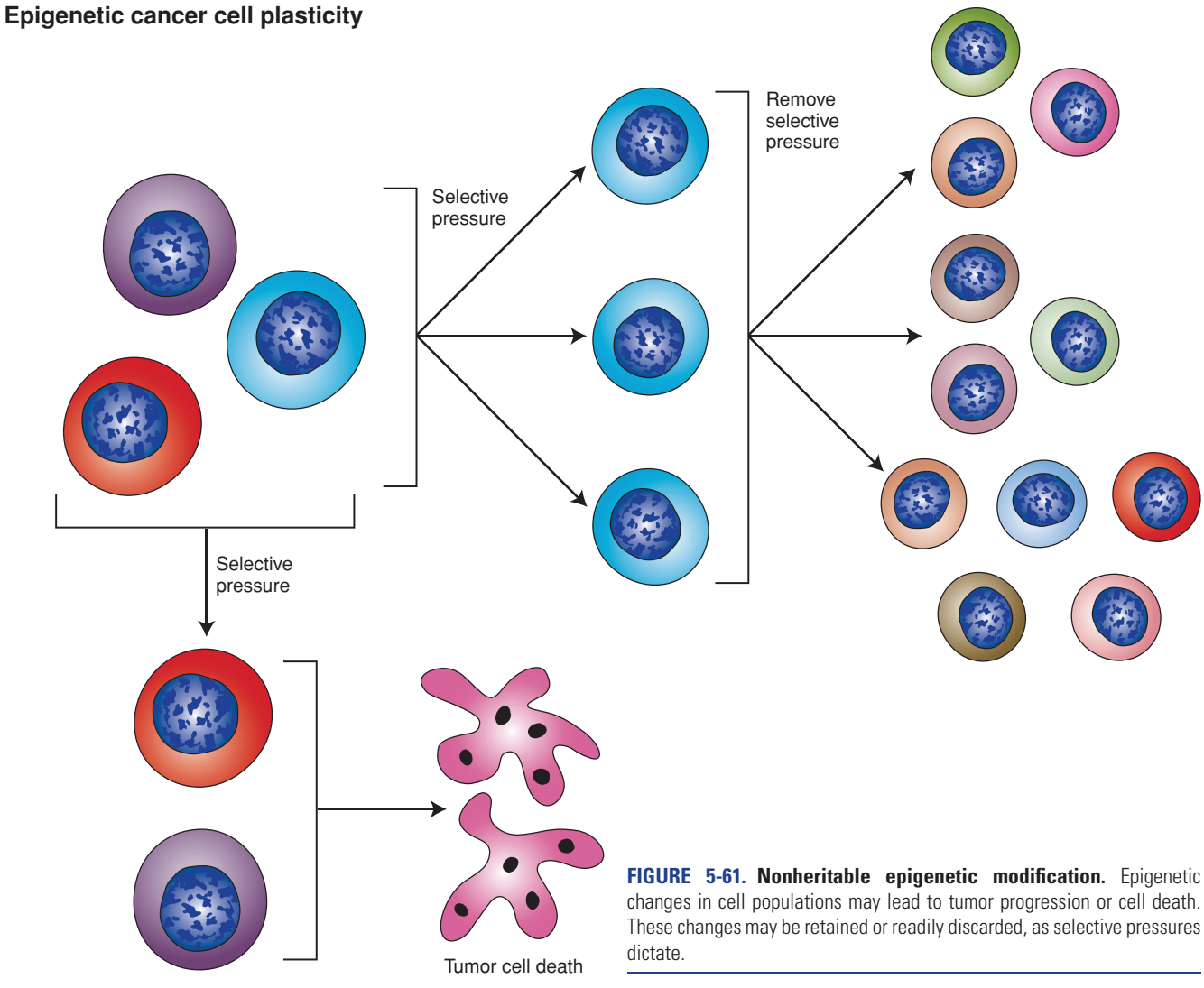


FIGURE 5-61. Nonheritable epigenetic modification. Epigenetic changes in cell populations may lead to tumor progression or cell death. These changes may be retained or readily discarded, as selective pressures dictate.

different epigenetic states, and so fluctuate between the ability to reconstitute a tumor (stem cell–like) and the lack of such ability. This type of deviousness allows for diverse populations of tumor cells to alternate between stemness—slowly dividing, tumor-reconstituting cells—and rapidly dividing, nonreconstituting cells. Furthermore, tumor cells may achieve such metamorphoses without incurring further mutations.

The implications of this phenomenon are substantial. Some malignant tumors may represent constantly shifting therapeutic targets, with incredible plasticity in adapting to a changing chemotherapeutic milieu via the ability to shift phenotypes rapidly to evade antineoplastic drugs, and then to shift back to reemerge from a defensive posture and reassert an aggressive nature.

The Significance of CSCs

CSCs are not merely an experimental curiosity. They are the cells from which many human tumors arise. They divide infrequently, which allows them to evade destruction by cytotoxic chemotherapeutic agents that preferentially kill rapidly dividing cells. Thus, while chemotherapy may destroy the bulk of the rapidly dividing cells in a malignant tumor mass, residual CSCs may survive to regenerate the cancer.

Even more significantly, CSCs in many ways are closer to their normal tissue counterparts than to the cells that make up the bulk of the tumor. They may be far more capable of, for example, repairing DNA damage than their more differentiated malignant derivative cells. Also, because they proliferate less, they rely less on mutant cell activation signaling pathways and resemble normal cells more than do their highly mitotically active progeny. Thus, the main determinants of their survival make them more likely to persevere through treatments, even kinase inhibitors, that kill the rapidly dividing cells that make up the vast majority of the tumor. Radiation of glioblastomas may kill the great majority of the tumor cells, but CSCs are radioresistant. Their numbers, as a percentage of remaining viable tumor cells, increase after radiation. They survive and repopulate the tumor. In this sense, therapy may destroy 99.9%, or 99.99%, of a tumor, shrinking its mass correspondingly, but may do little more than buy the patient a few months more of life.

CSCs have evolved to evade apoptosis and senescence and therefore are likely to be less sensitive to cancer therapies than their normal tissue counterparts. Thus, tumor stem cells are better suited to survive cytotoxic therapy that is likely to kill the normal tissue stem cells from which the CSCs probably derived.

It is therefore critical to bear in mind that the goal of tumor therapy is not to eliminate the bulk of the tumor, but to save the patient's life. This requires approaches that are effective against CSCs because it is these cells, and not the aggregate of their highly proliferating progeny, that will regenerate the tumor after cytoreductive or other therapy. The CSCs, then, are the true enemies; it is they that will kill the patient.

Tumors Are Heterogeneous

We have alluded above to phenotypic heterogeneity in tumor cell morphology and to divergent evolution after tumor cells arise from CSCs (Fig. 5-62). In fact, however, the term "tumor heterogeneity" has two basic and different meanings. **Intertumor heterogeneity** describes the variation (genetic, epigenetic, phenotypic) between tumors that develop in one patient and those that arise in others. **Intratumor heterogeneity** refers to variation in the same parameters among different tumor cells and areas, and between a primary tumor and its metastases, within one patient.

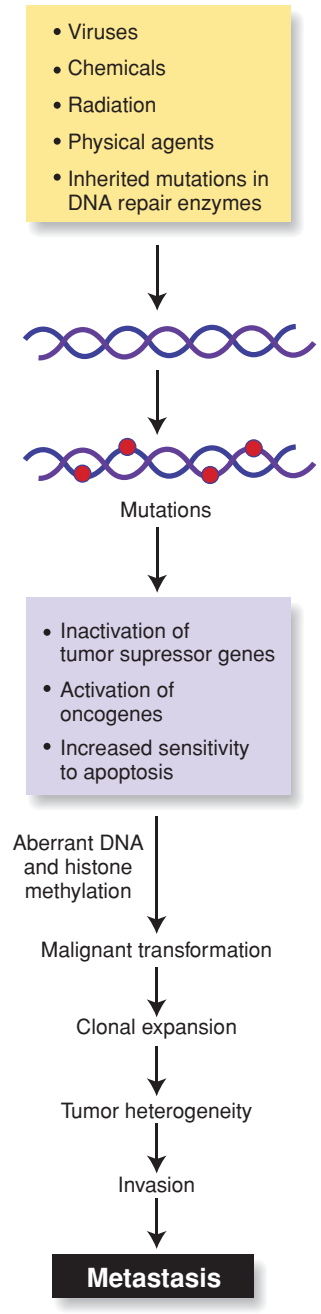


FIGURE 5-62. Summary of the general mechanisms of cancer.

Intertumor Heterogeneity

> Walter Donovan: "…We're on the verge of completing a quest that began [many] years ago. We're just one step away."
>
> Indiana Jones: "That's usually when the ground falls out from underneath your feet."
>
> *Indiana Jones and the Last Crusade*

There are distinct patterns of alterations that are both characteristic of certain tumor types and that offer useful therapeutic

targets, at least for hematologic malignancies. For example, almost every case of Burkitt lymphoma has chromosome rearrangements involving the *MYC* gene on chromosome 8. These tumors seem to follow the paradigmatic sequence shown in Fig. 5-59A: one initial set of mutations triggers the tumor and is needed to carry it through whatever follows. Similarly, almost all cases of chronic myeloid leukemia show the t(9;22) translocation to generate mutant bcr-abl protein. The successful targeting of bcr-abl is emblematic of the goal of developing agents that are specific for mutations that are necessary for tumor survival.

As comforting as this paradigm is for hematologic malignancies (at least selected ones), the situation for characteristic mutations in solid tumors has been more problematic. Even though some studies that have focused on selected individual genes have found mutational patterns, these studies in retrospect may have exercised such high levels of selectivity that many other, perhaps more important, mutations were not detected. More extensive genetic analysis of the protein-coding parts of the genome (whole exome sequencing) have shown wide diversity among individual solid tumors. One study of almost 200 lung cancers showed that only 4 genes were mutated (all SNPs or point mutations) in more than 10% of tumors, and 15% of tumors showed no mutations at all.

Our increasing awareness of the roles of untranslated RNAs in human cancer (see above) underscores this problem. The wider a net we cast, the more we find and the more restricted the applicability of the simple step-wise model shown in Fig. 5-59A appears to be. The extensive tumor-to-tumor diversity (e.g., Fig. 5-61) in patterns of genetic changes in solid tumors underscores the potential complexity of developing effective targeted therapies.

Intratumor Heterogeneity

In addition to variability of one tumor type from one person's tumor to someone else's tumor, all models predict that there will be variability within the tumor of a single individual. If a stochastic (i.e., random) mutation model (e.g., Fig. 5-61) applies to solid tumor evolution, rather than a linear step-wise model in which all cells are progressively derived from identically altered progenitors (as in Fig. 5-59A), one would expect the cells of any individual tumor to be highly heterogeneous, one to the other.

Although only a few such studies have been reported, they generally confirm our worst fears along these lines. It is clear that, at least for some solid tumors, variability is enormous. In one study of renal cell carcinomas, multiple biopsies of a single tumor mass showed that only 34% of protein-coding genetic alterations were concordant *between different pieces of that one tumor mass.* When analyses also included metastases, or comparison of pre- and posttreatment tumor samples, concordance was even lower.

Therefore, sophisticated tools to analyze tumors have not exactly allowed us to impose a man-made paradigm—either analytical or therapeutic—on the field of cancer biology. Rather, these technologies have illuminated the fact that cancers, especially solid ones, are highly diverse genetically and that each patient's individual tumor presents a vast, nonuniform array of mutations. When patient-to-patient variations in tumor genotypes are considered, it is clear that tumors are incredibly more complicated than we had imagined. We are only beginning to lift the veil on that heterogeneity.

Agents Implicated in Causing Cancer

VIRUSES AND HUMAN CANCER

Despite the existence of viral oncogenes and many viruses that are known to cause cancers in mice and other animals, only a few viruses demonstrably cause human cancers. Thus, viral infections are responsible for an estimated 15% of human cancers. The strongest associations between specific viruses and tumors in humans involve:

- Human T-cell leukemia virus type I (HTLV-I) **(RNA retrovirus)** and T-cell leukemia/lymphoma
- Hepatitis B virus (HBV, **DNA**) and hepatitis C virus (HCV, **RNA**) and primary hepatocellular carcinoma
- HPV **(DNA)** and carcinomas of the cervix, anus and vulva, and some oropharyngeal cancers
- Epstein-Barr virus (EBV, DNA) and certain forms of lymphoma and nasopharyngeal carcinoma
- Human herpes virus 8 (HHV8, **DNA**) and Kaposi sarcoma

Worldwide, infections with hepatitis B and C viruses and HPVs alone account for 80% of all virus-associated human cancers.

Human T-Cell Leukemia Virus Type I Is Lymphotropic

The one human cancer that has been firmly linked to infection with an RNA retrovirus is the rare adult T-cell leukemia, which is endemic in southern Japan and the Caribbean basin and occurs sporadically elsewhere. The etiologic agent, HTLV-I, is tropic for $CD4^+$ T lymphocytes and has also been incriminated in the pathogenesis of a number of neurologic disorders. It is estimated that leukemia develops in 3%–5% of people infected with HTLV-I and then only after a latency period of 30–50 years. A closely related virus, HTLV-II, has been associated with only a few cases of lymphoproliferative disorders.

The HTLV-I genome contains no known oncogene and does not integrate at specific sites in the host DNA. Viral oncogenicity appears to be mediated mainly by the viral transcriptional activator Tax. This protein not only drives transcription of the viral genome but also promotes the activity of other genes involved in cell proliferation, such as NFκB and IL-2 receptor. Tax also downregulates the cell cycle control protein, $p16^{INK4a}$ and p53. Lymphocyte transformation in vitro by HTLV-I is initially polyclonal and only later monoclonal. Tax therefore probably initiates transformation, but additional genetic events are required for the complete malignant phenotype.

Hepatitis B and C Viruses Are Responsible for Liver Carcinomas

Epidemiologic studies have established a strong link between primary hepatocellular carcinoma and chronic infection with HBV, a DNA virus, and HCV, an RNA virus (see Chapter 20). Two mechanisms have been invoked to explain the mechanism of carcinogenesis in virus-related liver cancer. One theory holds that the inability of some people to clear these infections leads to continued hepatocyte

proliferation owing to ongoing liver injury, and eventually causes malignant transformation. However, a small subset of patients with HBV infection develop hepatocellular carcinomas in noncirrhotic livers. A second theory implicates a virally encoded protein in the pathogenesis of HBV-induced liver cancer. Transgenic mice expressing HBx, a small viral regulatory protein, also develop liver cancer, but without evident preexisting liver cell injury and inflammation. The *HBx* gene product upregulates a number of cellular genes. It also binds to and inactivates p53. The underlying mechanisms in HBV-induced carcinogenesis are still controversial and require further investigation.

It has not been shown that HCV is directly oncogenic. Tumors, when they develop in HCV-infected patients, tend to do so 20 or more years after primary infection, and then usually in the context of cirrhosis and chronic liver injury. However, some data suggest that expression of HCV core protein may contribute to the pathogenesis of hepatocellular carcinoma, and one of the HCV nonstructural proteins activates NFκB.

DNA Viruses Encode Proteins That Bind Regulatory Proteins

Four DNA viruses (HPV, EBV, HBV, HHV8) are incriminated in human cancers. The transforming genes of oncogenic DNA viruses exhibit virtually no homology with cellular genes, but those of animal RNA retroviruses (oncogenes) are derived from, and are homologous with, their cellular counterparts (proto-oncogenes). As discussed above, oncogenic DNA viruses have genes that encode protein products that bind to, and inactivate, the products of tumor suppressor genes (e.g., *Rb, p53*).

Human Papilloma Virus

HPVs induce lesions in humans that progress to squamous cell carcinoma. They manifest a pronounced tropism for epithelial tissues, and their full productive life cycle occurs only in squamous cells. More than 140 distinct HPVs have been identified, and most are associated with benign lesions of squamous epithelium, including warts, laryngeal papillomas and condylomata acuminata (genital warts) of the vulva, penis and perianal region. Occasionally, condylomata acuminata and laryngeal papillomas undergo malignant transformation to squamous cell carcinoma. Although warts of the skin invariably remain benign, in a rare hereditary disease called **epidermodysplasia verruciformis,** HPV produces benign flat warts that commonly progress to squamous carcinoma. At least 20 HPV types are associated with cancer of the uterine cervix, especially HPV types 16 and 18 (see Chapter 18). This association holds for both ectocervical squamous carcinoma and endocervical adenocarcinoma. A newly available vaccine protects against infection with most oncogenic HPV types and is expected to reduce the incidence of cervical cancer.

In recent years, HPV, especially HPV-16, has been identified in many head and neck squamous cell carcinomas, especially those of the tonsils and oropharynx (see Chapter 29). Similar colocalization has been reported for non–small cell lung carcinomas. Some of these tumor types are also associated with cigarette smoking, and tumors that are HPV positive may also occur in smokers. In addition, high-risk strains of HPV are involved in about 6% of lung cancers that arise in smokers.

The major oncoproteins encoded by HPV are E5, E6 and E7. E6 binds to p53 and targets it for degradation. It also activates telomerase expression and promotes tumor development via other mechanisms that are independent of p53. E7 binds to Rb, thus releasing its inhibitory effect on E2F transcriptional activity and allowing cell cycle progression. E6 and E7 of non-cancer-causing strains of HPV do not have these activities. E5 has been shown to activate the epidermal growth factor receptor. During the last half century, a cell line derived from cervical cancer, *HeLa cells,* has maintained worldwide popularity in the study of cancer. Interestingly, these cells have been found to express HPV-18 E6 and E7, and inactivation of these oncoproteins results in growth arrest. Thus, after many years growing in vitro in innumerable laboratories, these cancer cells remain dependent on the expression of HPV proteins.

Epstein-Barr Virus

EBV is a human herpesvirus that is so widely disseminated that 95% of adults in the world have antibodies to it. EBV infects B lymphocytes, transforming them into lymphoblasts. In a small proportion of primary infections with EBV, this lymphoblastoid transformation is manifested as infectious mononucleosis (see Chapter 9), a short-lived benign lymphoproliferative disease. However, EBV is also intimately associated with the development of certain human cancers. A number of EBV genes are implicated in lymphocyte immortalization, including Epstein-Barr nuclear antigens (EBNAs); certain untranslated nuclear EBV RNAs, called EBER1 and EBER2; and latency-associated membrane proteins (LMPs). As well, about 40 miRNAs are encoded by EBV, some of which activate or repress specific cellular genes. LMP1 interacts with cellular proteins that normally transduce signals from the tumor necrosis factor (TNF) receptor, but it does not trigger apoptosis. Rather, it activates NFκB and other cell division–associated signaling molecules. Generally, EBV-related tumors are ascribed to the activities of the virus's latency-associated genes.

EBV-induced tumors tend to reflect the establishment of patterns of gene expression associated with viral latency. This may happen even in acute infection. EBV, in fact, is unusual in that virus-related lymphomas (see below) can occur during primary exposure. The known three different patterns of EBV latency (called latency I, II and III) have different associations with human malignancies. However, the human tumors that develop as a result all appear to entail viral orchestration of the same types of cancer hallmark traits (see above) that characterize sporadic cancers that occur independently of such infections.

BURKITT LYMPHOMA: EBV was the first virus to be unequivocally linked to the development of a human tumor. In 1958, Denis Burkitt described a form of childhood lymphoma in a geographical belt across equatorial Africa, which he suggested might have a viral etiology. A few years later, Epstein and Barr discovered viral particles in cell lines cultured from patients with Burkitt lymphoma (BL).

African BL is a B-cell tumor, in which the neoplastic lymphocytes invariably contain EBV and manifest EBV-related antigens (see Chapter 26). The tumor has also been recognized in non-African populations, but in those cases only about 20% carry EBV genomes. The localization of BL to equatorial Africa is not understood, but prolonged stimulation of the immune system by endemic malaria may be

important. Normally, EBV-stimulated B-cell proliferation is controlled by suppressor T cells. The lack of an adequate T-cell response often reported in chronic malarial infections might result in uncontrolled B-cell proliferation, thus providing a context for further genetic changes that may lead to the development of lymphoma. One of these is a translocation in which c-*myc* is being brought into proximity to an immunoglobulin promoter. In addition, EBV proteins inhibit apoptosis and activate signaling pathways involved in cell proliferation. Therefore, the multistep pathogenesis of African BL may be viewed as follows:

1. Infection and polyclonal lymphoblastoid transformation of B cells by EBV
2. Proliferation of B cells and inhibition of suppressor T cells induced by malaria
3. Deregulation of c-*myc* by translocation in a single transformed B cell, with effects on other signaling pathways
4. Uncontrolled proliferation of a malignant clone of B lymphocytes

NASOPHARYNGEAL CARCINOMA: Nasopharyngeal carcinoma is a variant of squamous cell carcinoma that is particularly common in certain parts of Asia. EBV DNA and EBNA are present in virtually all of these cancers. Epithelial cells may be exposed to EBV via infected lymphocytes traveling through lymphoid-rich epithelium. One of the EBV proteins in this tumor has been shown to activate the EGFR signaling. Fortunately, 70% of patients with this disease are cured by radiation therapy alone.

OTHER EBV-ASSOCIATED TUMORS: EBV markers have been identified in about half of cases of classical Hodgkin lymphoma, in which the virus infects Reed-Sternberg cells. A number of T-cell and NK lymphomas have also been found to harbor EBV, as well as 5% of gastric carcinomas.

POLYCLONAL LYMPHOPROLIFERATION IN IMMUNODEFICIENT STATES: Congenital or acquired immunodeficiency states can be complicated by the development of EBV-induced B-cell proliferative disorders. These lesions are clinically and pathologically indistinguishable from true malignant lymphomas, but most of them are polyclonal. Lymphoid neoplasia occurs in immunosuppressed renal transplant recipients 30–50 times more often than in the general population. Almost all congenital or acquired immunodeficiencies (especially AIDS) and lymphoproliferative diseases associated with organ transplantation involve EBV. Occasionally, a true monoclonal lymphoma may develop in the background of an EBV-induced lymphoproliferative disorder.

Human Herpesvirus 8

Kaposi sarcoma (KS) is a vascular tumor that was originally described in elderly eastern European men and later observed in sub-Saharan Africa (see Chapter 16). Kaposi sarcoma is today the most common neoplasm associated with AIDS. The neoplastic cells contain sequences of a novel herpesvirus, HHV8, also known as KS-associated herpesvirus (KSHV). HHV8 is present in virtually all specimens of Kaposi sarcoma, whether from HIV-positive or HIV-negative patients, and appears to be necessary—but not sufficient—for development of KS.

Other, unidentified, factors contribute. Many more people are HHV8 positive than ever develop KS. In the United States, about 6% of the population carries HHV8, and 60%–80% of the black population in sub-Saharan Africa is seropositive for HHV 8, but the risk of developing KS is miniscule compared to these percentages. Furthermore, among HIV-1–positive people, the risk of KS is greatest when HIV-1 infection was acquired via sexual transmission, rather than by transfusion or by a baby from an infected mother.

In addition to infecting the spindle cells of Kaposi sarcoma, HHV8 is lymphotropic and has been implicated in two uncommon B-cell lymphoid malignancies, namely, **primary effusion lymphoma** and **multicentric Castleman disease** (see Chapter 26).

Like other DNA viruses, the HHV8 viral genome encodes proteins that interfere with the p53 and Rb tumor suppressor pathways. Some viral proteins also inhibit apoptosis and act in multiple ways to accelerate cell cycle transit. HHV8 encodes an inhibitor of the normal regulator of NFκB (i.e., IκB). As a result, HHV8 infection is associated with unrestrained activation of NFκB. Development and progression of KS seems to entail interdependence between lytic HHV8 infection and latently infected cells. Thus, antiviral drugs that inhibit HHV8 lytic infection provide strong protection from the development of KS.

Other DNA Viruses

There have been intriguing claims that a recently discovered virus, **Merkel cell polyoma virus** (MCV), causes a very uncommon skin tumor, Merkel cell carcinoma. MCV genomes integrated into cellular DNA have been identified in 70% of these tumors. However, serologic evidence indicates that MCV infection is widespread in the general population, so the nature of the association of the virus with the rare tumor that gives MCV its name remains uncertain.

Other viruses have been claimed to be associated with human cancers over the years, but with little or no verifiable data to substantiate those assertions. SV40, which does cause tumors in some rodents, is a case in point. However, after extensive study, there are no reproducible experimental or epidemiologic data to support the contention that SV40 is oncogenic for humans.

Enormous interspecies differences in susceptibility to oncogenicity, and past experience, reinforce concern as to the dangers of falling prey to excessive gullibility and accepting seeming reasonableness as a substitute for hard data. One should be skeptical about such contentions and demand careful studies and independent verification before inculpating any agent as a cause of human cancer.

CHEMICAL CARCINOGENESIS

The field of chemical carcinogenesis originated some two centuries ago in descriptions of an occupational disease (this was not the first recognition of an occupation-related cancer, since a specific predisposition of nuns to breast cancer was appreciated even earlier). The English physician Sir Percival Pott gets credit for relating cancer of the scrotum in chimney sweeps to a specific chemical exposure, namely, soot. Today we realize that other products of the combustion of organic materials are responsible for a man-made epidemic of cancer, namely, lung cancer in cigarette smokers.

The experimental production of cancer by chemicals dates to 1915, when Japanese investigators produced skin cancers in rabbits with coal tar. Since that time, the list of

organic and inorganic carcinogens has grown exponentially. Yet a curious paradox existed for many years. Many compounds known to be potent carcinogens are relatively inert in terms of chemical reactivity. *The solution to this riddle became apparent in the early 1960s, when it was shown that most, although not all, chemical carcinogens require metabolic activation before they can react with cell constituents.* On the basis of those observations and the close correlation between mutagenicity and carcinogenicity, an in vitro assay using *Salmonella* organisms for screening potential chemical carcinogens—the Ames test—was developed a decade later. Subsequently, a variety of genotoxicity assays have been developed and are still used to screen chemicals and new drugs for potential carcinogenicity.

Chemical Carcinogens Are Mostly Mutagens

Associations between exposure to a specific chemical and human cancers have historically been established on the basis of epidemiologic investigations. These studies have numerous inherent disadvantages, including uncertainties in estimated doses, variability of the population, long and variable latency and dependence on clinical and public health records of questionable accuracy. As an alternative to epidemiologic studies, investigators turned to the use of studies involving animals. Indeed, such studies are legally required before the introduction of a new drug. Yet the logarithmic increase in the number of chemicals synthesized every year makes even this method prohibitively cumbersome and expensive. The search for rapid, reproducible and reliable screening assays for potential carcinogenic activity has centered on the relationship between carcinogenicity and mutagenicity.

*A **mutagen** is an agent that can permanently alter the genetic constitution of a cell.* The Ames test uses the appearance of frameshift mutations and base-pair substitutions in a culture of bacteria of a *Salmonella* sp. Mutations, unscheduled DNA synthesis and DNA strand breaks are also detected in rat hepatocytes, mouse lymphoma cells and Chinese hamster ovary cells. Cultured human cells are now used increasingly for assays of mutagenicity. About 90% of known carcinogens are mutagenic in these systems. Moreover, most, but not all, mutagens are carcinogenic. This close correlation between carcinogenicity and mutagenicity presumably occurs because both reflect damage to DNA. Although not infallible, in vitro mutagenicity assays have proved to be valuable tools in screening for the carcinogenic potential of chemicals.

Chemical Carcinogenesis Is a Multistep Process

Studies of chemical carcinogenesis in experimental animals have shed light on the distinct stages in the progression of normal cells to cancer. Long before the genetic basis of cancer was appreciated, it was demonstrated that a single application of a carcinogen to the skin of a mouse was not, by itself, sufficient to produce cancer. However, when a proliferative stimulus was then applied locally, in the form of a second, noncarcinogenic, irritating chemical (e.g., a phorbol ester), tumors appeared. The first effect was called **initiation**. The action of the second, noncarcinogenic chemical was called **promotion**. Subsequently, further experiments in rodent models of a variety of organ-specific cancers (liver, skin, lung, pancreas, colon, etc.) expanded the concept of a two-stage mechanism to our present understanding of *carcinogenesis as a multistep process that involves numerous mutations.*

From these studies, one can abstract four stages of chemical carcinogenesis:

1. **Initiation** likely represents a mutation in a single cell.
2. **Promotion** reflects the clonal expansion of the initiated cell, in which the mutation has conferred a growth advantage. During promotion, the altered cells remain dependent on the continued presence of the promoting stimulus. This stimulus may be an exogenous chemical or physical agent or may reflect an endogenous mechanism (e.g., hormonal stimulation [breast, prostate] or the effect of bile salts [colon]).
3. **Progression** is the stage in which growth becomes autonomous (i.e., independent of the carcinogen or the promoter). By this time, sufficient mutations have accumulated to immortalize cells.
4. **Cancer,** the end result of the entire sequence, is established when the cells acquire the capacity to invade and metastasize.

The morphologic changes that reflect multistep carcinogenesis in humans are best exemplified in epithelia, such as those of the skin, cervix and colon. Although initiation has no morphologic counterpart, *promotion and progression are represented by the sequence of hyperplasia, dysplasia and carcinoma in situ.*

Chemical Carcinogens Usually Undergo Metabolic Activation

The International Agency for Research in Cancer (IARC) has listed about 75 chemicals as human carcinogens. Chemicals cause cancer either directly or, more often, after metabolic activation. The direct-acting carcinogens are inherently reactive enough to bind covalently to cellular macromolecules. A number of organic compounds, such as nitrogen mustard, *bis*(chloromethyl)ether and benzyl chloride, as well as certain metals are included in this category. Most organic carcinogens, however, require conversion to an ultimate, more reactive compound. This conversion is enzymatic and, for the most part, is effected by the cellular systems involved in drug metabolism and detoxification. Many cells in the body, particularly liver cells, possess enzyme systems that can convert procarcinogens to their active forms. Yet each carcinogen has its own spectrum of target tissues, often limited to a single organ. The basis for organ specificity in chemical carcinogenesis is not well understood.

POLYCYCLIC AROMATIC HYDROCARBONS: The polycyclic aromatic hydrocarbons, originally derived from coal tar, are among the most extensively studied carcinogens. In this class are such model compounds as benzo(a)pyrene, 3-methylcholanthrene and dibenzanthracene. These compounds have a broad range of target organs and generally produce cancers at the site of application. The specific type of cancer produced varies with the route of administration and includes tumors of the skin, soft tissues and breast. Polycyclic hydrocarbons have been identified in cigarette smoke, and so it has been suggested, but not proved, that they are involved in the production of lung cancer.

Polycyclic hydrocarbons are metabolized by cytochrome P450–dependent mixed function oxidases to electrophilic epoxides, which in turn react with proteins and nucleic acids. The formation of the epoxide depends on the presence of an unsaturated carbon–carbon bond. For example,

vinyl chloride, the simple two-carbon molecule from which the widely used plastic polyvinyl chloride is synthesized, is metabolized to an epoxide, which is responsible for its carcinogenic properties. Workers exposed to the vinyl chloride monomer in the ambient atmosphere later developed hepatic angiosarcomas.

ALKYLATING AGENTS: Many chemotherapeutic drugs (e.g., cyclophosphamide, cisplatin, busulfan) are alkylating agents that transfer alkyl groups (methyl, ethyl, etc.) to macromolecules, including guanines within DNA. Although such drugs destroy cancer cells by damaging DNA, they also injure normal cells. Thus, alkylating chemotherapy carries a significant risk of solid and hematologic malignancies at a later time.

AFLATOXIN: Aflatoxin B_1 is a natural product of the fungus *Aspergillus flavus.* Like the polycyclic aromatic hydrocarbons, aflatoxin B_1 is metabolized to an epoxide, which can bind covalently to DNA. Aflatoxin B_1 is among the most potent liver carcinogens recognized, producing tumors in fish, birds, rodents and primates. Since *Aspergillus* sp. are ubiquitous, contamination of vegetable foods exposed to the warm moist conditions, particularly peanuts and grains, may result in the formation of significant amounts of aflatoxin B_1. It has been suggested that in addition to hepatitis B and C, aflatoxin-rich foods may contribute to the high incidence of cancer of the liver in parts of Africa and Asia. In rodents exposed to aflatoxin B_1, the resulting liver tumors exhibit a specific inactivating mutation in the *p53* gene (G:C→T:A transversion at codon 249). Interestingly, human liver cancers in areas of high dietary concentrations of aflatoxin carry the same *p53* mutation.

AROMATIC AMINES AND AZO DYES: Aromatic amines and azo dyes, in contrast to the polycyclic aromatic hydrocarbons, are not ordinarily carcinogenic at the point of application. However, they commonly produce bladder and liver tumors, respectively, when fed to experimental animals. Both aromatic amines and azo dyes are primarily metabolized in the liver. The activation reaction undergone by aromatic amines is N-hydroxylation to form the hydroxylamino derivatives, which are then detoxified by conjugation with glucuronic acid. In the bladder, hydrolysis of the glucuronide releases the reactive hydroxylamine. Occupational exposure to aromatic amines in the form of aniline dyes has resulted in bladder cancer.

NITROSAMINES: Carcinogenic nitrosamines are a subject of considerable study because it is suspected that they may play a role in human gastrointestinal neoplasms and possibly other cancers. The simplest nitrosamine, dimethylnitrosamine, produces kidney and liver tumors in rodents. Nitrosamines are also potent carcinogens in primates, although unambiguous evidence of cancer induction in humans is lacking. However, the extremely high incidence of esophageal carcinoma in the Hunan province of China (100 times higher than in other areas) has been correlated with the high nitrosamine content of the diet. There is concern that nitrosamines may also be implicated in other gastrointestinal cancers because nitrites, commonly added to preserve processed meats and other foods, may react with other dietary components to form nitrosamines. In addition, tobacco-specific nitrosamines have been identified, although a contribution to carcinogenesis has not been proved. Nitrosamines are activated by hydroxylation, followed by formation of a reactive alkyl carbonium ion.

METALS: A number of metals or metal compounds can induce cancer, but the carcinogenic mechanisms are unknown. Divalent metal cations, such as nickel (Ni^{2+}), lead (Pb^{2+}), cadmium (Cd^{2+}), cobalt (Co^{2+}) and beryllium (Be^{2+}), are electrophilic and can, therefore, react with macromolecules. In addition, metal ions react with guanine and phosphate groups of DNA. A metal ion such as Ni^{2+} can depolymerize polynucleotides. Some metals can bind to purine and pyrimidine bases through covalent bonds or pi electrons of the bases. These reactions all occur in vitro, and the extent to which they occur in vivo is not known. Most metal-induced cancers occur in an occupational setting (see Chapter 8).

Endogenous and Environmental Factors Influence Chemical Carcinogenesis

Chemical carcinogenesis in experimental animals involves consideration of genetic aspects (species and strain, age and sex of the animal), hormonal status, diet and the presence or absence of inducers of drug-metabolizing systems and tumor promoters. A similar role for such factors in humans has been postulated on the basis of epidemiologic studies.

METABOLISM OF CARCINOGENS: Mixed-function oxidases are enzymes whose activities are genetically determined, and a correlation has been observed between the levels of these enzymes in various strains of mice and their sensitivity to chemical carcinogens. Since most chemical carcinogens require metabolic activation, agents that enhance the activation of procarcinogens to ultimate carcinogens should lead to greater carcinogenicity, while those that augment the detoxification pathways should reduce the incidence of cancer. In general, this is the case experimentally. Since humans are exposed to many chemicals in the diet and environment, such interactions are potentially significant.

SEX AND HORMONAL STATUS: These factors are important determinants of susceptibility to chemical carcinogens but are highly variable and in many instances not readily predictable. In experimental animals, there is sex-linked susceptibility to the carcinogenicity of certain chemicals. However, the effects of sex and hormonal status on chemical carcinogenesis in humans are not clear.

DIET: The composition of the diet can affect the level of drug-metabolizing enzymes. Experimentally, a low-protein diet, which reduces the hepatic activity of mixed-function oxidases, is associated with decreased sensitivity to hepatocarcinogens. In the case of dimethylnitrosamine, the decreased incidence of liver tumors is accompanied by an increased incidence of kidney tumors, an observation that emphasizes the fact that the metabolism of carcinogens may be regulated differently in different tissues.

PHYSICAL CARCINOGENESIS

The physical agents of carcinogenesis discussed here are UV light, asbestos and foreign bodies. Radiation carcinogenesis is discussed in Chapter 8.

Ultraviolet Radiation Causes Skin Cancers

Among fair-skinned people, a glowing tan is commonly considered the mark of a successful holiday. However, this overt manifestation of the alleged healthful effects of the sun conceals underlying tissue damage. The harmful effects

of solar radiation were recognized by ladies of a bygone era, who shielded themselves from the sun with parasols to maintain a "roses-and-milk" complexion and to prevent wrinkles. The more recent fad for a tanned complexion has been accompanied not only by cosmetic deterioration of facial skin but also by an increased incidence of the major skin cancers.

Cancers attributed to sun exposure, namely, basal cell carcinoma, squamous carcinoma and melanoma, occur predominantly in people of the white race. The skin of people of the darker races is protected by the increased concentration of melanin pigment, which absorbs UV radiation. In fair-skinned people, the areas exposed to the sun are most prone to develop skin cancer. Moreover, there is a direct correlation between total exposure to sunlight and the incidence of skin cancer.

UV radiation is the short-wavelength portion of the electromagnetic spectrum adjacent to the violet region of visible light. It appears that only certain portions of the UV spectrum are associated with tissue damage, and a carcinogenic effect occurs at wavelengths between 290 and 320 nm. *The effects of UV radiation on cells include enzyme inactivation, inhibition of cell division, mutagenesis, cell death and cancer.*

The most important biochemical effect of UV radiation is the formation of **pyrimidine dimers** in DNA, a type of DNA damage that is not seen with any other carcinogen. Pyrimidine dimers may form between thymine and thymine, between thymine and cytosine or between cytosine pairs alone. Dimer formation leads to a cyclobutane ring, which distorts the phosphodiester backbone of the double helix in the region of each dimer. Unless efficiently eliminated by the nucleotide excision repair pathway, genomic injury produced by UV radiation is mutagenic and carcinogenic.

Xeroderma pigmentosum, an autosomal recessive disease, exemplifies the importance of DNA repair in protecting against the harmful effects of UV radiation. In this rare disorder, sensitivity to sunlight is accompanied by a high incidence of skin cancers, including basal cell carcinoma, squamous cell carcinoma and melanoma. Both the neoplastic and nonneoplastic disorders of the skin in xeroderma pigmentosum are attributed to an impairment in the excision of UV-damaged DNA.

Asbestos Causes Mesothelioma

Pulmonary asbestosis and asbestosis-associated neoplasms are discussed in Chapter 12. Here we review possible mechanisms of carcinogenesis attributed to asbestos. In this context, it is not conclusively established whether the cancers related to asbestos exposure should be considered examples of chemical carcinogenesis or of physically induced tumors, or both.

Asbestos, a material widely used in construction, insulation and manufacturing, is a family of related fibrous silicates, which are classed as "serpentines" or "amphiboles." Serpentines, of which chrysotile is the only example of commercial importance, occur as flexible fibers; the amphiboles, represented principally by crocidolite and amosite, are firm narrow rods.

The characteristic tumor associated with asbestos exposure is ***malignant mesothelioma*** *of the pleural and peritoneal cavities.* This cancer, which is exceedingly rare in the general population, has been reported to occur in 2%–3% (in some studies even more) of heavily exposed workers. The latent period (i.e., the interval between exposure and the appearance of a tumor) is usually about 20 years but may be twice that figure. It is reasonable to surmise that mesotheliomas of both pleura and peritoneum reflect the close contact of these membranes with asbestos fibers transported to them by lymphatic channels.

The pathogenesis of asbestos-associated mesotheliomas is obscure. Thin crocidolite fibers are associated with a considerably greater risk of mesothelioma than shorter and thicker chrysotile fibers. There is increasing evidence that the surface properties of asbestos fibers are important in their carcinogenic properties.

An association between cancer of the lung and asbestos exposure is clearly established in smokers. A slight increase in the prevalence of lung cancer has been reported in nonsmokers exposed to asbestos, but the small number of cases renders an association questionable. Claims that exposure to asbestos increases the risk of gastrointestinal cancer have not withstood statistical analysis of the collected data. In any case, the widespread adoption of strict safety standards will undoubtedly relegate the hazards of asbestos to historical interest.

Foreign Bodies Produce Experimental Cancer

The implantation of inert materials induces sarcomas in certain experimental animals. However, *humans are resistant to foreign body carcinogenesis, as evidenced by the lack of cancers following the implantation of prostheses constructed of plastics and metals.* A few reports of cancer developing in the vicinity of foreign bodies in humans probably reflect scar formation, which in some organs seems to be associated with an increased incidence of cancers. Despite numerous contrary claims in lawsuits, there is no evidence that a single traumatic injury can lead to any form of cancer.

The general mechanisms underlying the development of neoplasia are summarized in Fig. 5-62.

Dietary Influences on Cancer Development Are Highly Controversial

About a quarter of a century ago, respected epidemiologists suggested that approximately one third of cancers in the United States could be prevented by changes in diet. Numerous epidemiologic studies have attempted to identify possible relationships between dietary factors and the occurrence of a variety of cancers. Such investigations have particularly emphasized the roles of dietary fats, red meat and fiber. The results of studies comparing different ethnic groups or societies across international borders have often not been accepted as accurate and in fact have sometimes yielded misleading conclusions. Prospective, cohort studies comparing like populations are usually more reliable.

Some such cohort studies have indicated correlations between consumption of animal (but not vegetable) fat and increased risk of breast cancer. This relationship was limited to premenopausal women, and there is a suggestion that nonlipid components of food containing animal fats may be involved.

In the case of colon cancer, consumption of red meat has been associated with increased risk; total fat and animal fat intake are not correlated independently of red meat intake. At one time, it was thought that intake of dietary fiber protected from colorectal cancer and other malignancies, but these conclusions have not withstood the test of time.

An association between the risk of aggressive (but not indolent) prostate cancer and the consumption of red meat has been claimed. However, further studies of this matter are needed.

Despite claims that eating fruits and vegetables helps to prevent cancer, there is little evidence that these dietary constituents protect from tumor development. Although there is a popular notion that high intake and blood concentrations of vitamin D may be associated with a lower incidence of some cancers, a recent review indicates that this is not the case. Several epidemiologic studies have provided preliminary data suggesting that a folate-rich diet decreases the risk of colorectal cancer.

In conclusion, the beneficial effects of dietary constituents on cancer risk are at best limited and are often controversial. The consequences of a specific type of diet on longevity are largely limited to reduced cardiovascular disease.

Physical activity and obesity are closely correlated with diet, and the dissection of independent effects of these influences by epidemiologic techniques has proven to be exceedingly difficult. The best evidence that physical activity decreases the risk of developing cancer exists for breast and colon malignancies. The same is true for obesity, which adds risk for endometrial, esophageal and kidney cancer. However, it is generally agreed that the evidence for these associations is not sufficient to allow for specific recommendations for changes in lifestyle in order to decrease cancer risk.

SYSTEMIC EFFECTS OF CANCER ON THE HOST

The symptoms of cancer are, for the most part, referable to local effects of the primary tumor or its metastases. However, in a minority of patients, cancer produces remote effects that are not attributable to tumor invasion or to metastasis, and are collectively called **paraneoplastic syndromes**. Such effects are rarely lethal, but in some cases they dominate the clinical course. It is important to recognize these syndromes for several reasons. First, signs and symptoms of the paraneoplastic syndrome may be the first clinical manifestation of a malignant tumor. Second, the syndromes may be mistaken for those produced by advanced metastatic disease and may, therefore, lead to inappropriate therapy. Third, the paraneoplastic syndrome itself may be disabling, and treatment that alleviates those symptoms may have important palliative effects. Finally, certain tumor products that result in paraneoplastic syndromes provide a means of monitoring recurrence of the cancer in patients who have had surgical resections or are undergoing chemotherapy or radiation therapy.

We discuss here systemic paraneoplastic manifestations. Those mainly manifesting as involvement of one or another organ are addressed in the chapters specific for individual organs.

Fever

It is not uncommon for cancer patients to present initially with fever of unknown origin that cannot be explained by an infectious disease. Fever attributed to cancer correlates with tumor growth, disappears after treatment and reappears on recurrence. The cancers in which this most commonly occurs are Hodgkin disease, renal cell carcinoma and osteogenic sarcoma, although many other tumors are occasionally complicated by fever. Tumor cells may themselves release pyrogens or the inflammatory cells in the tumor stroma can produce IL-1.

Anorexia and Weight Loss

A paraneoplastic syndrome of anorexia, weight loss and cachexia is very common in patients with cancer, often appearing before its malignant cause becomes apparent. For example, a small asymptomatic pancreatic cancer may be suspected only on the basis of progressive and unexplained weight loss. Although cancer patients often decrease their caloric intake because of anorexia and abnormalities of taste, restricted food intake does not explain the profound wasting so common among them. The mechanisms responsible for this phenomenon are poorly understood. It is known, however, that unlike starvation, which is associated with a lowered metabolic rate, cancer is often accompanied by an elevated metabolic rate. It has been demonstrated that TNF-α and other cytokines (interferons, IL-6) can produce a wasting syndrome in experimental animals.

EPIDEMIOLOGY OF CANCER

The mere compilation of raw epidemiologic data is of little use unless they are subjected to careful analysis. In evaluating the relevance of epidemiologic observations to cancer causation, the Hill criteria are germane:

- Strength of the association
- Consistency under different circumstances
- Specificity
- Temporality (i.e., the cause must precede the effect)
- Biological gradient (i.e., there is a dose-response relationship)
- Plausibility
- Coherence (i.e., a cause-and-effect relationship does not violate basic biological principles)
- Analogy to other known associations

It is not mandatory that a valid epidemiologic study satisfy all these criteria, nor does adherence to them guarantee that the hypothesis derived from the data is necessarily true. However, as a guideline they remain useful.

Cancer accounts for one fifth of the total mortality in the United States and is the second-leading cause of death after ischemic cardiovascular diseases. For most cancers, death rates in the United States have largely remained flat for more than half a century, with some notable exceptions (Fig. 5-63). The death rate from cancer of the lung among men has risen dramatically from 1930, when it was an uncommon tumor, to the present, when it is by far the most common cause of death from cancer in men. As discussed in Chapter 8, the entire epidemic of lung cancer deaths is attributable to smoking. Among women, smoking did not become fashionable until World War II. Considering the time lag needed between starting to smoke and the development of cancer of the lung, it is not surprising that the increased death rate from lung cancer in women did not become significant until after 1965. In the United States, the death rate from lung cancer in women now exceeds that for breast cancer, and it is now, as in men, the most common fatal cancer. By contrast, for reasons difficult to fathom, cancer of the stomach, which in 1930 was by far the most common cancer in men and was more

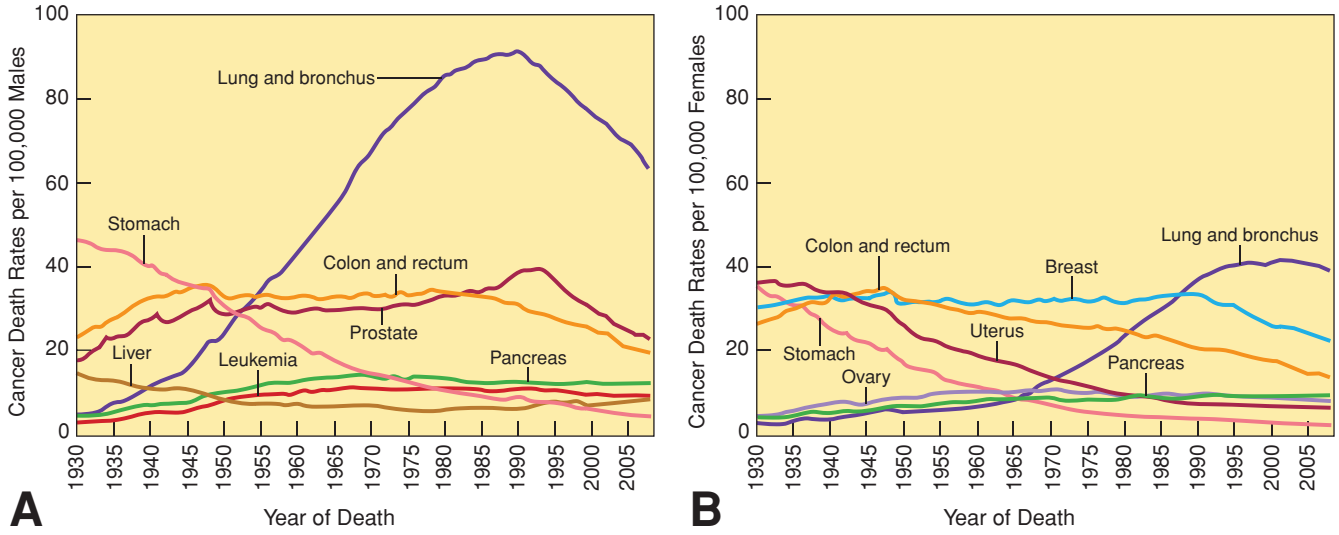


FIGURE 5-63. Cancer death rates in the United States, 1930–2009, among men (**A**) and women (**B**).

common than breast cancer in women, has shown a remarkable and sustained decline in frequency. Similarly, there has been a conspicuous decline in the death rate from cancer of the uterine corpus and cervix, possibly explained by better screening, diagnostic techniques and therapeutic methods. Overall, after decades of steady increases, the age-adjusted mortality as a result of all cancers has now reached a plateau. The ranking of the incidence of tumors in men and women in the United States is shown in Table 5-11.

Individual cancers have their own age-related profiles, but for most, increased age is associated with an increased incidence. The most striking example of the dependency on age is carcinoma of the prostate, in which the incidence increases 30-fold between men ages 50 and 85 years. Certain neoplastic diseases, such as acute lymphoblastic leukemia in children and testicular cancer in young adults, show different age-related peaks of incidence (Fig. 5-64).

TABLE 5-11
MOST COMMON TUMOR TYPES IN MEN AND WOMEN

Tumor Type	% of Cases	Tumor Type	% of Cases
Men		**Women**	
Prostate	29	Breast	29
Lung and bronchus	14	Lung and bronchus	14
Colon and rectum	9	Colon and rectum	9
Urinary bladder	7	Uterine corpus	6
Melanoma (cutaneous)	5	Thyroid	5
Kidney and renal pelvis	5	Melanoma (cutaneous)	4
Non-Hodgkin lymphoma	4	Non-Hodgkin lymphoma	4
Oral cavity	3	Kidney and renal pelvis	3
Leukemia	3	Ovary	3
Pancreas	3	Pancreas	3
All other sites	18	All other sites	20

Source: American Cancer Society, estimates for 2012.

Geographic and Ethnic Differences Influence Cancer Incidence

NASOPHARYNGEAL CANCER: Nasopharyngeal cancer is rare in most of the world except for certain regions of China, Hong Kong and Singapore.

ESOPHAGEAL CARCINOMA: The range in incidence of esophageal carcinoma varies from extremely low in Mormon women in Utah to a value some 300 times higher in the female population of northern Iran. Particularly high rates of esophageal cancer are noted in a so-called Asian esophageal cancer belt, which includes the great land mass stretching from Turkey to eastern China. Interestingly, throughout this region, as the incidence rises, the proportional excess in males decreases; in some of the areas of highest incidence there is even a female excess. The disease is also more common in certain regions of sub-Saharan Africa and among blacks in the United States. The causes of esophageal cancer are obscure, but it is known that it disproportionately affects the poor in many areas of the world, and the combination of alcohol abuse and smoking is associated with a particularly high risk.

STOMACH CANCER: The highest incidence of stomach cancer occurs in Japan, where the disease is almost 10 times as frequent as it is among American whites. A high incidence has also been observed in Latin American countries, particularly Chile. Stomach cancer is also common in Iceland and eastern Europe.

COLORECTAL CANCER: The highest incidence of colorectal cancer is found in the United States, where it is three or four times more common than in Japan, India, Africa and Latin America. It had been theorized that the high fiber content of the diet in low-risk areas and the high fat content in the United States are related to this difference, but this concept has been seriously questioned.

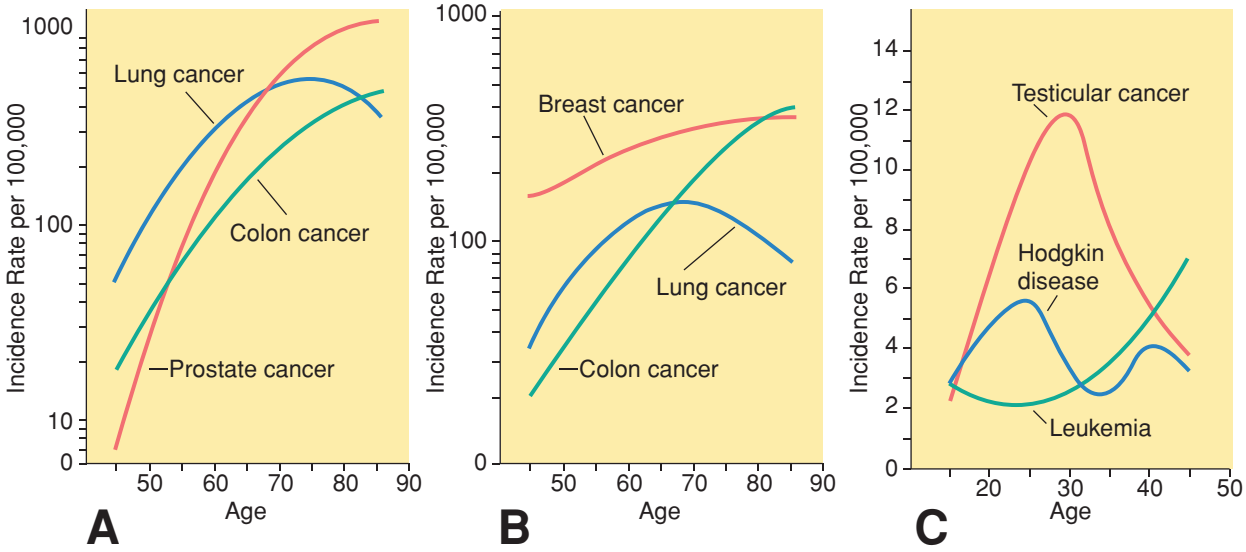


FIGURE 5-64. Incidence of specific cancers as a function of age. A. Men. **B.** Women. **C.** Testicular cancer in men and Hodgkin disease and leukemia in both sexes. The incidence of these cancers in **C** peaks at younger ages than do those in **A** and **B**.

LIVER CANCER: There is a strong correlation between the incidence of primary hepatocellular carcinoma and the prevalence of hepatitis B and C. Endemic regions for both diseases include large parts of sub-Saharan Africa and most of Asia, Indonesia and the Philippines. It must be remembered that levels of aflatoxin B_1 are high in the staple diets of many of the high-risk areas.

SKIN CANCER: As noted above, the rates for skin cancers vary with skin color and exposure to the sun. Thus, particularly high rates have been reported in northern Australia, where the population is principally of English origin and sun exposure is intense. Increased rates of skin cancer have also been noted among the white population of the American Southwest. The lowest rates are found among people with pigmented skin (e.g., Japanese, Chinese and Indians). The rates for African blacks, despite their heavily pigmented skin, are occasionally higher than those for Asians because of the higher incidence of melanomas of the soles and palms in the former population.

BREAST CANCER: Adenocarcinoma of the breast, the most common female cancer in many parts of Europe and North America, shows considerable geographic variation. The rates in African and Asian populations are only one fifth to one sixth of those prevailing in Europe and the United States. Epidemiologic studies have contributed little to our understanding of the etiology of breast cancer.

CERVICAL CARCINOMA: Striking differences in the incidence of squamous carcinoma of the cervix exist between ethnic groups and different socioeconomic levels. For instance, the very low rate in Ashkenazi Jews of Israel contrasts with a 25 times greater rate in the Hispanic population of Texas. In general, groups of low socioeconomic status have a higher incidence of cervical cancer than the more prosperous and better educated. This cancer is also directly correlated with early sexual activity and multiparity, and is rare among women who are not sexually active, such as nuns. It is also uncommon among women whose husbands are circumcised. A strong association with infection by HPVs has been demonstrated, and cervical cancer should be classed as a venereal disease.

CHORIOCARCINOMA: Choriocarcinoma, an uncommon cancer of trophoblastic differentiation, is found principally in women, following a pregnancy, although it can occur in men as a testicular tumor. The rates of this disease are particularly high in the Pacific rim of Asia (Singapore, Hong Kong, Japan and the Philippines).

PROSTATIC CANCER: Very low incidences of prostatic cancer are reported for Asian populations, particularly Japanese, while the highest rates described are in American blacks, in whom the disease occurs some 25 times more often. The incidence in American and European whites is intermediate.

TESTICULAR CANCER: An unusual aspect of testicular cancer is its universal rarity among black populations. Interestingly, although the rate in American blacks is only about one-fourth that in whites, it is still considerably higher than the rate among African blacks.

CANCER OF THE PENIS: This squamous carcinoma is virtually nonexistent among circumcised men of any race but is common in many parts of Africa and Asia. It is usually associated with HPV infection.

CANCER OF THE URINARY BLADDER: The rates for transitional cell carcinoma of the bladder are fairly uniform. Squamous carcinoma of the bladder, however, is a special case. Ordinarily far less common than transitional cell carcinoma, it has a high incidence in areas where schistosomal infestation of the bladder (bilharziasis) is endemic.

BURKITT LYMPHOMA: Burkitt lymphoma, a disease of children, was first described in Uganda, where it accounts for half of all childhood tumors. Since then, a high frequency has been observed in other African countries, particularly in hot, humid lowlands. It has been noted that these are areas where malaria is also endemic. High rates have been recorded in other tropical areas, such as Malaysia and New Guinea, but European and American cases are encountered only sporadically.

MULTIPLE MYELOMA: This malignant tumor of plasma cells is uncommon among American whites but displays a three to four times higher incidence in American and South African blacks.

CHRONIC LYMPHOCYTIC LEUKEMIA: Chronic lymphocytic leukemia is common among elderly people in Europe and North America but is considerably less common in Japan.

Studies of Migrant Populations Give Clues to Cancer Development

Although planned experiments on the etiology of human cancer are hardly feasible, certain populations have unwittingly performed such experiments by migrating from one environment to another. Initially at least, the genetic characteristics of such people remained the same, but the new environment differed in climate, diet, infectious agents, occupations and so on. *Consequently, epidemiologic studies of migrant populations have provided many intriguing clues to the factors that may influence the pathogenesis of cancer.* The United States, which has been the destination of one of the greatest population movements of all time, is the source of most of the important data in this field.

COLORECTAL, BREAST, ENDOMETRIAL, OVARIAN AND PROSTATIC CANCERS: Emigrants from low-risk areas in Europe and Japan to the United States exhibit an increased risk of colorectal cancer in the United States. Moreover, their offspring continue at higher risk and reach the incidence levels of the general American population. This rule for colorectal cancer also prevails for cancers of the breast, endometrium, ovary and prostate.

CANCER OF THE LIVER: As noted above, primary hepatocellular carcinoma is common in Asia and Africa, where it has been associated with hepatitis B and C. In American blacks and Asians, however, the neoplasm is no more common than in American whites, a situation that presumably reflects the relatively low prevalence of chronic viral hepatitis in the United States.

HODGKIN DISEASE: In general, in poorly developed countries the childhood form of Hodgkin disease is the one reported most often. In developed Western countries, by contrast, the disease is most common among young adults, except in Japan. Such a pattern is characteristic of certain viral infections. Further evidence for an environmental influence is the higher incidence of Hodgkin disease in Americans of Japanese descent than that in Japan.