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Neoplasia

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A neoplasm (Greek, *neo*, new + *plasma*, thing formed) is the autonomous growth of tissues that have escaped normal restraints on cell proliferation and exhibit varying degrees of fidelity to their precursors. However, in some instances, for example follicular lymphoma (see Chapter 20), the accumulation of neoplastic cells reflects an aberration in programmed cell death (apoptosis). The structural resemblance of the neoplastic cell to its cell of origin usually enables specific conclusions about its source and potential behavior. In view of their space-

occupying properties, solid neoplasms are termed **tumors** (Gr., *swelling*). Tumors that remain localized are considered **benign**, whereas those that spread to distant sites are termed **malignant**, or **cancer**. The neoplastic process entails not only cellular proliferation but also a 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 greater longevity in modern times necessarily enlarges the population at risk. For this reason alone, the overall incidence of cancer is increasing. In previous generations, on average, 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. Despite assertions that contemporary society is or will be subject to an “epidemic” of cancer, the epidemiologic data do not support such a concept. If all deaths from cancers caused by tobacco smoke are removed from the statistics, there has been no increase in the overall age-adjusted cancer death rate in men in the past half-century, and there has been a continually decreasing rate in women. However, the age-adjusted incidence of specific cancers has fluctuated over this time period.

In general, neoplasms are irreversible, and their growth is, for the most part, autonomous. Several observations are important:

- Neoplasms are derived from cells that normally maintain a proliferative capacity. Thus, mature neurons and cardiac myocytes do not give rise to tumors.
- A tumor may express varying degrees of differentiation, from relatively mature structures that mimic normal tissues to a collection of cells so primitive that the cell of origin cannot be identified.
- The stimulus responsible for the uncontrolled proliferation may not be identifiable; in fact, it is not known for most human neoplasms.
- Neoplasia arises from mutations in genes that regulate cell growth, apoptosis, or DNA repair.

Benign versus Malignant Tumors

By definition, benign tumors 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. As a rule, benign tumors are more differentiated than malignant ones—that is, they more closely resemble their tissue of origin. *By contrast, malignant tumors, or cancers, have the added property of invading contiguous tissues and metastasizing to distant sites, where subpopulations of malignant cells take up residence, grow anew, and again invade.*

In common usage, the terms **benign** and **malignant** refer to the overall biological behavior of a tumor rather than to its morphologic characteristics. In most circumstances, malignant tumors kill, whereas 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 kill suddenly by blocking the mitral valve orifice. In certain locations, the 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 are curable by surgical resection. In this category are many cancers of breast, and some malignant tumors of connective tissue (e.g., fibrosarcoma).

A number of tumors are difficult to classify because they do not fit all the criteria for either benign or malignant neoplasms. The best-known example is basal cell carcinoma of the skin, which is histologically malignant (i.e., it invades aggressively) but only rarely has been reported to metastasize to distant sites. Similarly, the local growth of a pleomorphic adenoma of a salivary gland, which is classified as benign, may be so aggressive that it defies surgical cure.

Classification of Neoplasms

In any language, the classification of objects and concepts is pragmatic and useful only insofar as its general acceptance permits effective communication. 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.

Benign Tumors Carry the Suffix “oma”

The primary descriptor of any tumor, benign or malignant, is its cell or tissue of origin. The classification of benign tumors is the basis for the names of their malignant variants. *The suffix “oma” for benign tumors is preceded by reference to the cell or tissue of origin.* For example, a benign tumor that resembles chondrocytes is called a **chondroma** (Fig. 5-1). If the tumor resembles the precursor of the chondrocyte, it is labeled **chondroblastoma**. When a chondroma is located entirely within the bone, it is designated **enchondroma**.

Tumors of epithelial origin are given a variety of names based on what is believed to be their outstanding characteristic. Thus, a benign tumor of the squamous epithelium may be called simply **epithelioma** or, when branched and exophytic, may be termed **papilloma**. Benign tumors arising from glandular epithelium, such as in the colon or the endocrine glands, are named

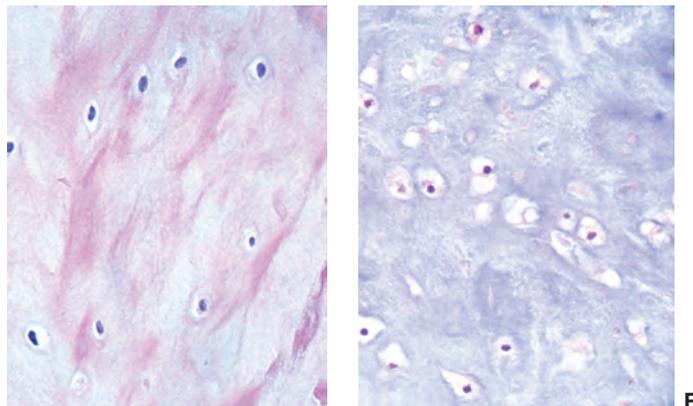


FIGURE 5-1. Benign chondroma. A. Normal cartilage. B. A benign chondroma closely resembles normal cartilage.

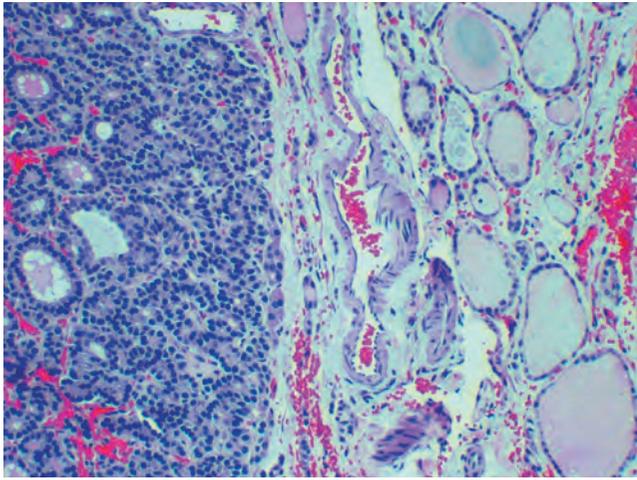


FIGURE 5-2. Benign thyroid adenoma. The follicles of a thyroid adenoma (left) contain colloid and resemble those of the normal thyroid tissue (right).

adenoma. Accordingly, we refer to a **thyroid adenoma** (Fig. 5-2) or a **pancreatic islet cell adenoma**. In some instances, the predominating feature is the gross appearance, in which case we speak, for example, of an **adenomatous polyp** of the colon.

Benign tumors that arise from germ cells and contain derivatives of different germ layers are labeled **teratoma**. These tumors occur principally in the gonads and occasionally in the mediastinum and may contain a variety of structures, such as skin, neurons and glial cells, thyroid, intestinal epithelium, and cartilage. Localized, disordered differentiation during embryonic development results in a **hamartoma**, a disorganized caricature of normal tissue components (Fig. 5-3). Such tumors, which are not strictly neoplasms, contain varying combinations of cartilage, ducts or bronchi, connective tissue, blood vessels,

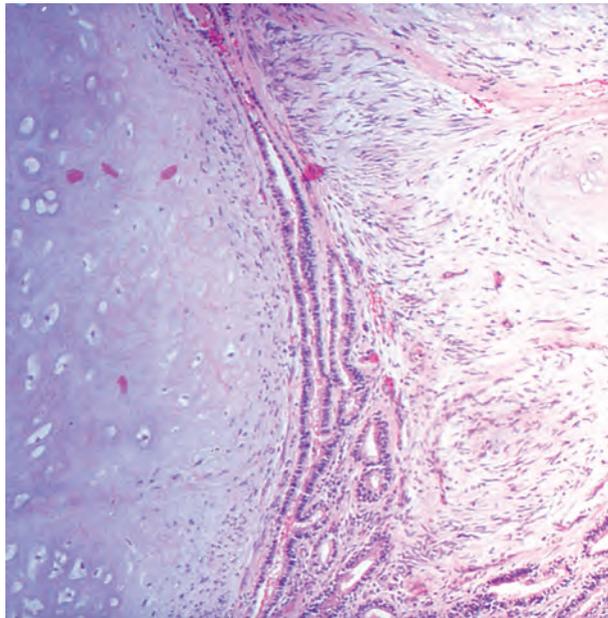


FIGURE 5-3. Hamartoma of the lung. The tumor contains islands of hyaline cartilage and clefts lined by cuboidal epithelium embedded in a fibromuscular stroma.

and lymphoid tissue. Ectopic islands of normal tissue, called **choristoma**, may also be mistaken for true neoplasms. These small lesions are represented by pancreatic tissue in the wall of the stomach or intestine, adrenal rests under the renal capsule, and nodules of splenic tissue in the peritoneal cavity. Certain benign growths, recognized clinically as tumors, are not truly neoplastic but rather represent overgrowth of normal tissue elements. Examples are vocal cord polyps, skin tags, and hyperplastic polyps of the colon.

Malignant Tumors Are Mostly Carcinomas or Sarcomas

In general, the malignant counterparts of benign tumors usually carry the same name, except that the suffix “**carcinoma**” is applied to epithelial cancers and “**sarcoma**” to those of mesenchymal origin. For instance, a malignant tumor of the stomach is a **gastric adenocarcinoma** or **adenocarcinoma of the stomach** (Fig. 5-4). **Squamous cell carcinoma** is an invasive tumor of the skin or other organs lined by a squamous epithelium (e.g., the esophagus). In addition, squamous cell carcinoma arises in the metaplastic squamous epithelium of the bronchus or endocervix. **Transitional cell carcinoma** is a malignant neoplasm of the bladder or ureters. By contrast, we speak of **chondrosarcoma** (Fig. 5-5) or **fibrosarcoma**. Sometimes the name of the tumor suggests the tissue type of origin, as in **osteogenic sarcoma** or **bronchogenic carcinoma**. Some tumors display neoplastic elements of different cell types but are not germ cell tumors. For example, **fibroadenoma** of the breast, composed of epithelial and stromal elements, is benign, whereas, as the name implies, **adenosquamous carcinoma** of the uterus or the lung is malignant. A rare malignant tumor that contains intermingled carcinomatous and sarcomatous elements is known as **carcinosarcoma**.

The persistence of certain historical terms adds a note of confusion. **Hepatoma** of the liver, **melanoma** of the skin, **seminoma** of the testis, and the lymphoproliferative tumor, **lymphoma**, are all highly malignant. Tumors of the hematopoietic system are a special case in which the relationship to the blood is

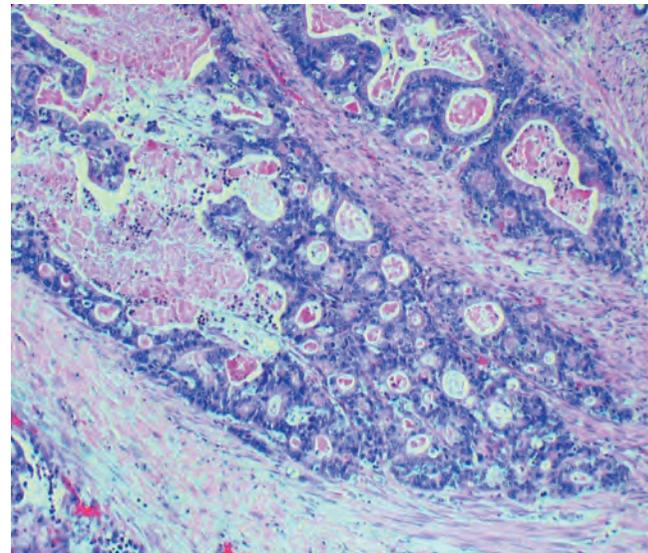


FIGURE 5-4. Adenocarcinoma of the stomach. Irregular neoplastic glands infiltrate the gastric wall.

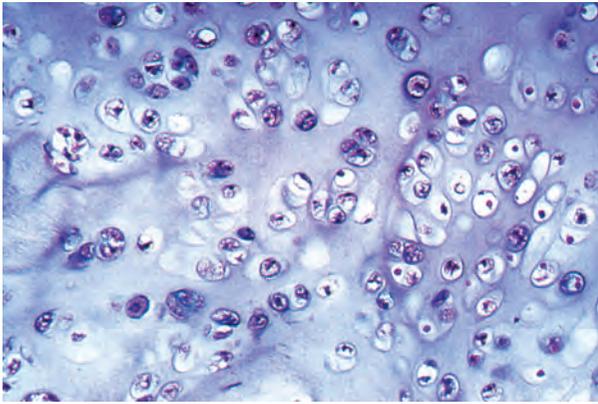


FIGURE 5-5. Chondrosarcoma of bone. The tumor is composed of malignant chondrocytes, which have bizarre shapes and irregular hyperchromatic nuclei, embedded in a cartilaginous matrix. Compare with Figure 5-1.

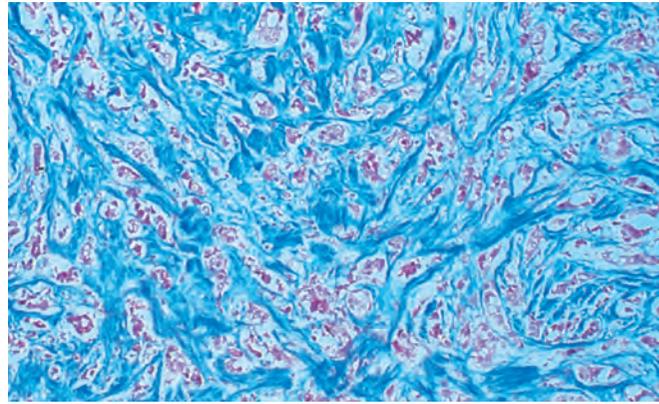


FIGURE 5-7. Scirrhous adenocarcinoma of the breast. A trichrome stain shows nests of cancer cells (red) embedded in a dense fibrous stroma (blue).

indicated by the suffix “emia.” Thus, **leukemia** refers to a malignant proliferation of leukocytes.

Secondary descriptors (again, with some inconsistencies) refer to a tumor’s morphologic and functional characteristics. For example, the term **papillary** describes a frondlike structure (Fig. 5-6). **Medullary** signifies a soft, cellular tumor with little connective tissue stroma, whereas **scirrhous** or **desmoplastic** implies a dense fibrous stroma (Fig. 5-7). **Colloid** carcinomas secrete abundant mucus, in which float islands of tumor cells. **Comedocarcinoma** is an intraductal neoplasm in which necrotic material can be expressed from the ducts. Certain visible secretions of the tumor cells lend their characteristics to the classification—for example, production of mucin or serous fluid. A further designation describes the gross appearance of a cystic mass. From all these considerations we derive such common terms as **papillary serous cystadenocarcinoma** of the ovary, **comedocarcinoma** of the breast, **adenoid cystic carcinoma** of the salivary glands, **polypoid adenocarcinoma** of the stomach, and **medullary carcinoma** of the thyroid. Finally, tumors in which the histogenesis is poorly understood are often given an eponym—for example, Hodgkin disease, Ewing sarcoma of bone, or Brenner tumor of the ovary.

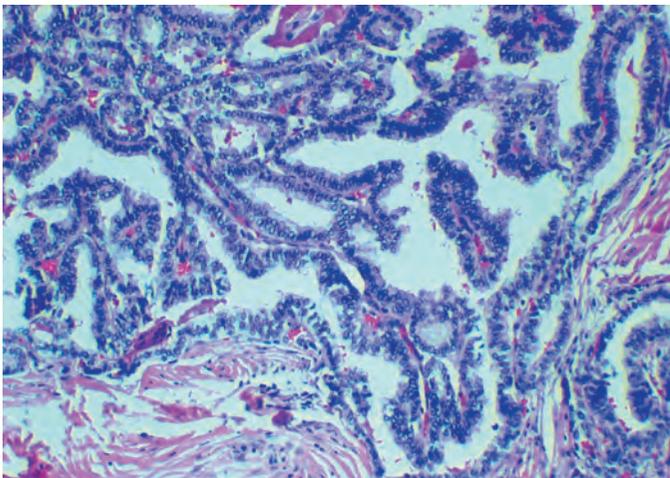


FIGURE 5-6. Papillary adenocarcinoma of the thyroid. The tumor exhibits numerous fronds lined by malignant epithelial cells.

Histologic Diagnosis of Malignancy

There are no reliable molecular indicators of malignancy, and the “gold standard” for diagnosis of cancer remains routine microscopy. The distinction between benign and malignant tumors is, from a practical point of view, the most important diagnostic challenge faced by the pathologist. In most cases, the differentiation poses few problems; in a few, careful study is required before an accurate diagnosis is secure. However, there remain tumors that defy the diagnostic skills and experience of any pathologist; in these cases, the correct diagnosis must await the 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 a historical correlation of histologic and cytologic patterns with clinical outcomes. Although general criteria for malignancy are recognized, they must be used with caution in specific cases. For example, a reactive proliferation of connective cells termed **nodular fasciitis** (Fig. 5-8) has a more alarming histologic appearance than many fibrosarcomas, and misdiagnosis can lead to unnecessary surgery. Conversely, many well-differentiated endocrine adenocarcinomas are histologically indistinguishable from benign adenomas.

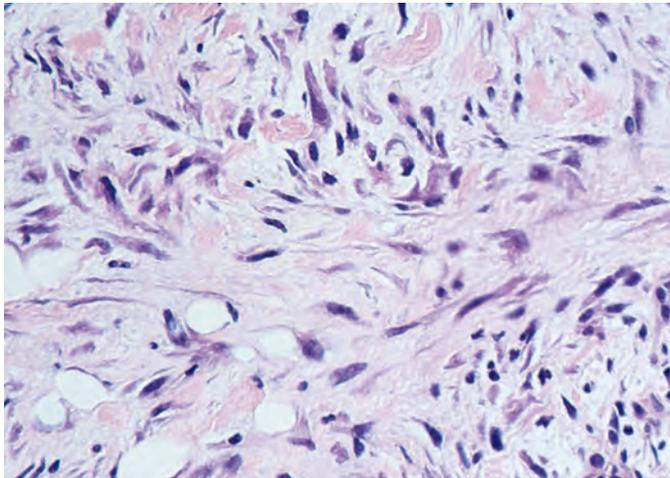


FIGURE 5-8. Nodular fasciitis. This cellular reactive lesion contains atypical and bizarre fibroblasts, which may be mistaken for a fibrosarcoma.

Benign Tumors Resemble Their Parent Tissue

Benign tumors tend to be histologically and cytologically similar to their tissues of origin. For example, **lipomas**, despite their often lobulated gross appearance, seem to be composed of normal adipocytes (Fig. 5-9). **Fibromas** are composed of mature fibroblasts and a collagenous stroma. **Chondromas** exhibit chondrocytes dispersed in a cartilaginous matrix. **Thyroid adenomas** form acini and produce thyroglobulin. The gross structure of a benign tumor may depart from the normal and assume papillary or polypoid configurations, as in papillomas of the bladder and skin and adenomatous polyps of the colon. *However, the lining epithelium of a benign tumor resembles that of the normal tissue.* Although many benign tumors are circumscribed by a connective tissue capsule, many equally benign neoplasms are not encapsulated. Unencapsulated benign tumors include papillomas and polyps of the visceral organs, hepatic adenomas, many endocrine adenomas, and hemangiomas. *Remember that the definition of a benign tumor resides above all in its inability to invade adjacent tissue and to metastasize.*

Malignant Tumors Depart from the Parent Tissue Morphologically and Functionally

Despite the histologic divergence of malignant tumors from their tissue of origin, an accurate identification of their source depends not only on the location but also on a morphologic resemblance to a normal tissue. Some of the histologic features that favor malignancy include the following:

- **Anaplasia or cellular atypia:** These terms refer to the lack of differentiated features in a cancer cell. In general, the degree of anaplasia correlates with the aggressiveness of the tumor. Cytologic evidence of anaplasia includes (1) variation in the size and shape of cells and cell nuclei (**pleomorphism**); (2) enlarged and hyperchromatic nuclei with coarsely clumped

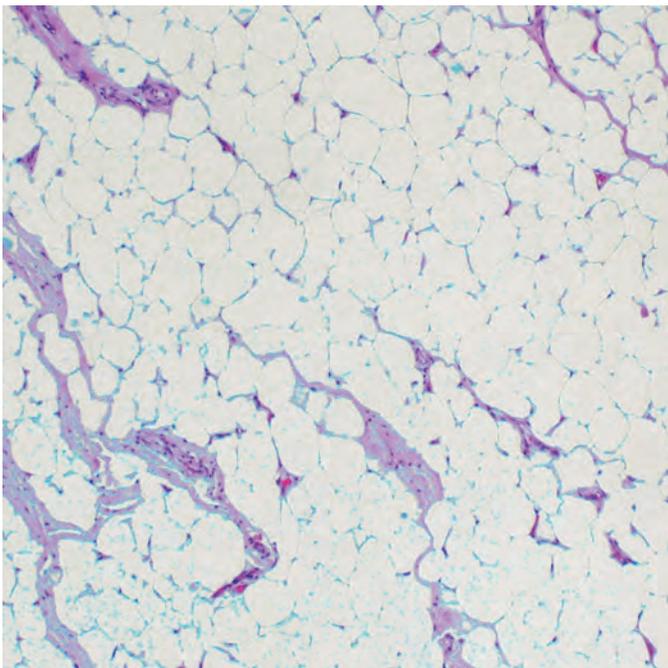


FIGURE 5-9. Lipoma. This subcutaneous, nodular tumor of adipocytes is grossly and microscopically indistinguishable from normal fat.

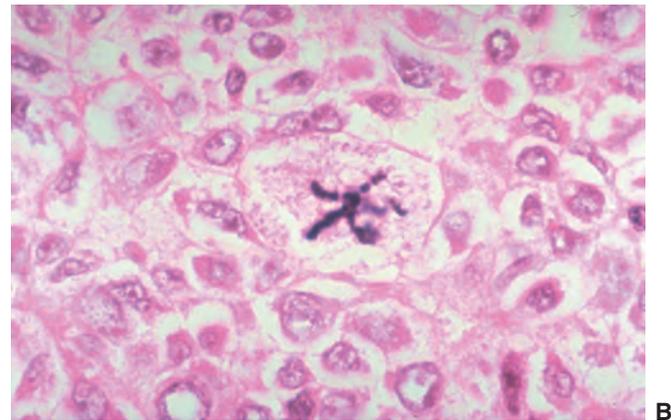
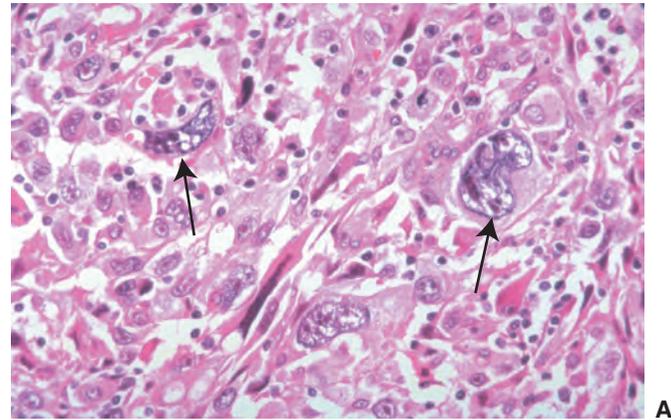


FIGURE 5-10. Anaplastic features of malignant tumors. **A.** The cells of this anaplastic carcinoma are highly pleomorphic (i.e., they vary in size and shape). The nuclei are hyperchromatic and are large relative to the cytoplasm. Multinucleated tumor giant cells are present (arrows). **B.** A malignant cell in metaphase exhibits an abnormal mitotic figure.

chromatin and prominent nucleoli; (3) atypical mitoses; and (4) bizarre cells, including tumor giant cells (Fig. 5-10). Many of these features are preceded by a preneoplastic dysplastic epithelium, which may lead to carcinoma in situ (see Chapter 1).

- **Mitotic activity:** Abundant mitoses are characteristic of many malignant tumors but are not a necessary criterion. However, in some cases (e.g., leiomyosarcomas), the diagnosis of malignancy is based on the finding of even a few mitoses.
- **Growth pattern:** In common with many benign tumors, malignant neoplasms often exhibit a disorganized and random growth pattern, which may be expressed as uniform sheets of cells, arrangements around blood vessels, papillary structures, whorls, rosettes, and so forth. Malignant tumors often outgrow their blood supply and display 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 on the basis of local invasion.
- **Metastases:** The presence of metastases identifies a tumor as malignant. In metastatic disease that was not preceded by a clinically diagnosed primary tumor, the site of origin is often

not readily apparent from the morphologic characteristics of the tumor. In such cases, electron microscopic examination and the demonstration of specific tumor markers may establish the correct origin.

Electron Microscopy of Undifferentiated Tumors May Identify the Source

There are no specific determinants of malignancy or even of neoplasia itself that can be detected by electron microscopy. However, this technique may aid in the diagnosis of poorly differentiated cancers, whose classification is problematic by routine light microscopy. For example, carcinomas often exhibit desmosomes and specialized junctional complexes, structures that are not typical of sarcomas or lymphomas. The presence of melanosomes signifies a melanoma, whereas small, membrane-bound granules with dense cores are features of endocrine neoplasms (Fig. 5-11). Another example of a diagnostically useful granule is the characteristic crystal-containing granule of an insulinoma derived from the pancreatic islets.

Immunohistochemical Tumor Markers Are Antigens That Point to the Origin of Neoplasms

Tumor markers are products of malignant neoplasms that can be detected in the cells themselves or in body fluids. The ultimate tumor marker would be one that allows the unequivocal distinction between benign and malignant cells, but unfortunately no such marker exists. Nevertheless, some markers are often useful in identifying the cell of origin of a metastatic or poorly differentiated primary tumor. Metastatic tumors may be so undifferentiated microscopically as to preclude even the distinction between an epithelial and a mesenchymal origin. Tumor markers rely on the preservation of characteristics of the progenitor cell or the synthesis of specialized proteins by the neoplastic cell to make this distinction. The determination of cell lineage of undifferentiated tumors is more than an academic exercise, because therapeutic decisions may be based on their appropriate identification. For example, the treatment of carcinomas usually involves surgery, whereas malignant lymphomas are treated with radiation therapy and chemotherapy. Among these diagnostically useful markers are such diverse products as immunoglobulins, fetal proteins, enzymes, hormones, and cytoskeletal and junctional proteins.

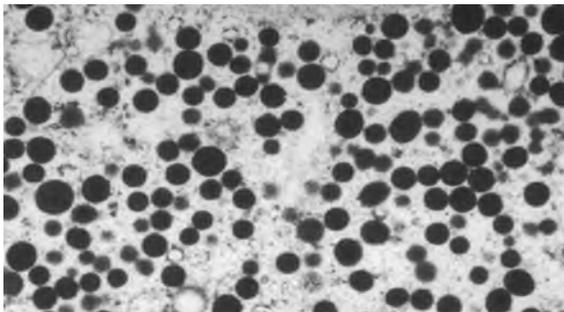


FIGURE 5-11. Electron micrograph of a metastatic cancer of the adrenal medulla (pheochromocytoma). The neuroendocrine origin of this poorly differentiated tumor was identified by the presence of characteristic cytoplasmic secretory granules.

- **Carcinomas** uniformly express **cytokeratins**, which are intermediate filaments belonging to a multigene family of proteins. Lineage-associated markers are often useful in establishing the origin of a poorly differentiated carcinoma. For example, prostatic carcinomas consistently express the glycoprotein **prostate-specific antigen (PSA)** and **prostate-specific acid phosphatase (PSAP)**. By contrast, colon cancers are negative for these markers, but most of them express **carcinoembryonic antigen (CEA)**. Some thyroid carcinomas demonstrate **thyroglobulin**, and breast cancers frequently show nuclear receptors for **estrogen** and **progesterone**. Expression of the sialated form of the **Lewis a antigen** (cancer antigen [CA] 19-9) has been associated with pancreatic and gastrointestinal cancers, whereas **CA 125** is a sensitive marker for ovarian cancers.
- **Neuroendocrine tumors** share the positivity for cytokeratins with other carcinomas. However, they can be identified by their content of **chromogranins**, a family of proteins found in neurosecretory granules. Neuron-specific enolase is another, albeit less specific, marker for neuroendocrine cells. Other markers for neuroendocrine differentiation are **synaptophysin** and **Leu-7 (CD57)**. Specific antibodies exist for a number of peptide hormones, such as gastrin, bombesin, corticotrophic hormone (adrenocorticotrophic hormone [ACTH]), insulin, glucagon, somatostatin, and serotonin.
- **Malignant melanomas** may be unpigmented and appear similar to other poorly differentiated carcinomas. They can often be distinguished by immunohistochemical studies (Fig. 5-12). Melanomas express **HMB-45** and **S-100 protein**, but unlike most carcinomas, they are not positive for cytokeratins.
- **Soft tissue sarcomas** express the intermediate filament **vimentin**. Since this marker is also present in numerous non-mesenchymal tumors, its expression is meaningful only in concert with other markers and morphologic criteria. **Desmin**, another useful intermediate filament, is present in benign and malignant neoplasms originating from either smooth or striated muscle fibers. **Muscle-specific actin** is another marker for muscle tissue. **Neurofilament proteins** are excellent markers for tumors originating from neurons, including neuroblastomas and ganglioneuroma. **Neuron-specific enolase (NSE)** also shows a strong association with neurogenic tissue and is found in almost all neuroblastomas. **Glial fibrillary acidic protein (GFAP)**, the first intermediate filament discovered, is strongly expressed on astrocytes and in most glial cell neoplasms.
- **Malignant lymphomas** are generally positive for **leukocyte common antigen (LCA, CD45)**. Markers for lymphomas and leukemias are grouped by so-called cluster designations (CDs), at present numbering over 200. Markers for CD antigens help to discriminate between T and B lymphocytes, monocytes, and granulocytes and the mature and immature variants of these cells. B cell malignancies, including plasmacytomas, manifest immunoglobulin light-chain restriction. A single B cell expresses κ or λ light chains. The presence of both κ - and λ -positive B cells argues against malignancy, whereas the demonstration of only one type of light chain on the lymphocytes strongly suggests a monoclonal B cell lymphoma.
- **Vascular tumors** derived from endothelial cells, including hemangiomas and hemangiosarcomas, are identified by antibodies against **factor VIII-related antigen** or by the binding of certain lectins.

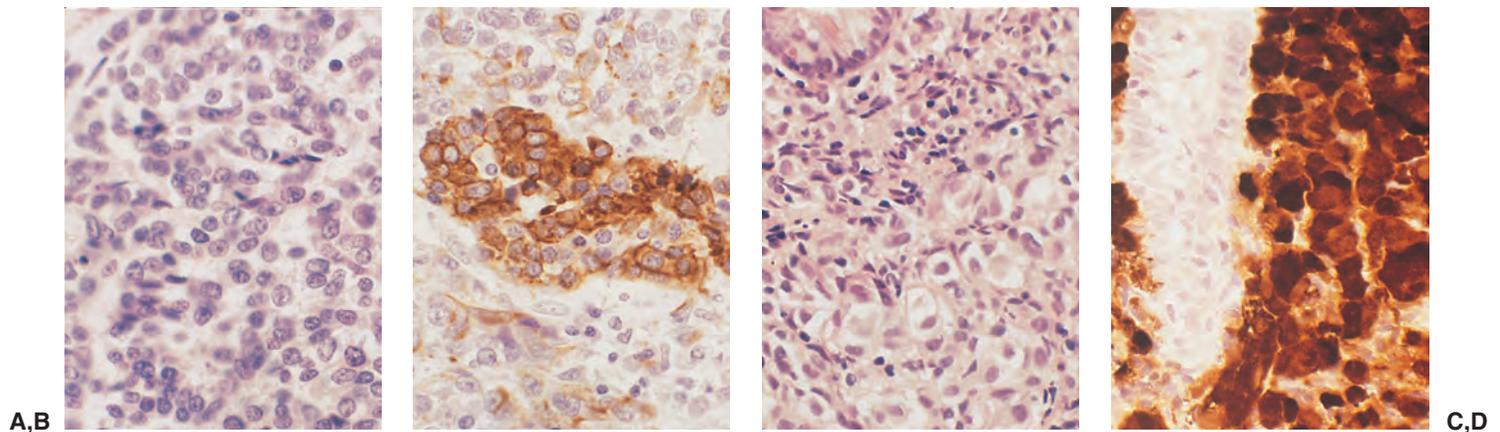


FIGURE 5-12. Tumor markers in the identification of undifferentiated neoplasms. **A.** A poorly differentiated metastatic bladder cancer is difficult to identify as a carcinoma with the hematoxylin and eosin stain. **B.** A section of the tumor depicted in **A** is positive for cytokeratin with an immunoperoxidase 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.

- **Proliferating cells** display **Ki-67** and **proliferating cell nuclear antigen (PCNA)**. Although the presence of proliferating cells alone does not establish a diagnosis of malignancy, the presence of cycling cells at sites in which cell growth is normally absent frequently suggests a cancer.

Serum tumor markers are not disease-specific, but they allow monitoring of tumor recurrence after surgery. For example, high serum levels of CEA are associated with carcinomas of the gastrointestinal tract and some carcinomas of the breast. Increased levels of **serum α -fetoprotein (AFP)** suggest liver cancer or a yolk sac tumor. **Human chorionic gonadotropin (hCG)** is used for monitoring the recurrence of malignant trophoblastic tumors. Elevated **CA 19-9** serum titers are found in patients with pancreatic or gastrointestinal cancers, and high **CA 125** levels are associated with ovarian carcinomas. Increased serum levels of PSA accompany prostatic cancers. Elevated titers of human placental alkaline phosphatase (HPAP) occur with seminomas. Table 5-1 lists many of the commonly used tumor markers.

Invasion and Metastasis

The 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 is generally amenable to surgical extirpation.

Direct Extension Damages the Involved Organ and Adjacent Tissues

Most carcinomas begin as localized growths confined to the epithelium in which they arise. As long as these early cancers do not penetrate the basement membrane on which the epithelium rests, such tumors are termed **carcinoma in situ** (Fig. 5-13). In this stage, it is unfortunate that in situ tumors are asymptomatic, because they are invariably curable. When the in situ tumor acquires invasive potential and extends directly through the underlying basement membrane, it 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.

Malignant tumors characteristically grow within the tissue of origin, where they enlarge and infiltrate normal structures. They may also extend directly 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 the intestinal obstruction produced by cancer of the colon (Fig. 5-14).

The invasive growth pattern of cancers often leads to their direct extension outside the tissue of origin, in which case the tumor may secondarily impair the function of an adjacent organ. Squamous carcinoma of the cervix frequently grows beyond the genital tract to produce vesicovaginal fistulas and obstruct the ureters. 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. 5-15).

Metastatic Spread Is the Most Common Cause of Cancer Death

Metastasis refers to the transfer of malignant cells from one site to another not directly connected with it. The invasive properties of malignant tumors bring them into contact with blood and lymphatic vessels. In the same way that they can invade parenchymal tissue, neoplastic cells can also penetrate vascular and lymphatic channels, through which they are disseminated to distant sites. In general, metastases resemble the primary tumor histologically, although they are occasionally so anaplastic that their cell of origin is obscure.

TABLE 5-1

Frequently Used Markers to Identify Tumors

Marker	Target Cells	Marker	Target Cells
<i>Epithelial Cells</i> Cytokeratins (CK) CK7 CK20	Carcinomas, mesothelioma Many adenocarcinomas Gastrointestinal and ovarian carcinomas, urothelial carcinomas, Merkel cell tumor	Placental alkaline phosphatase (PLAP) Human chorionic gonadotropin (hCG) CA19-9	Seminoma Trophoblastic tumors Pancreatic and gastrointestinal carcinomas
Epithelial membrane antigen (EMA) Ber-Ep4	Carcinomas, mesothelioma, some large cell lymphomas Most adenocarcinomas, but not in mesothelioma	CA125 Calcitonin	Ovarian carcinoma Medullary carcinoma of the thyroid
B72.3 (tumor-associated)	Many adenocarcinomas, but not in mesothelioma	<i>CD Markers</i> CD1	Some T-cell leukemias; Langerhans cell proliferations T cells, T-cell malignancies
Carcinoembryonic antigen (CEA) CD15	Many adenocarcinomas, but not in mesothelioma Many adenocarcinomas, but not in mesothelioma	CD2 CD3 CD4	T cells, T-cell malignancies T cells, T-cell malignancies; Monocytes, monocytic malignancies
<i>Mesothelial Cells</i> Cytokeratins CK5/6 Vimentin HBME Calretinin	Mesothelioma Mesothelioma Mesothelioma Mesothelioma	CD5 CD8	T cells; some B-cell malignancies Suppressor T cells; some T-cell malignancies
<i>Melanocytes</i> HMB-45 S-100 protein Mel A	Malignant melanoma Malignant melanoma Malignant melanoma	CD10 (common ALL antigen, CALLA) CD15	Acute lymphoblastic leukemia; some B-cell lymphomas; Renal cell carcinomas Hodgkin disease; some myeloid leukemias
<i>Neuroendocrine and Neural Cells</i> Chromogranins Synaptophysin Neuron-specific enolase CD57	Neuroendocrine tumors Neuroendocrine tumors Neuroendocrine tumors Neuroendocrine tumors	CD19 CD20 CD30	B cells, B-cell malignancies B cells, B-cell malignancies Hodgkin disease; Anaplastic large cell lymphoma
<i>Glial Cells</i> Glial fibrillary acidic protein (GFAP)	Astrocytoma and other glial tumors	CD33 CD34	Myeloid leukemias Acute myeloid or lymphoblastic leukemias; Some spindle cell tumors
<i>Mesenchymal Cells</i> Vimentin Desmin Muscle-specific actin CD99	Most sarcomas Muscle tumors Muscle tumors Ewing sarcoma, peripheral neuroectodermal tumors (PNET)	<i>Non-CD Leukemia/Lymphoma Markers</i> κ-Light chain λ-Light chain TdT Bcl-1 Bcl-2	B-cell malignancies B-cell malignancies Acute lymphoblastic leukemia Mantle cell lymphoma Follicular lymphoma
<i>Specific Organs</i> Prostate-specific antigen (PSA) Prostate-specific alkaline phosphatase (PSAP) Thyroglobulin α-Fetoprotein (AFP)	Prostatic cancer Prostatic cancer Thyroid cancer Hepatocellular carcinoma, yolk sac tumor	<i>Endothelial Markers</i> von Willebrand factor (vWF) CD31 CD34 Lectins	Vascular neoplasms Vascular neoplasms Vascular neoplasms Vascular neoplasms
HepPar 1 CEA	Hepatocellular carcinoma Gastrointestinal cancers		

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

Hematogenous Metastases

Cancer cells commonly invade capillaries and venules, whereas thicker-walled arterioles and arteries are relatively resistant. Before they can form viable metastases, circulating tumor cells must lodge in the vascular bed of the metastatic site (Fig. 5-16). Here they presumably attach to the walls of blood vessels, either to endothelial cells or to naked basement membranes. For many tumors this sequence of events explains why the liver and the lung are so frequently the sites of metastases. Because abdomi-

nal tumors seed the portal system, they lead to hepatic metastases; other tumors penetrate systemic veins that eventually drain into the vena cava and hence to the lungs. In this respect, some tumor cells released into the venous system survive passage through the microcirculation and are thus transported to more distant organs. For instance, tumor cells may traverse the liver and produce pulmonary metastases, and neoplastic cells may also survive passage through the pulmonary microcirculation to reach the brain, bones (Fig. 5-17), and other organs

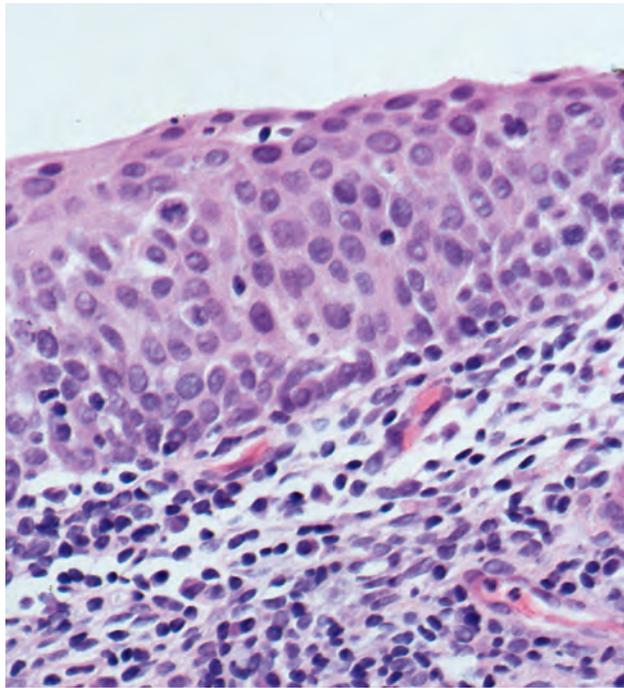


FIGURE 5-13. Carcinoma in situ. A section of the uterine cervix shows neoplastic squamous cells occupying the full thickness of the epithelium and confined to the mucosa by the underlying basement membrane.



FIGURE 5-15. Peritoneal carcinomatosis. The mesentery attached to a loop of small bowel is studded with small nodules of metastatic ovarian carcinoma.

through arterial dissemination. Neoplastic cells arrested in the microcirculation penetrate the vessel walls at the site of metastasis by use of the same mechanisms by which the primary tumor invades.

Lymphatic Metastases

A historical dogma of metastatic spread held that epithelial tumors (carcinomas) preferentially metastasize through lymphatic channels, whereas mesenchymal neoplasms (sarcomas) are distributed hematogenously. This distinction is no longer considered valid because of clinical observations of metastatic patterns and the demonstration of numerous connections between the lymphatic and vascular systems. Tumors arising in tissues that have a rich lymphatic network (e.g., the breast) often metastasize by this route, although the particular properties of specific neoplasms may play a role in the route of spread.

Basement membranes envelop only large lymphatic channels; they are lacking in lymphatic capillaries. Thus, invasive tumor cells may penetrate lymphatic channels more readily than blood vessels. Once in lymphatic vessels, the cells are carried to the regional draining lymph nodes, where they initially lodge in the marginal sinus and then extend throughout the node. Lymph



FIGURE 5-14. Adenocarcinoma of the colon with intestinal obstruction. The lumen of the colon at the site of the cancer is narrow. The colon above the obstruction is dilated.

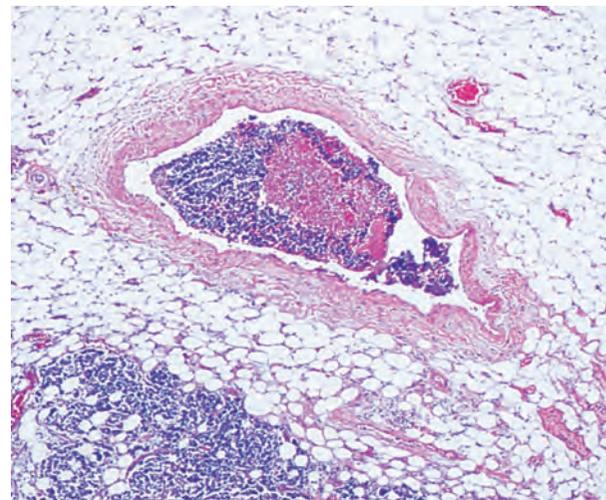


FIGURE 5-16. Hematogenous spread of cancer. A malignant tumor (bottom) has invaded adipose tissue and penetrated into a small vein.

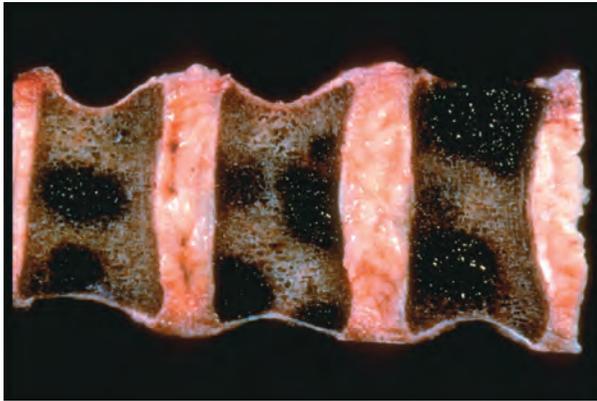


FIGURE 5-17. Multiple pigmented metastases in the vertebral bodies in a patient who died of malignant melanoma.

nodes bearing metastatic deposits may be enlarged to many times their normal size, often exceeding the diameter of the primary lesion. The cut surface of the lymph node usually resembles that of the primary tumor in color and consistency and may also exhibit the necrosis and hemorrhage commonly seen in primary cancers (Fig. 5-18).

The regional lymphatic pattern of metastatic spread 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.

Lymphatic metastases are occasionally found in lymph nodes distant from the site of the primary tumor; these are termed **skip metastases**. For example, abdominal cancers may initially be signaled by the appearance of an enlarged supraclavicular node, the so-called **sentinel node**. A graphic example of the relationship of lymphatic anatomy to the spread of ma-



FIGURE 5-18. Metastatic carcinoma in periaortic lymph nodes. The aorta has been opened and the nodes bisected.

lignant tumors is afforded by cancers of the testis. Rather than metastasizing to the regional 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, are observed. Similar to tissue culture, tumor in these sites grows in masses and often produces fluid (e.g., ascites, pleural fluid), sometimes in very large quantities. Mucinous adenocarcinoma may also secrete copious amounts of mucin in these locations.

Invasion and Metastasis Are Multistep Events

Several steps are required for malignant cells to establish a metastasis (Fig. 5-19):

1. Invasion of the basement membrane underlying the tumor
2. Movement through extracellular matrix
3. Penetration of vascular or lymphatic channels
4. Survival and arrest within circulating blood or lymph
5. Exit from the circulation into a new tissue site
6. Survival and growth as a metastasis, a process that involves angiogenesis

Most cancers originate from the malignant transformation of a single cell (**monoclonal origin of tumors**). Nevertheless, the inherent genetic instability of the malignant phenotype leads to the appearance of subpopulations with diverse biological characteristics and profound variations in their metastatic potential (**tumor heterogeneity**). The demonstration of tumor heterogeneity has led to the concept that at each step of the metastatic cascade, only the fittest cells survive. Thus, the metastatic process can be viewed as a competition in which a subpopulation of cells within the primary cancer ultimately prevails as a metastasis.

Invasion

Inherent in the definition of a malignant cell is the capacity to invade surrounding tissue. In epithelial tumors, invasion requires disruption of, and penetration through, the underlying basement membrane and passage through the extracellular matrix. Similarly, circulating cells destined to establish metastases must reproduce these same events to exit from the vascular or lymphatic compartment and establish residence at a distant site.

Adhesion Molecules

The entire metastatic sequence, from the initial binding of the tumor cell to the underlying extracellular matrix to the growth in a distant location, depends on the expression of numerous adhesion molecules by the malignant cells. The display of such surface molecules varies with (1) the type of tumor, (2) the individual clone (tumor heterogeneity), (3) the stage of the malignant progression, and (4) the specific step in the metastatic process.

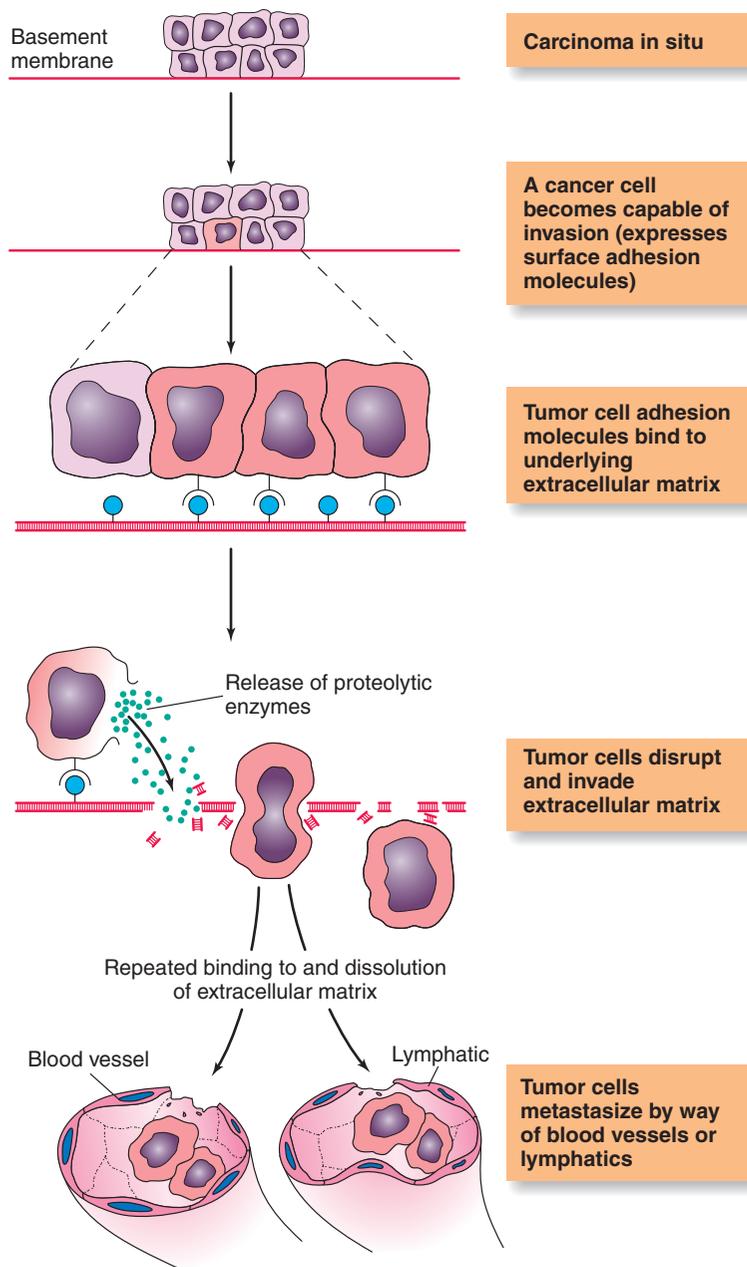


FIGURE 5-19. Mechanisms of tumor invasion and metastasis. The mechanism by which a malignant tumor initially penetrates a confining basement membrane and then invades the surrounding extracellular environment involves several steps. The tumor first acquires the ability to bind components of the extracellular matrix. These interactions are mediated by the expression of a number of adhesion molecules. Proteolytic enzymes are then released from the tumor cells, and the extracellular matrix is degraded. After moving through the extracellular environment, the invading cancer penetrates blood vessels and lymphatics by the same mechanisms.

INTEGRINS: Integrins are transmembrane receptors, each consisting of two α and two β subunits, which together confer substrate specificity on the receptor (see Chapter 3). These adhesion receptors mediate cell–matrix and cell–cell attachment. The binding of integrins to their ligands also stimulates intracellular signaling and gene expression, which play a role in cell migration, proliferation, differentiation, and survival. In addition, integrins affect the expression, localization, and activation of collagenases (matrix metalloproteinases [MMPs]; see below) and can guide these enzymes to their targets in the extracellular matrix, where they degrade connective tissue and pave the way for the spread of tumor cells.

IMMUNOGLOBULIN SUPERGENE FAMILY: A number of intercellular adhesion molecules belong to this superfamily,

including intercellular adhesion molecule-1 (ICAM-1), MUC18, and vascular cell adhesion molecule-1 (VCAM-1). The expression of ICAM-1 correlates positively with the aggressiveness of a variety of tumor cell types.

CADHERINS AND CATENINS: Cadherins are a family of cell–cell adhesion molecules, which are calcium (Ca^{2+})-dependent transmembrane glycoproteins. The best-characterized of the cadherins, E-cadherin, 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 suppress invasion and metastasis. The expression of

both E-cadherin and catenins is reduced or lost in most carcinomas, an effect that permits individual malignant cells to leave the main tumor mass and metastasize. Interestingly, β -catenin also binds to the adenomatous polyposis coli (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 13).

Growth Factors and Cytokines

Growth factors and cytokines orchestrate cellular responses during development, differentiation, and repair. Aberrant production of growth factors by tumors contributes to neoangiogenesis and attraction of inflammatory cells as well as enhances proliferation, migration, and invasive properties of the tumor cells. A notable example is **autocrine motility factor** (AMF), a molecule that belongs to a family of tumor cell cytokines that stimulate motility via a receptor-mediated signaling pathway. AMF not only regulates motility but also modulates the expression of cell surface integrins. The expression of the AMF receptor (gp78) in normal cells is regulated by cell contact, whereas in many cancer cells, it is constitutively expressed.

Proteolytic Enzymes

A breach of the basement membrane that separates an epithelium from the underlying mesenchymal compartment is the first event in tumor cell invasion. The basement membrane is composed of a number of extracellular matrix components, including type IV collagen, laminin, and proteoglycans (see Chapter 3). Malignant cells and stromal cells associated with cancers elaborate a variety of proteases that degrade one or more of the basement membrane components. Such enzymes include the urokinase-type plasminogen activator (u-PA) and MMPs, including collagenases.

u-PA converts serum plasminogen to plasmin, a serine protease that degrades laminin and activates type IV procollagenase. u-PA activity is balanced by plasminogen activator inhibitor (PAI); changes in the expression of u-PA, the u-PA receptor and PAI have been reported in different cancers.

The MMPs comprise a family of zinc-dependent endopeptidases that are susceptible to tissue inhibitors of MMPs (TIMPs). MMPs include interstitial collagenases, stromelysins, gelatinases, and membrane-type MMPs. These enzymes are synthesized and secreted by normal cells under conditions associated with physiologic tissue remodeling, such as wound healing and placental implantation. Under these circumstances, a balance between MMPs and TIMPs is strictly regulated. By contrast, the invasive and metastatic phenotypes of cancer cells are characterized by dysregulation of this balance.

A direct correlation between increased expression of MMPs and augmented invasive capacity or metastatic potential of tumor cells has been observed in many cancers. In addition, many of these same tumors exhibit decreased TIMP expression. MMPs are present in either tumor cells or surrounding stromal cells or both, depending on the particular neoplasm. In some instances, MMPs secreted by stromal cells are bound to integrins on the surface of the tumor cells, thereby providing a particularly high local concentration of protease activity at the site of tumor invasion. Deregulated MMP activity permits entry of cancer cells into, and their passage through, the extracellular matrix.

Metastasis

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

1. **Invasion of the circulation:** After invading interstitial tissue, malignant cells penetrate lymphatic or vascular channels. 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 less than 0.1% remain to establish a new colony.
2. **Escape from the circulation:** Circulating tumor cells may arrest mechanically in capillaries and venules, where they attach to endothelial cells. This adherence causes retraction of the endothelium, thereby exposing the underlying basement membrane to which tumor cells now bind. Clumps of tumor cells may also arrest in arterioles, where they grow within vascular lumens. In both situations, tumor cells eventually extravasate by mechanisms similar to those responsible for local invasion.
3. **Local growth:** In a hospitable site, the extravasated cancer cells grow in response to autocrine and possibly local growth factors produced by the host tissue. However, a new vascular supply is necessary for the tumor to grow to a diameter greater than 0.5 mm. Thus, many tumors secrete polypeptides (e.g., fibroblast growth factor [FGF], vascular endothelial growth factor [VEGF], transforming growth factor- β [TGF- β], and platelet-derived growth factor [PDGF]), which together trigger and regulate the process of **angiogenesis** (see below). The newly established metastatic colony must also escape detection and destruction by the host immune defenses (see below). The metastasis can metastasize again, either within the same organ or to distant sites.

The establishment of a metastatic colony does not mean that it inevitably enlarges. It is well known clinically that tumors may recur locally or at metastatic sites many years after the primary cancer has been surgically removed. For example, patients treated for breast cancer or malignant melanoma may be apparently cured for 20 or more years, only to have the tumor suddenly recur. The molecular basis for this phenomenon, termed **tumor dormancy**, is not well understood (see below).

Target Organs in Metastatic Disease

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 microenvironment factors in the secondary site (the soil). By contrast, others have argued that metastatic spread depends solely on anatomical factors and the blood flow to an organ. Today, there is evidence that both mechanisms operate, depending on the tumor. 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. Yet for many cancers, the vascular anatomy unquestionably influences the pattern of metastatic spread. Malignant tumors of the gastrointestinal tract commonly metastasize to the first capillary bed they encounter, namely the liver. Similarly, lung cancers often spread to the brain. An additional factor that regulates homing

of malignant cells may be the expression of complementary adhesion molecules, either by the cancer cells or those of the organ to which they home.

The Grading and Staging of Cancers

In an attempt to predict the clinical behavior of a malignant tumor and to establish criteria for therapy, many cancers are classified according to cytologic and histologic grading schemes or by staging protocols that describe the extent of spread.

Cancer Grading Reflects Cellular Characteristics

Low-grade tumors are **well differentiated**; high-grade ones tend to be **anaplastic**. 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 glandlike structures in adenocarcinomas or epithelial pearls in squamous carcinomas. The presence of such characteristics identify a tumor as well differentiated. By contrast, the cells of “poorly differentiated” malignancies bear little resemblance to their normal counterparts. Evidence of rapid or abnormal 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. 5-20). 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.

Cancer Staging Refers to the Extent of Spread

The choice of a surgical approach or the selection of treatment modalities is influenced more by the stage of a cancer than by its cytologic grade. Moreover, most statistical data related to cancer survival are based on the stage rather than the cytologic grade of the tumor. Clinical staging is independent of cytologic grading.

The significant criteria used for staging vary with different organs. Commonly used criteria include:

- Tumor size
- Extent of local growth, whether within or without the organ
- Presence of lymph node metastases
- Presence of distant metastases

These criteria have been codified in the international **TNM cancer staging system**, in which “T” refers to the size of the primary tumor, “N” to regional node metastases, and “M” to the presence and extent of distant metastases. The definitions of numerical scores for T, N, and M (e.g., T1–T4, N1–N3) vary according to specific tumor types.

In some cases, the distinction between benign and malignant tumors is based solely on size. For example, on the basis of clinical experience with papillary renal cancers, tumors smaller than 0.5 cm in diameter are generally considered benign adenomas, whereas those of larger size are labeled renal cell carcinomas. The choice of surgical therapy is often influenced by size alone. For instance, a primary breast cancer smaller than 2 cm in diameter can be treated with local excision and radiation therapy; larger masses often necessitate mastectomy. Local extension can also be used to estimate prognosis, as in the Dukes classification of colorectal cancer. Penetration of the tumor into the muscularis and serosa of the bowel is associated with a poorer prognosis than that of a more superficial tumor. Clearly, the presence of lymph node metastases mandates more aggressive treatment than does their absence, whereas the presence of distant metastases is generally a contraindication to surgical intervention other than for palliation.

The Clonal Origin of Cancer

Studies of human and experimental tumors have provided strong evidence that most cancers arise from a single transformed cell. This theory has been most thoroughly examined in connection with proliferative disorders of the hematopoietic system. The most common piece of clinical evidence in its favor is the production by neoplastic plasma cells of a single immunoglobulin unique to

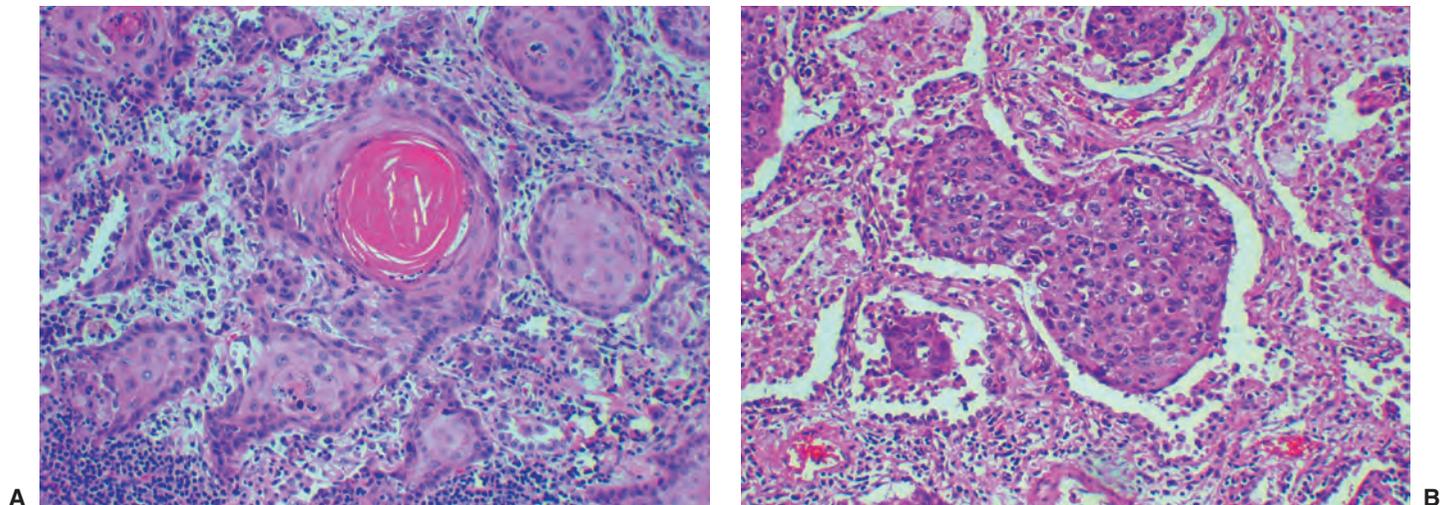


FIGURE 5-20. Cytologic grading of squamous cell carcinoma of the lung. A. Well-differentiated (grade 1) squamous cell carcinoma. The tumor cells bear a strong resemblance to normal squamous cells and synthesize keratin, as evidenced by epithelial pearls. **B.** Poorly differentiated (grade 3) squamous cell carcinoma. The malignant cells are difficult to identify as being of squamous origin.

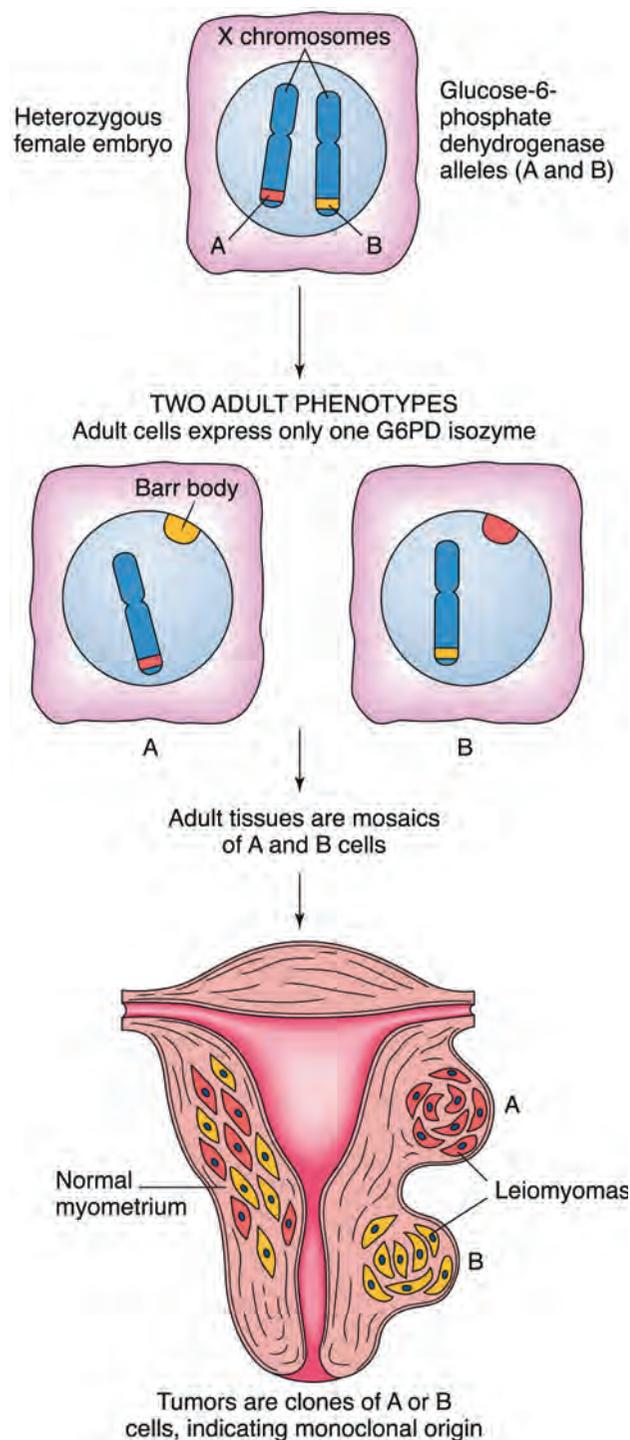


FIGURE 5-21. Monoclonal origin of human tumors. Some females are heterozygous for the two alleles of glucose-6-phosphate dehydrogenase (G6PD) on the long arm of the X chromosome. Early in embryogenesis, one of the X chromosomes is randomly inactivated in every somatic cell and appears cytologically as 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.

an individual patient with multiple myeloma. Indeed, such a “monoclonal spike” in the serum electrophoresis from a patient with suspected myeloma is regarded as conclusive evidence of the disease. Similarly, cell surface markers have been used to establish a monoclonal origin for many other hematopoietic malignant disorders. For example, B-cell lymphomas are composed of cells that exclusively display either κ or λ light chains on their surface, whereas polyclonal lymphoid proliferations exhibit both types of cells. Monoclonality has also been demonstrated in the individual metastases of a number of solid tumors.

One of the most important observations in regard to the monoclonal origin of cancer was derived from the study of glucose-6-phosphate dehydrogenase in women who were heterozygous for its two isozymes, A and B (Fig. 5-21). These isozymes are encoded by genes located on the X chromosome. Since one X chromosome is randomly inactivated, only one of these genes is expressed in any given cell. Thus, although the genotypes of all cells are the same, their phenotypes vary with regard to the expression of isozyme A or B. An examination of benign uterine smooth muscle tumors (leiomyomas, or “fibroids”) revealed that all the cells in an individual tumor expressed either A or B but not both, indicating that each tumor was derived from a single progenitor cell.

Cancer As Altered Differentiation

In many cancers, the malignant phenotype reflects, at least in part, defects in the normally strict control of cell proliferation. However, in some cancers, it is thought that the malignant cells result from a maturation arrest in the sequence of development from a stem or progenitor cell to a fully differentiated cell. According to this theory, tumor cells accumulate because the mechanisms that control the total number of cells in the fully differentiated compartment of some tissues do not apply when less-differentiated precursor cells fail to mature.

SQUAMOUS CELL CARCINOMA: In many tumors most of the neoplastic cells are outside the cell cycle and thus do not contribute to malignancy of the tumor. For example, as noted above, fewer than 3% of the cells in a squamous carcinoma maintain the tumor’s malignant potential, and most differentiate and die spontaneously. When such terminally differentiated tumor cells are transplanted into appropriate hosts, they do not grow, whereas their undifferentiated counterparts from the same tumor form typical squamous carcinomas. Such observations support the theory that the initial step in the development of some cancers is a failure of the stem cell to differentiate normally to complete the sequence of cell differentiation.

TERATOCARCINOMA: Further evidence to support the concept of cancer as a failure of differentiation has come from the study of experimental malignant germ cell tumors (teratocarcinomas). A single embryonal carcinoma cell—the stem cell of a teratocarcinoma—when transplanted into a mouse, gives rise to a tumor that contains cells derived from all three germ layers. Clearly, the progeny of the original transplanted tumor cell differentiate into more mature cells that express recognizable phenotypes of more fully differentiated tissues. When these differentiated tissues of the teratocarcinoma are separated from the malignant embryonal cells and transplanted into compatible hosts, they not only survive but also function with no detriment to the host. These cells are clearly benign, and the dogma “once a cancer cell, always a cancer cell” does not hold in this case.

A further refinement of this approach involves the transplantation of a single teratocarcinoma stem cell from a mouse into an early mouse embryo. At term the entirely normal pup is a mosaic composed of cells derived from both the embryo proper and the embryonal carcinoma. The progeny of the malignant cell, under the influence of normal developmental controls, has differentiated into mature tissue elements. Thus, the unregulated growth of the cancer cells may be converted into normal patterns of growth and differentiation.

Clinical analogies to the experimental situation do exist. The best known is the rare spontaneous conversion of a malignant neuroblastoma to its better-differentiated, benign counterpart, ganglioneuroma.

LEUKEMIAS AND LYMPHOMAS: The most comprehensive systematic analysis of human neoplasia from the perspective of developmental biology has come from the study of leukemias and lymphomas. During normal B- and T-lymphocyte maturation, there are well-documented sequential changes of membrane antigens and rearrangements of immunoglobulin and T-cell receptor genes. For example, in acute lymphoblastic leukemia of childhood, the neoplastic cells exhibit only partial assembly of the cell surface receptor molecules that characterize mature lymphocytes. In other words, the leukemic cell phenotype bears a strong resemblance to lymphocytes that appear transiently during the developmental sequence of the normal lymphocyte. Thus, the leukemic cells appear to be “frozen” in the act of receptor gene assembly and expression.

Acute myeloid leukemia is similar to acute lymphoblastic leukemia in that the malignant cells express phenotypes of transient, immature myeloid populations. Likewise, studies of chronic lymphocytic leukemias and lymphomas have revealed that these malignant disorders represent clonal expansions of lymphocyte populations corresponding to subsets found in normal lymphoid tissue.

In normal hematopoietic maturation, differentiation is tightly coupled to proliferation—that is, terminally differentiated cells are continually lost, to be replaced by newly proliferated and differentiated cells. By contrast, the data reviewed above suggest that certain leukemias and lymphomas are not truly proliferative disorders but rather reflect an uncoupling of differentiation from proliferation, with resulting accumulation of cells that have not attained terminal differentiation. According to this theory, leukemia and lymphoma may represent the stabilization of a phenotype that is also expressed, though only transiently, in developing normal cells.

RETINOIDS: The view that certain cancers may reflect impaired differentiation has led to a search for drugs that commit cancer cells to terminal differentiation and, therefore, apoptosis. The interest in the retinoids derives from experiments showing that administration of excess vitamin A or its derivatives inhibits chemically induced carcinogenesis in skin, lung, bladder, colon, and mammary gland.

A dramatic response to all-*trans*-retinoic acid is generated in acute promyelocytic leukemia, in which the administration of this agent induces a complete remission in most patients. In this disease, the reciprocal translocation between chromosomes 15 and 17 results in a fusion gene consisting of the retinoic acid receptor and the promyelocytic leukemia gene (PML) gene. The chimeric protein blocks myeloid differentiation at the promyelocyte stage, a process that is reversed by retinoic acid. Other forms of retinoic acid have shown limited activity against

a variety of tumors. In patients with acute promyelocytic leukemia who are refractory to therapy with retinoic acid, arsenic trioxide, a compound that induces partial nonterminal differentiation of leukemic cells, is surprisingly effective.

The Growth of Cancers

Historically, cancer was considered to result from a totally unregulated growth of cells, and a logical corollary was that neoplastic cells divide at a faster rate than normal ones. *It is now clear that tumor cells do not necessarily proliferate more rapidly than their normal counterparts.* Tumor growth depends on other factors, such as the growth fraction (proportion of cycling cells) and the rate of cell death. In normal proliferating tissues (e.g., intestine and bone marrow), an exquisite balance between cell renewal and cell death is strictly maintained. *By contrast, the major determinant of tumor growth is clearly the fact that more cells are produced than die in a given time.* Such an effect can reflect either an excess of cell proliferation over programmed cell death or also normal rates of cell renewal in the face of reduced apoptosis.

Tumor Growth Rates May Be Expressed As Doubling Times

Tumor doubling time is the time taken for the number of cells in the mass to double. Internal cancers are not usually detected before they attain a size of about 1 cm³ (1 g), which corresponds to 10⁸ to 10⁹ cells. The origin of most tumors from a single cell implies that the mass has doubled at least 30 times to reach this size. If the cancer is neglected and enlarges to the impressive size of 1 kg, it now contains 10¹² cells. Yet, the growth from 1 g to 1 kg (assuming no cell death) can be achieved by only 10 population doublings. Thus, when cancers are initially detected clinically, they are already far advanced in their natural history. Because of the variable death rate of tumor cells and differences in cell cycle kinetics, the actual doubling time of human tumors is highly unpredictable.

The doubling time is not necessarily correlated with the growth fraction (i.e., the proportion of cells that are within the cell cycle). Since the duration of mitosis in cancer cells is often prolonged, the increased number of mitoses in a histologic section can be misleading as an indicator of overall growth. For example, a doubling in the time required for mitosis results in twice as many visible mitoses without any real increase in the rate of growth. In most cases, the theoretical tumor doubling time, calculated from the growth fraction and the cell cycle time, bears little relation to the actual clinical situation. To give a particular example, if a tumor weighing 1 g (often the smallest size clinically detectable) produces 2 new cells per 1000 cells in each mitotic cycle, the theoretical net increase would be a staggering 10⁶ cells per hour, a figure totally at variance with the experience with most solid tumors. *Because of this difference between the theoretical and observed growth of tumors, it has been estimated that in human skin tumors, as many as 97% of proliferated cells die spontaneously.* The causes of tumor cell death are not precisely defined but probably include such factors as programmed cell death (apoptosis); inadequate blood supply, with consequent ischemia; a paucity of nutrients; and vulnerability to specific and nonspecific host defenses. From a practical point of view, the duration of a malignant tumor cannot be reasonably estimated from its size when it is first discovered.

Tumor Angiogenesis Refers to the Sprouting of New Capillaries

In the absence of new vessels to supply nutrients and remove waste products, malignant tumors do not grow larger than 1 to 2 mm in diameter. In this context, the density of capillaries within the primary tumor (e.g., cancers of the breast, prostate, and colon) correlates directly with metastases and decreased survival. Importantly, tumor angiogenesis occurs in non-neoplastic host tissue and is comparable to that in wound healing and other physiologic circumstances (see Chapter 3). Neovascularization of the evolving cancer may appear at various stages of tumor development and probably is related to phenotypic and genetic changes in the tumors. However, it is still unclear whether tumor angiogenesis is fundamentally a response to tissue hypoxia or to a distinct angiogenic tumor phenotype by which neoplastic cells secrete angiogenic factors. The latter is supported by the observation that neovascularization can begin even in premalignant lesions.

A number of factors can stimulate angiogenesis, including FGF, TGF- α and TGF- β , TNF- α , VEGF, PDGF, and epidermal growth factor (EGF). Some factors act directly on endothelial cells (e.g., VEGF), whereas others stimulate inflammatory cells to promote the formation of new blood vessels (e.g., PDGF). As the new vessels mature, these factors support the recruitment of pericytes and smooth muscle cells, and support the deposition of extracellular matrix. Inactivation of tumor suppressor genes may also play a role in regulating tumor angiogenesis (see below).

VEGF and FGF-2 are thought to be the most important angiogenic factors. Tumor vessels overexpress the tyrosine kinase receptors for VEGFs (VEGFR1 and VEGFR2). The role of such angiogenic factors is underscored by the growth suppression of many solid tumors by both endogenous and synthetic inhibitors of angiogenesis factors, some of which are in clinical use. Tumor angiogenesis may also be influenced by variations in the production of angiogenic inhibitors, such as thrombospondin, TIMPs, platelet factor 4, and interferons α and β . Other factors that have been documented to influence angiogenesis include adhesion molecules, matrix MMPs, and plasmin.

Tumor Dormancy Accounts for the Interval before the Appearance of Metastases

Metastatic disease is often not detectable at the time of the removal of a primary cancer. With some tumors, notably breast cancer and melanoma, metastases may remain dormant for many years, only to become apparent without any obvious cause. It is not clear whether tumor dormancy represents a balance between cell growth and cell death or whether the tumor cells are in cell cycle arrest. Clinically, most patients who have undergone a resection for a primary cancer do not evidence any detectable metastases either radiologically or pathologically. Thus, so-called micrometastases consist of single tumor cells or very small clusters. In the case of dormant tumor cells, it is not known whether they remain in G₀ phase of the cell cycle for prolonged periods of time or whether they do not grow because of interference with angiogenesis, unresponsiveness to growth factors, or the presence of immune growth restraints.

The Molecular Genetics of Cancer

The belief that cancer has a genetic basis, embodied in the concept of “cancer genes,” has been prevalent for more than half a

century and was rooted in the recognition of four factors: (1) hereditary predisposition, (2) the presence of chromosomal abnormalities in neoplastic cells, (3) a correlation between impaired DNA repair and cancer occurrence, and (4) the close association between carcinogenesis and mutagenesis. *It is now recognized that the unregulated growth of cancer cells results from the sequential acquisition of somatic mutations in genes that control cell growth, differentiation, and apoptosis, or that maintain genomic integrity.* Similar mutations may also be present in the germ line of persons with hereditary cancer predispositions. Mutations can be produced by environmental mutagens such as chemical carcinogens or radiation (see below). They can also arise during normal cellular metabolism, particularly from the formation of activated oxygen species (see Chapter 1).

It is likely that the most common mechanism of mutagenesis relates to spontaneous errors in DNA replication and repair. Considering that 10¹⁷ mitoses occur during an average human lifetime, corresponding to incorporation of the more than 10²⁶ nucleotides into nascent DNA, it is impossible for this much DNA replication to occur without the introduction of unrepaired errors (mutations), particularly with the added burden of environmental mutagenic stresses. Since the body is composed of some 10¹⁴ cells and the mutation rate is roughly 10⁷ per gene per cell division, it is inevitable that everyone is a somatic mosaic at many genetic loci. Most such mutations are of no consequence, because they either do not affect the function of the cell or are lost as a result of the death of the cell. However, if the mutation involves genes that control growth or stabilize the genome, it may give rise to a clone of cells that possess a growth advantage over their normal neighbors. Successive mutations in similar genes result in increasingly aberrant clones until a malignant phenotype eventually emerges. *In a sense, the emergence of malignancy may be viewed as an evolutionary process wherein we see only the surviving clones.*

Transformed Cells Share Common Attributes

The precise definition of cell transformation is difficult, but it is generally accepted that malignant transformation involves somatic mutations that confer a set of common properties. It is estimated that a minimum of 4 to 7 mutated genes are required for transformation of a normal cell into a malignant phenotype. This **multistep** process takes place over a period of years, an observation that accounts, at least in part, for the fact that the incidence of cancer increases with age. Although mutations in hundreds of genes have been implicated in the pathogenesis of cancer, individual cancers exhibit unique profiles of genetic alterations. Nevertheless, the disruption of a limited number of regulatory pathways in the cell that leads to deregulation of cell proliferation and suppression of apoptosis confers a neoplastic phenotype to diverse cell types. Metazoans must allow cell proliferation upon demand. *Thus, from a teleological perspective, cancer reflects the failure to suppress the deregulated growth of mutated cells.*

Cancer cells are remarkably heterogeneous in appearance, growth rate, invasiveness, and metastatic potential, presumably owing to the interplay between diverse acquired mutations and the inherent gene expression of specific cell lineages. Nevertheless, transformed cells share certain biological features:

- Autonomous generation of mitogenic signals
- Insensitivity to exogenous antigrowth signals
- Resistance to apoptosis
- Limitless replicative potential (immortalization)
- Blocked differentiation

- Ability to sustain angiogenesis
- Capacity to invade surrounding tissues
- Potential to metastasize

Normal genes are mutated in various cancers, including cell cycle regulators, signal transduction factors, transcriptional factors, DNA-binding proteins, growth factor receptors, adhesion molecules, effectors of apoptosis, and telomerase. Thus, the concept of specific “cancer genes” is fanciful. The transforming genes can be grouped into three categories:

- **Oncogenes** are altered versions of normal genes, termed **protooncogenes**, that regulate normal cell growth, differentiation, and survival. Gain-of-function (dominant) mutations activate protooncogenes to become oncogenes and are positive effectors of the neoplastic phenotype.
- **Tumor suppressor genes** are normal genes whose products inhibit cellular proliferation. Loss-of-function (recessive) mutations inactivate inhibitory activities of tumor suppressor genes, thereby permitting unregulated cell growth.
- **Mutator genes (DNA mismatch repair genes)** normally maintain the integrity of the genome and the fidelity of DNA replication. Inactivating mutations of these genes allow the successive accumulation of further mutations.

Cell Cycle Control

The replication of a eukaryotic cell follows a tightly orchestrated program. Myriad intracellular signal transduction pathways connect extracellular signals (growth factors and cytokines) with genes that regulate the cell cycle machinery. Upon growth factor stimulation, a cell leaves the quiescent state (G_0) and enters G_1 (Fig. 5-22). DNA replication occurs during S phase, followed by G_2 and ultimately mitosis (M phase). Cells progress directly from M phase into G_1 in actively dividing cells.

During G_1 , the commitment to enter the S phase occurs at a **restriction (R) point** wherein the cell monitors its internal and external environment and “decides” whether to proceed with replication. The R point is regulated by **cyclins**, so named for their cyclic expression and degradation during the cell cycle. Cyclins complex with and activate a family of related serine/threonine protein kinases, termed **cyclin-dependent kinases (CDKs)**. CDK 2, 4, and 6 phosphorylate a family of **retinoblastoma proteins (pRb)**. Retinoblastoma (Rb) phosphorylation unleashes transcription factors of the **E2F family**. E2F drives the cell past the R point. Other cyclins and CDKs regulate S to G_2 and G_2 to M transitions.

CDKs are also regulated by **cyclin-dependent kinase inhibitors (CKIs)**. The expression of CKIs can be induced by senescence, contact inhibition, extracellular antimetabolic factors (e.g., TGF) and the tumor suppressor protein p53 (see below).

Loss of R point control deregulates progression through the cell cycle. Cancer cells often display loss of R point control through mechanisms such as (1) amplification/overexpression of cyclins/CDKs, (2) loss of CKIs, and (3) mutational inactivation of pRb or p53 proteins. For example, decreased levels of the CKI P27 are associated with a poor prognosis in adenocarcinoma of the colon and certain cancers of the lung. Conversely, a number of malignant tumors overexpress several cyclins and CDKs. Loss of Rb is a feature of many malignancies.

Cell cycle progression depends upon regulatory mechanisms that involve “check points,” which ensure that the cell does not progress to mitosis until the S phase has been com-

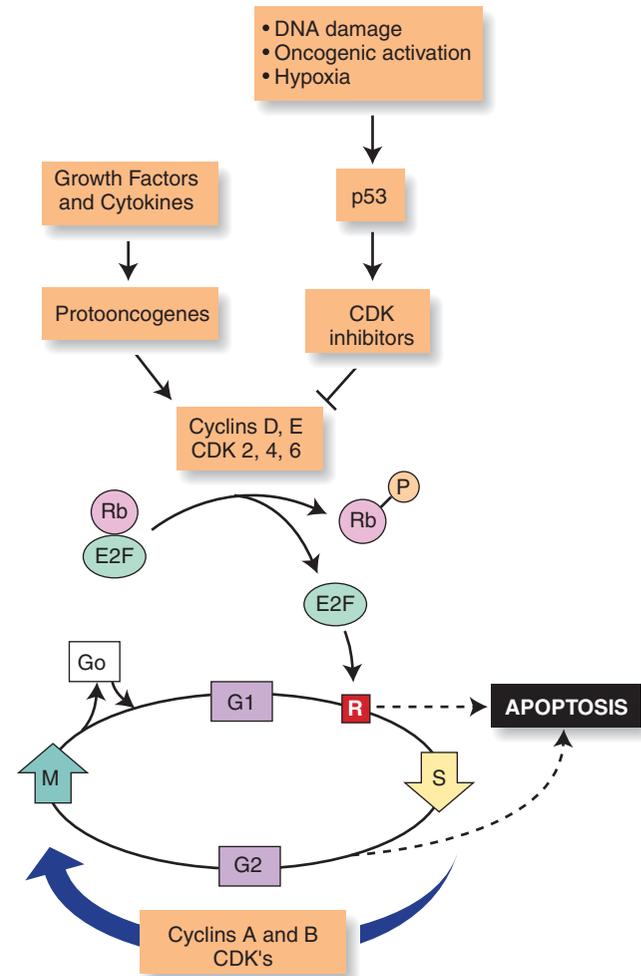


FIGURE 5-22. Regulation of the cell cycle. Cells are stimulated to enter G_1 from G_0 by growth factors and cytokines via protooncogene activation. A critical juncture in the transition of cells from G_1 to S phase is the restriction point (R). A major regulatory event in this process is the phosphorylation of retinoblastoma (Rb) by cyclin-dependent kinases (CDKs), which causes the release of the transcriptional activator E2F. CDKs are suppressed by CDK inhibitors (CKIs) that are regulated by p53. Tumor suppressor proteins block cell cycle progression largely within G_1 . Interruption of cell cycle progression during G_1 and G_2 may lead to apoptosis as a default pathway. S, G_2 , and M phases are also regulated by cyclins, CDKs, and CKIs.

pleted and that any DNA damage has been repaired. Blocks in cell cycle progression in G_1 and G_2 often lead to apoptosis as a default pathway. This phenomenon accounts for induction of tumor cell death in response to various chemotherapeutic agents.

Oncogenes Are Counterparts of Normal Genes

The concept of oncogenes was originally derived from studies of animal tumor viruses. Early research on transforming retroviruses showed that a limited number of viral genes could impart a neoplastic phenotype to virally infected cells. It was subsequently demonstrated that the transfer of specific genes from human tumor cells (**oncogenes**) into rodent cells *in vitro* could transform the recipient cells. The transforming genes were discovered to be mutant versions of normal genes involved in growth regulation and were termed **protooncogenes**. Transforming viral oncogenes

were termed *v-onc* genes, and their cellular counterparts (*c-*) were individual normal genes (e.g., *c-myc*, *c-jun*, *c-src*).

Mechanisms of Activation of Cellular Oncogenes

There are three general mechanisms by which protooncogene activation is accomplished:

- A mutation of a protooncogene leads to the constitutive production of an abnormal protein.
- An increase in the expression of the protooncogene causes overproduction of a normal gene product.
- The activation of protooncogenes is regulated by numerous auto-inhibitory mechanisms, which operate as a safeguard against inappropriate activity. Thus, many mutations in protooncogenes lead to insensitivity to normal auto-inhibitory and regulatory constraints.

Activation by Mutation

Mutations by which protooncogenes are converted to oncogenes may involve (1) point mutations, (2) deletions, or (3) chromosomal translocations. The first oncogene identified in a human tumor was activated *c-ras* from a bladder cancer. This gene was found to have a remarkably subtle alteration, namely, a point mutation in codon 12, a change that results in the substitution of valine for glycine in the ras protein. Subsequent studies of other cancers have revealed point mutations involving other codons of the *ras* gene, suggest-

ing that these positions are critical for the normal function of the ras protein. Since the discovery of mutations in *c-ras*, alterations in other growth-regulatory genes have been described.

Activating, or gain-of-function, mutations in protooncogenes are usually somatic rather than germ line alterations. Germ line mutations in protooncogenes, 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.

Activation by Chromosomal Translocation

Chromosomal translocations (i.e., the transfer of a portion of one chromosome to another) have 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 (Fig. 5-23). The *c-abl* protooncogene 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

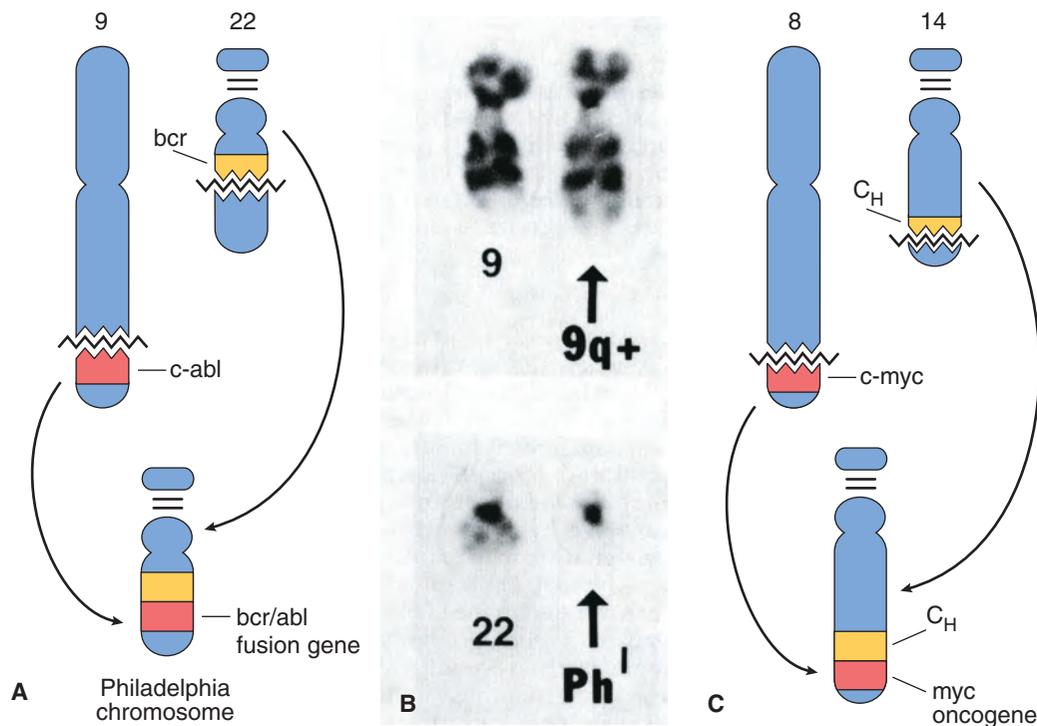


FIGURE 5-23. Oncogene activation by chromosomal translocation. **A.** Chronic myelogenous leukemia. Breaks at the ends of the long arms of chromosomes 9 and 22 allow reciprocal translocations to occur. The *c-abl* protooncogene on chromosome 9 is translocated to the breakpoint region (*bcr*) of chromosome 22. The result is the Philadelphia chromosome, (Ph¹) which contains a new fusion gene coding for a hybrid oncogenic protein (*bcr-abl*), presumably involved in the pathogenesis of chronic myelogenous leukemia. **B.** Karyotypes of a patient with chronic myelogenous leukemia showing the results of reciprocal translocations between chromosomes 9 and 22. The Philadelphia chromosome is recognized by a smaller-than-normal chromosome 22 (22q⁻). One chromosome 9 (9q⁺) is larger than its normal counterpart. **C.** 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.

antiapoptotic signals. The chromosomal translocation that produces the Philadelphia chromosome is an example of oncogene activation by formation of a chimeric (fusion) protein.

In 75% of patients with Burkitt lymphoma (a type of B-cell lymphoma; see Chapter 20), there is a translocation of *c-myc*, a protooncogene involved in cell cycle progression, from its site on chromosome 8 to a position on chromosome 14 (see Fig. 5-23C). This translocation places *c-myc* adjacent to genes that control transcription of the immunoglobulin heavy chains. As a result, the *c-myc* protooncogene 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* protooncogene 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 Gene Amplification

Chromosomal alterations that result in an increased number of gene copies (i.e., gene amplification) have been found primarily in human solid tumors. Such aberrations are recognized as (1) **homogeneous staining regions (HSRs)** (Fig. 5-24A); (2) **abnormal banding regions** on chromosomes; or (3) **double minutes**, which are visualized as multiple, paired, small, cytoplasmic bodies (see Fig. 5-24B). In some cases, gene amplification involves protooncogenes. For example, HSRs derived from the *N-myc* protooncogene may be seen in neuroblastomas. The presence of *N-myc* HSRs is associated with up to a 700-fold amplification of this gene and is a marker of advanced disease with a poor prognosis. Activation of *myc*-family protooncogenes by means of gene amplification has also been demonstrated in small cell carcinoma of the lung, Wilms tumor, and hepatoblastoma.

The *erb B* protooncogene is amplified in up to a third of breast and ovarian cancers. *Erb B2* gene (also designated *HER2/neu*) encodes a receptor-type tyrosine kinase that shows close structural similarity to the EGF receptor. Amplification of *erb B2* in breast and ovarian cancer 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.

Mechanisms of Oncogene Action

Oncogenes can be classified according to the roles of their normal counterparts (protooncogenes) in the biochemical pathways that regulate growth and differentiation. These include the following (Fig. 5-25 and Fig. 5-26):

- Growth factors
- Cell surface receptors
- Intracellular signal transduction pathways

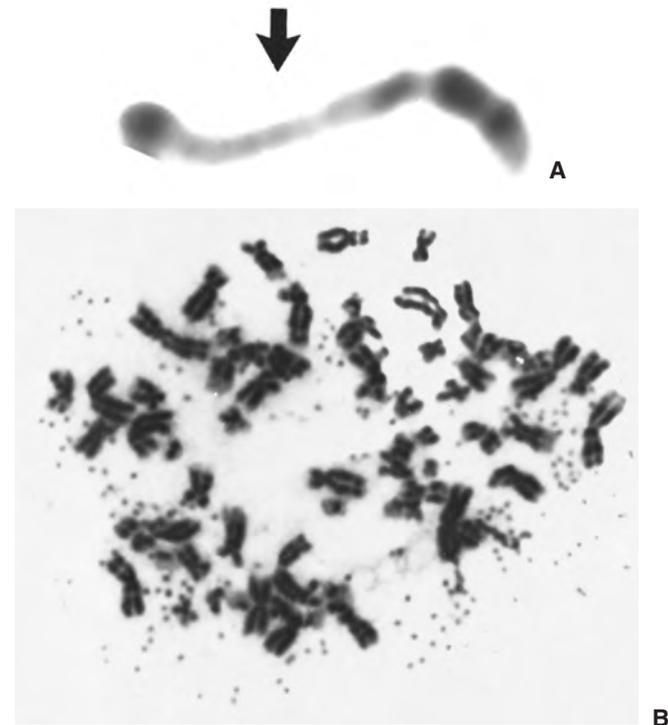


FIGURE 5-24. Chromosomal alterations in human solid tumors. A. Homogeneously staining region (HSR; arrow) in a chromosome from an ovarian carcinoma. **B.** Double minutes in a karyotype of a soft tissue sarcoma appear as multiple small bodies.

- DNA-binding nuclear proteins (transcription factors)
- Cell cycle proteins (cyclins and cyclin-dependent protein kinases)
- Inhibitors of apoptosis (bcl-2)

Oncogenes and Growth Factors

The binding of soluble extracellular growth factors to their specific surface receptors initiates signaling cascades that eventuate in entry of the cell into the mitotic cycle. A few protooncogenes encode growth factors that stimulate tumor cell growth. In some instances a growth factor acts upon the same cell that produces it (**autocrine stimulation**). Other growth factors act upon the receptors of neighboring cells (**paracrine stimulation**). Examples of growth factors involved in neoplastic transformation include PDGF and FGF.

PDGF is the protein product of the *c-sis* protooncogene and is a potent mitogen for fibroblasts, smooth muscle cells, and glial cells. Cells derived from human sarcomas and glioblastomas (malignant glial cell tumors) produce PDGF-like polypeptides; their normal counterparts do not. Thus, a normal human gene (*c-sis*) that encodes a growth factor (PDGF) acquires transforming capacity when it is constitutively expressed in a cell that responds to this signal.

An oncogene (*HST*) that codes for a protein with homology to FGF has been identified in human stomach cancer and Kaposi sarcoma. In rodent models, neoplastic cells often express TGF.

Mutational activation of growth factor genes is not well characterized in human cancers. Nevertheless, whether caused by genetic or epigenetic mechanisms, cancer cells generally produce a mixture of growth factors with autocrine or paracrine

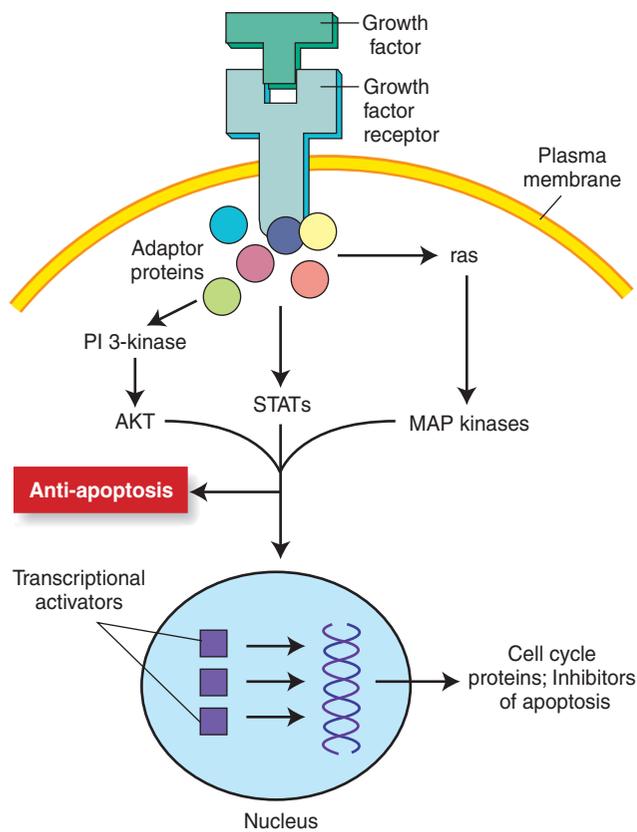


FIGURE 5-25. Signaling pathways controlling proliferation and apoptosis. The activation of growth factor receptors by their ligands causes the binding of adaptor proteins and the activation of a series of intracellular signaling molecules leading to transcriptional activation, the induction of cell cycle proteins, and inhibition of apoptosis. Key targets include *ras*, mitogen activated protein (MAP) kinases, signal transducer and activator transcription factors (STATs), phosphatidylinositol 3-kinase (PI 3-kinase), and the serine/threonine kinase AKT.

activity, including insulin-like growth factor-I (IGF-I), PDGF, TGF- α , FGF, colony-stimulating factor-1 (CSF-1), and hepatocyte growth factor (HGF).

Oncogenes and Growth Factor Receptors

Many growth factors stimulate cellular proliferation by interacting with a family of cell surface receptors that are integral membrane proteins with tyrosine kinase activity. In fact, the regulation of the functional responses to growth factors—including cell proliferation, differentiation, and survival—depends principally on the expression of, and relative balance between, various growth factor receptors. Binding of a ligand to the extracellular domain of its receptor stimulates an intrinsic kinase activity in the cytoplasmic domain of the receptor that phosphorylates tyrosine residues on intracellular signaling molecules. Thus, because growth factor receptors can generate potent mitogenic signals, they harbor a latent oncogenic potential, which when activated, overrides the normal controls of signaling pathways.

The most common mechanism by which growth factors participate in oncogenesis is overexpression of a normal receptor by enhanced activation of promoters or gene amplification. Under normal circumstances, transient binding of a growth factor to its receptor leads to activation of the cytoplasmic tyrosine kinase domain, after which the receptor reverts to its resting state. Certain mutations of growth factor receptors, including truncation

of the extracellular or intracellular domains, point mutations, and deletions, result in unrestrained (constitutive) activation of the receptor, independent of ligand binding. The following examples deserve mention.

- The *c-met* protooncogene encodes a receptor for HGF. Point mutations in the intracellular catalytic domain convert the *c-met* protooncogene to an oncogene that is involved in papillary renal cancers.
- Germline point mutations in *c-ret* lead to constitutive activation of the receptor and are associated with the multiple endocrine neoplasia (MEN) syndromes and familial medullary thyroid carcinoma (see Chapter 21).
- Patients with germ line mutations in the catalytic domain of the *c-kit* tyrosine kinase tend to develop gastrointestinal stromal tumors (GISTs).
- Another abnormality of a growth factor receptor can result from chromosomal translocations that produce hybrid proteins with constitutive tyrosine kinase activity. In the case of the PDGF receptor, a chromosomal translocation [t(5;12)] generates a fusion protein between the cytoplasmic domain of the PDGF receptor and a motif encoded by *c-tel*. The abnormal receptor has been found in patients with myelomonocytic leukemia.

Epigenetic changes that result in increased synthesis of growth factors and their receptors are equally important as mutations and overexpression of growth factor receptors in the pathogenesis of human cancers. In some human malignancies (e.g., breast, ovarian, and stomach cancers), amplification of *HER2/neu* results in autocrine activation that is mediated by overexpression of this growth factor receptor. Of greater importance in human cancers are epigenetic changes that cause increased synthesis of growth factors and receptors.

Oncogenes and Nonreceptor Protein Kinases

A number of proteins that possess tyrosine kinase activity are loosely associated with the inner aspect of the plasma membrane. Although they possess tyrosine kinase activity, they are neither integral membrane proteins nor growth factor receptors. The prototype of a viral oncogene that codes for mutant forms of these protein kinases is *v-src* (see Fig. 5-26). A number of other oncogenes (*abl*, *lck*, *yes*, *fgr*, *fps*, *fes*) belong to the *src* family. The homologous *c-src* protooncogene product is expressed in most cells, whereas other members of the *src* family are expressed in specialized cell types, such as hematopoietic cells and epithelia. The *src* enzymes are activated by most receptor tyrosine kinases and influence cell proliferation, survival, and invasiveness.

The only member of the *src* family that has been implicated in human tumorigenesis is *c-abl*. As discussed above, in chronic myelogenous leukemia this protooncogene, which codes for a cytoplasmic tyrosine kinase, is translocated from chromosome 9 to the breakpoint cluster region (*bcr*) of chromosome 22. The *bcr-abl* fusion gene encodes a mutant protein with conspicuously elevated tyrosine kinase activity, which is necessary for the oncogenic action of the chimeric protein.

Soluble cytoplasmic oncoproteins (*raf*, *mos*, *pim-1*) that phosphorylate serine/threonine residues have also been described. The best studied of the soluble cytoplasmic oncoproteins is *raf*, which plays a role in the signal transduction cascade that converts ligand binding by cell surface receptors into nuclear transcriptional activation. Point mutations in *c-raf* occur in up to 10% of human cancers.

Receptor and nonreceptor tyrosine kinases are dephosphorylated and thereby inactivated by a variety of phosphatases. In this context mutations in the phosphatase PTEN (phosphatase and tensin homolog), the product of a tumor suppressor gene (see below), have been linked to a variety of human malignancies.

Ras Oncogenes

Ras is an effector molecule in the signal transduction cascade that couples the activation of growth factor receptors to changes in nuclear gene transcription. The *ras* protooncogene codes for a product, p21, that belongs to a family of small cytoplasmic proteins (G proteins) that bind guanosine triphosphate (GTP) and guanosine diphosphate (GDP). The ras protein, p21, is distinct from the integral membrane G proteins that are involved in receptor-mediated signal transduction (Fig. 5-27). The protein p21 is active when it binds GTP and is inactive when it binds GDP. Bound GTP is converted to GDP by the intrinsic GTPase activity of p21. This enzyme activity is normally very low but is stimulated more than 100-fold by a GTPase-activating protein (GAP). Thus, the inactivating switch for the ras protein is the p21 GTPase.

The discovery of an activated version of the *ras* protooncogene in bladder cancer cells was the first demonstration of a human oncogene. The substitution of valine for glycine at position 12 in p21 was the first mutation characterized in a human oncogene. It is now evident that activation of *ras* genes (*Ha-ras*, *Ki-ras*, or *N-ras*) is the most frequent dominant mutation in human cancers.

The mutant forms of p21 are characterized by persistence of GTP binding, which maintains the protein in its active conformation. Point mutations in the *ras* protooncogene interfere with the hydrolysis of GTP to GDP by rendering p21 resistant to the action of GAP. In addition, some mutations decrease the intrinsic ATPase activity of the ras protein. The persistence of the GTP-bound state results in uncontrolled stimulation of *ras*-related functions, because p21 is locked in the “on” position.

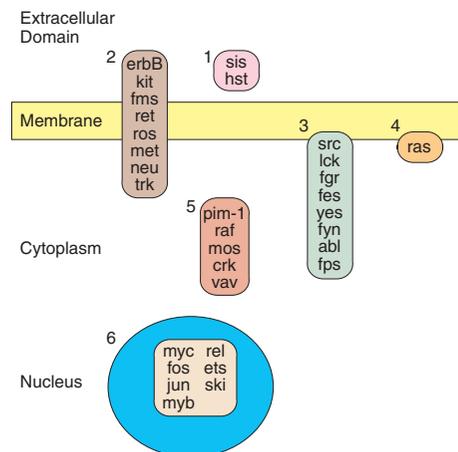


FIGURE 5-26. Cellular compartments in which oncogene or protooncogene products reside. (1) Growth factors, (2) transmembrane growth factor receptors (tyrosine kinase), (3) membrane associated kinases, (4) *ras* GTPase family, (5) cytoplasmic kinases, (6) nuclear transcriptional regulators. GTP = guanosine triphosphate.

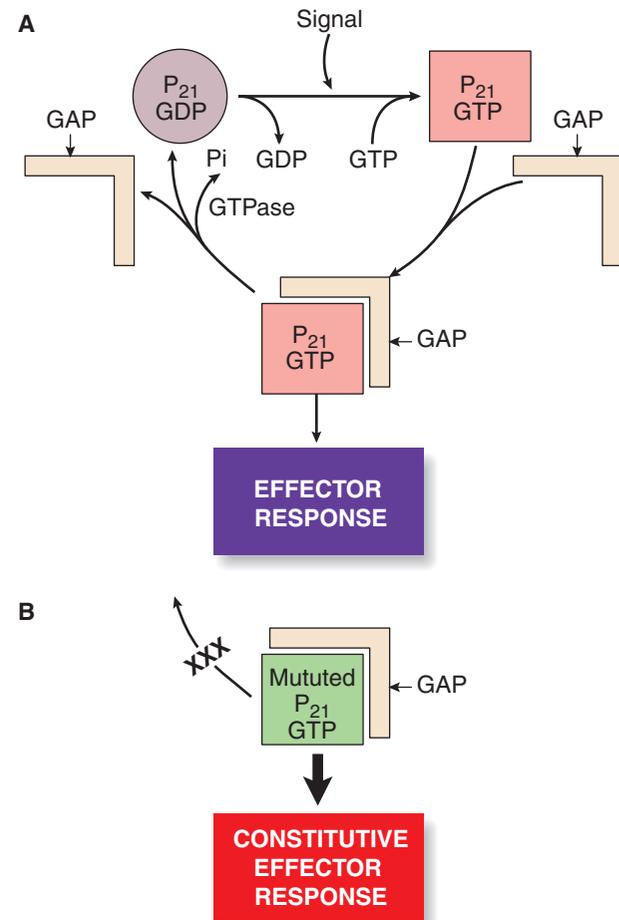


FIGURE 5-27. Mechanism of action of ras oncogene. **A.** Normal. The ras protein p21 exists in two conformational states, determined by the binding of either guanosine diphosphate (GDP) or guanosine triphosphate (GTP). Normally, most of the p21 is in the inactive GDP-bound state. An external stimulus, or signal, triggers the exchange of GTP for GDP, an event that converts p21 to the active state. Activated p21, 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 p21 GTP stimulates by about 100-fold the intrinsic GTPase activity of p21, thereby promoting the hydrolysis of GTP to GDP and the return of p21 to its inactive state. **B.** 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.

Oncogenes and Nuclear Regulatory Proteins

A number of nuclear proteins encoded by protooncogenes are intimately involved in the sequential expression of genes that regulate cellular proliferation and differentiation. Many of these proteins can bind to DNA, where they regulate the expression of other genes. The transitory expression of several protooncogenes is necessary for the cells to pass through specific points in the cell cycle. For example, the binding of PDGF to cultured fibroblasts causes the cells to leave G_0 and enter the G_1 phase of the cell cycle. Shortly thereafter, several genes, including *c-myc*, *c-fos*, and *c-jun*, are expressed. Protooncogenes that are expressed early in the cell cycle, such as *myc* and *fos*, render the cells competent to receive the final signals for mitosis and are, therefore, termed **competence genes**. In general, competence

genes play a role in (1) progression from G₁ to S phase in the cell cycle, (2) stability of the genome, (3) apoptosis, and (4) positive or negative effects on cellular maturation. However, the cells are not yet fully programmed to divide after the expression of these genes and will enter S phase and mitosis only after further stimulation by other factors, such as EGF or IGF-I (**progression factors**).

The proteins encoded by *c-fos* and *c-jun* are components of AP-1, a transcription factor that activates the expression of a variety of genes. Mutations of the *jun* protein eliminate a negative regulatory domain, thereby prolonging its half-life and stimulating progression through G₁. Few mutations of *c-jun* are described in human tumors, but overexpression of the protein has been described in lung and colorectal cancers.

Although nuclear proteins encoded by protooncogenes can promote cellular proliferation, in some circumstances they stimulate differentiation. A rapid increase in *c-fos* expression follows the induction of differentiation in a variety of cells in vitro, including several hematopoietic cell lines and teratocarcinomas.

c-Myc is a nuclear protein that binds to a variety of other proteins and DNA to regulate gene transcription. Among other proteins such targets include p53 and ornithine decarboxylase. As discussed above, the translocation characteristic of Burkitt lymphoma (t(8;14) constitutively activates *c-myc* expression. *c-Myc* is also overexpressed in many human malignant tumors (e.g., adenocarcinoma of lung and breast).

Bcl-2 and Apoptosis

Normal tissue requires an exquisite balance between cell proliferation and cell death (apoptosis, see Chapter 1). Programmed cell death is affected through molecular cascades that reflect two major mechanisms. The **mitochondrial pathway** involves the release of cytochrome c, which serves to trigger caspase activation, thereby leading to cell death. A **death receptor pathway** is unleashed by the binding of certain ligands (e.g., Fas, TNF) to cell surface receptors, which results in caspase activation. There is substantial cross-talk between the mitochondrial and the death receptor pathways.

Tumor cells can escape apoptosis by dismantling virtually every aspect of the apoptotic machinery. The most prominent example of suppression of apoptosis in a tumor cell is the upregulation of the antiapoptotic protein bcl-2 in B cell neoplasia. Bcl-2 and its family regulate the permeability of mitochondrial membranes. Bcl-2 itself exerts an antiapoptotic effect by preventing the release of cytochrome c, thereby protecting the cell from the mitochondrial apoptotic pathway.

Follicular B-cell lymphomas (see Chapter 20) display a characteristic chromosomal translocation, t(14;18), in which the *bcl-2* gene on chromosome 18 is brought under the transcriptional control of the immunoglobulin light-chain gene promoter, thereby causing overexpression of *bcl-2*. As a result of the antiapoptotic properties of bcl-2, the neoplastic clone accumulates in the affected lymph nodes. Since its demonstration in follicular lymphomas, *bcl-2* expression has been observed in a variety of other human cancers and nonneoplastic conditions, although the contribution of *bcl-2* to the disease process in these cases is not defined. Many human cancers show other abnormalities in the apoptotic cascades, including the increased expression of endogenous decoys of the death receptors, overexpression of proteins that block caspase activation, inactivating mutations of proapoptotic proteins, and numerous other mechanisms.

Tumor Suppressor Genes Negatively Regulate Cell Growth

The concept of oncogenes postulates a dominant genetic alteration that results in overproduction of a normal gene product or the synthesis of an abnormally active mutant protein (“**gain of function mutations**”). A second general mechanism by which a genetic alteration contributes to carcinogenesis is a mutation that creates a deficiency of a normal gene product (**tumor suppressors** or “**gate keepers**”) that exerts a negative regulatory control of cell growth and thereby suppresses tumor formation (“**loss of function mutations**”). Such genes encode negative transcriptional regulators of the cell cycle, signal-transducing molecules, and cell surface receptors.

Since both alleles of tumor suppressor genes must be inactivated to produce the deficit that allows the development of a tumor, it is inferred that the normal suppressor gene is dominant. In this circumstance, the heterozygous state is sufficient to protect against cancer. The **loss of heterozygosity** (LOH) in a tumor suppressor gene by deletion or somatic mutation of the remaining normal allele predisposes to tumor development.

The Role of Tumor Suppressor Genes in Carcinogenesis

Tumor suppressor genes are increasingly being incriminated in the pathogenesis of both hereditary and spontaneous cancers in humans. Two such genes have been particularly well studied. The Rb and p53 gene products serve to restrain cell division in many tissues, and their absence or inactivation is linked to the development of malignant tumors. In this context, oncogenic DNA viruses encode products that interact with these suppressor proteins, thereby inactivating their functions. *Thus, the mechanisms underlying the development of some tumors associated with germ line and somatic mutations and infections with DNA viruses involve the same cellular gene products.*

Retinoblastoma Gene

Retinoblastoma, a rare childhood cancer, is the prototype of a human tumor whose origin is attributed to the inactivation of a specific tumor suppressor gene. About 40% of cases are associated with a germ line mutation; the remainder are not hereditary. In patients with hereditary retinoblastoma, all somatic cells carry one missing or mutated allele of a gene (the *Rb* gene) located on the long arm of chromosome 13. By contrast, both alleles of the *Rb* gene are inactive in all the retinoblastoma cells. Thus, the *Rb* gene exerts a tumor suppressor function, and the development of hereditary retinoblastoma has been attributed to two genetic events (Knudson’s “two-hit” hypothesis) (Fig. 5-28). As mentioned above, the nuclear protein p105Rb is phosphorylated by the activated cyclin/CDK complex, thereby inducing the release of E2F transcription factor, which allows G₁-S phase transition. Additionally, certain products of human DNA viruses (e.g., human papillomavirus [HPV]) inactivate p105Rb by binding to it. *The function of Rb genes is the most critical checkpoint in the cell cycle, and inactivating mutations in Rb permit unregulated cell proliferation.*

An affected child inherits one defective *Rb* allele together with one normal gene. This heterozygous state is not associated with any observable changes in the retina, presumably because 50% of the *Rb* gene product is sufficient to prevent the development of retinoblastoma. If the remaining normal *Rb* allele is inactivated by deletion or mutation (LOH), the missing suppressor function allows the appearance of a retinoblastoma. Thus, the susceptibil-

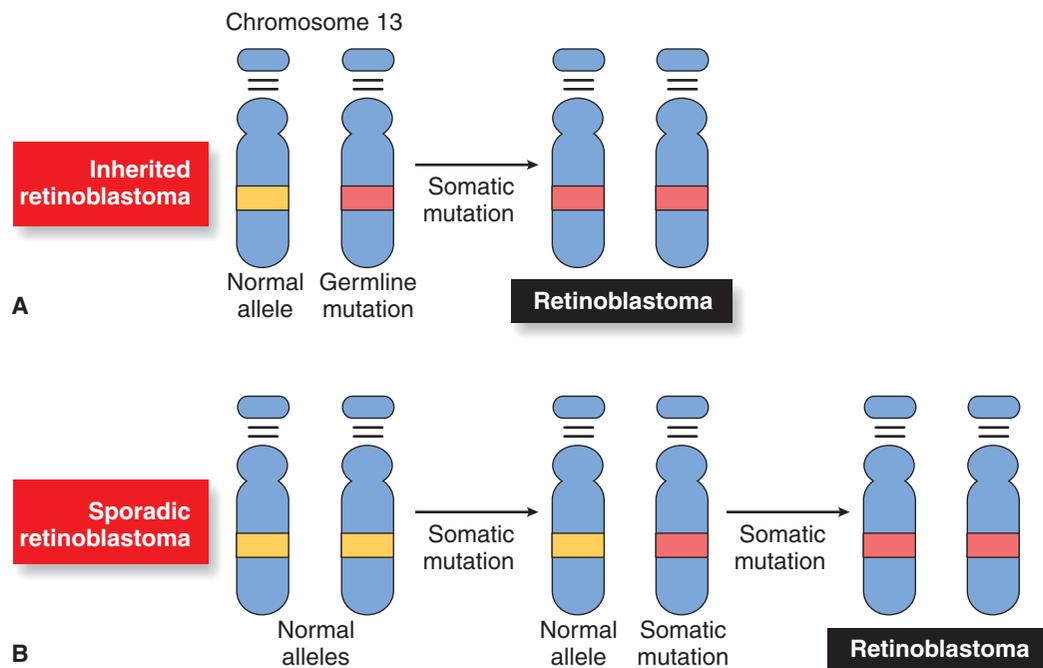


FIGURE 5-28. The “two-hit” origin of retinoblastoma. A. A child with the inherited form of retinoblastoma is born with a germ line mutation in one allele of the retinoblastoma gene located on the long arm of chromosome 13. A second somatic mutation in the retina leads to the inactivation of the functioning *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.

ity to retinoblastoma is inherited in a dominant fashion; that is, the heterozygote develops the disease. Paradoxically, the genetic defect in the tumor itself is recessive. In sporadic cases of retinoblastoma, the child begins life with two normal *Rb* alleles in all somatic cells, but both are inactivated by postzygotic mutations in the retina. Since somatic mutations in the *Rb* gene are uncommon, the incidence of sporadic retinoblastoma is very low (1/30,000).

Children who inherit a mutant *Rb* gene also suffer a 200-fold increased risk of developing mesenchymal tumors in early adult life. More than 20 different cancers have been described, with osteosarcoma being by far the most common. Chromosomal analysis has demonstrated abnormalities of the *Rb* locus in 70% of cases of osteosarcoma and in many instances of small cell lung cancer, carcinomas of the breast, bladder, pancreas, and other human tumors. Many types of *Rb* mutations have been described, including point mutations, insertions, deletions, and translocations. In addition, epigenetic events, such as promoter hypermethylation, may inactivate *Rb*.

The *p53* Gene Family

The *p53* tumor suppressor gene is a principal mediator of growth arrest, senescence, and apoptosis (Fig. 5-29). 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, thereby allowing time for DNA repair to take place. In this manner, *p53* acts as a “guardian of the genome” by restricting uncontrolled cellular proliferation under circumstances in which cells with abnormal DNA might propagate.

The *p53* protein is a transcriptional factor that promotes both the expression of a number of other genes involved in the control of 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 in-

activates cyclin/CDK complexes, thereby 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 they may undergo apoptosis. The stimulation of gene transcription by *p53* results in the synthesis of proteins (CIP1, GADD45) (see Fig. 5-29) that enhance DNA repair by binding to PCNA. In this manner, the upregulation of *p53* has two important and related consequences, namely, arrest of cell cycle progression and promotion of DNA repair.

The *p53* gene is located on the small arm of chromosome 17, and its protein product is present in virtually all normal tissues. This gene is deleted or mutated in 75% of cases of colorectal cancer and frequently in breast cancer, small cell carcinoma of the lung, hepatocellular carcinoma, astrocytoma, and numerous other tumors. *In fact, mutations of *p53* seem to be the most common genetic change in human cancer.* Inactivating mutations of *p53* allow cells with damaged DNA to progress through the cell cycle. Many human cancers exhibit deletion of both *p53* alleles, in which case the cell contains no *p53* gene product. By contrast, in some cancers, the malignant cells express one normal *p53* allele and one mutant version. In these cases, the mutant *p53* protein forms complexes with the normal *p53* protein and thereby inactivates the function of the normal suppressor gene. When a mutant allele inactivates the normal one, the mutant allele is said to be a **dominant negative** gene. Theoretically, a cell containing one mutant *p53* allele (i.e., a heterozygote) might have a growth advantage over the normal cells, a situation that would increase the number of cells at risk for a second mutation (loss of heterozygosity) and the development of cancer.

Negative regulation of *p53* is principally accomplished by its binding to MDM2 (murine double minute) protein. The formation of the MDM2-*p53* complex not only inhibits the function of *p53* but also targets it for degradation via the ubiqu-

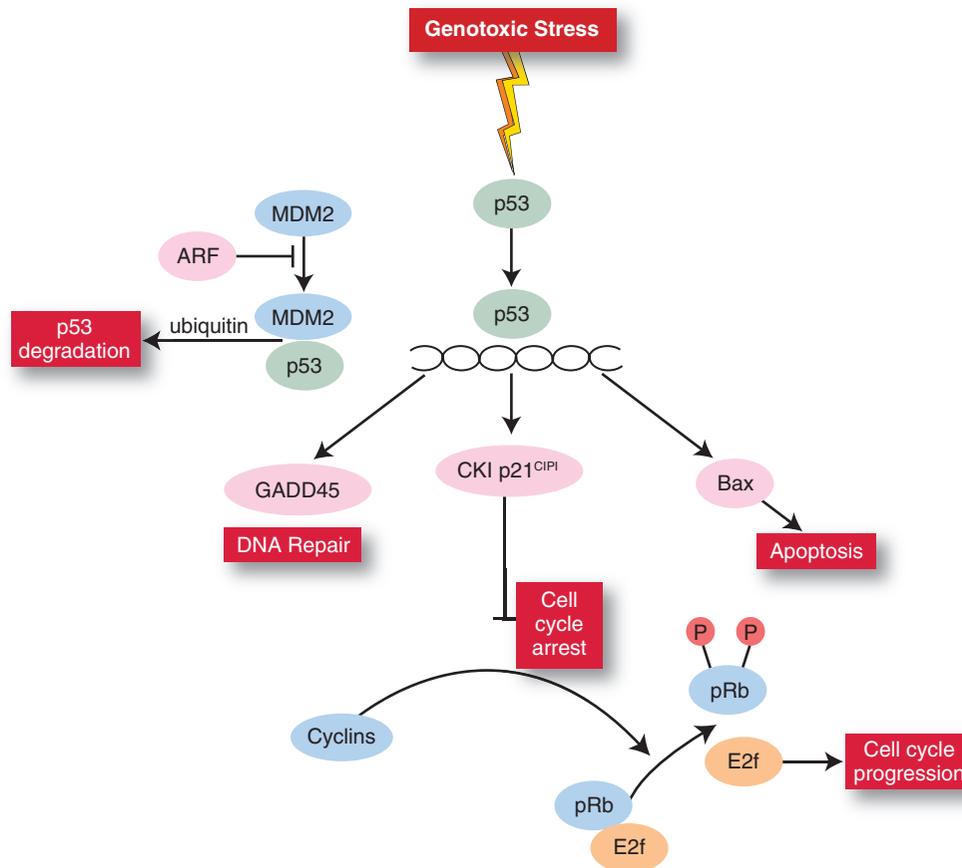


FIGURE 5-29. P53 regulation of genomic integrity. In response to genotoxic stress (e.g., ionizing radiation, carcinogens, mutagens), p53 binds to DNA and up-regulates transcription of several genes. Cyclin-dependent kinase inhibitor (CKI) p21^{CIP1} induces cell cycle arrest by preventing the release of E2F from retinoblastoma protein (pRb). GADD45 promotes DNA repair. Bax induces apoptosis, particularly if DNA repair fails. Importantly, cells with loss or mutation of p53 do not undergo cell cycle arrest and DNA repair, but rather proliferate, generate additional mutations, and increase the risk for the development of malignant tumors. MDM2 (murine double minute) binds to p53 and targets it for ubiquitin-mediated degradation. ARF inhibits MDM2/p53 binding. Reduction in p53 levels in some malignant neoplasms is associated with overexpression of MDM2 or absence of ARF.

uitin pathway. In turn, MDM2 is inhibited by binding to ARF (p14), a protein that is upregulated by any oncogenic stimulus (e.g., *myc*, *ras*, loss of *Rb*) that induces *Rb* phosphorylation and enhances E2F activity. The function of ARF, which maintains the integrity of p53, establishes *ARF* as another tumor suppressor gene. Some cancers in which both p53 alleles are normal overexpress MDM2, whereas others do not express functional ARF. As in the case of *Rb*, certain DNA tumor viral products, including HPV E6, promote p53 degradation. Thus, most human cancers display either inactivating mutations of p53 or abnormalities in the proteins that regulate p53 activity.

Li-Fraumeni syndrome refers to an inherited predisposition to develop cancers in many organs owing to germ line mutations of p53. Persons with this condition carry germ line mutations in one p53 allele, but their tumors display mutations at both alleles. This situation is similar to that determining inherited retinoblastoma and is another example of the two-hit hypothesis (see Fig. 5-28) and LOH.

Other Tumor Suppressor Genes

A number of unrelated syndromes have now been shown to harbor germ line mutations in various tumor suppressor genes:

- **APC gene:** This gene is implicated in the pathogenesis of familial adenomatous polyposis coli and most sporadic colorectal cancers (see Chapter 13). The APC gene product

binds to, and inhibits, the function of β -catenin, an intracellular protein that transmits signals from E-cadherin cell surface adhesion proteins. β -catenin activates certain transcription factors (e.g., *tcf/crf-1*) that activate several genes, including *myc* and cyclin D, which are involved in cell cycle progression. The products of mutant APC genes do not bind to β -catenin and are unable to downregulate its activity. As a result, the expression of *myc* and cyclin D1 is not appropriately repressed, thereby promoting cell proliferation. Further evidence for this mechanism of action comes from the observation that many colorectal tumors in which the APC gene is intact exhibit activating mutations in the β -catenin gene. Mutations in both APC and β -catenin genes have also been described in other malignant tumors, including malignant melanoma and ovarian cancer.

- **WT-1 gene:** The tumor suppressor gene *WT-1* is deleted in hereditary Wilms tumor (WT) and is essential for the normal development of the urogenital tract. It encodes a nuclear DNA-binding protein that represses transcription of a variety of genes whose products promote growth and survival, including *PDGF*, *IGF-I*, and *bcl-2*. Loss of *WT-1* gene expression also occurs in many breast cancers and a few other tumors.
- **NF-1 gene:** Neurofibromatosis (NF) type 1 is related to germ line mutations of the *NF-1* gene, which encodes *neurofibromin*,

a negative regulator of *ras*. Inactivation of *NF-1* permits unopposed *ras* function and thereby promotes cell growth. Patients with neurofibromatosis-1 are at a substantial risk for the development of neurogenic sarcomas.

- **VHL gene:** The inactivation of the von Hippel-Lindau (*VHL*) gene causes the von Hippel-Lindau (VHL) syndrome, which is associated with renal cell carcinoma, hemangioblastoma of the brain, and pheochromocytoma. It is also a major gene involved in the pathogenesis of sporadic renal carcinomas. The normal VHL protein complexes with and inhibits elongin, a molecule that promotes transcriptional elongation of growth-promoting genes by RNA polymerases B and C.
- **FHIT gene:** The fragile histidine triad (FHIT) protein is a dinucleoside phosphate hydrolase that is a tumor suppressor. Deletions within the *FHIT* gene, which is found within a fragile chromosome region that is highly susceptible to DNA damage, are associated with cancers of kidney, lung, digestive tract, and other organs. The mechanism by which loss of FHIT activity contributes to tumorigenesis remains to be elucidated, although the normal protein has been shown to be proapoptotic and growth suppressive.
- **p15 and p16 genes:** Inactivation of these genes has been identified primarily in breast, pancreas, and prostate tumors, has been detected in many other malignancies. The gene products are CKIs that serve as negative regulators of the cell cycle, and their loss removes a brake on cellular proliferation.
- **DPC4 gene:** Some 90% of pancreatic carcinomas feature allelic loss or inactivating mutations in the *DPC4* (deleted in pancreatic cancer) gene. The normal *DPC4* product is a transcriptional activator that mediates the growth inhibitory response to TGF- β .
- **BRCA1 and BRCA2 genes:** These breast (BR) cancer (CA) susceptibility genes, which are also incriminated in some ovarian cancers, are tumor suppressors that are involved in checkpoint functions of the cell cycle related to progression of the cell cycle into S phase, particularly by inducing the CKI p21. BRAC1 and BRAC2 are also thought to promote DNA repair by binding to RAD51, a molecule that mediates DNA double-strand break repairs, thereby functioning as DNA repair genes (see below).
- **PTEN gene:** Termed the phosphatase and tensin homologue deleted on chromosome 10, this gene is mutated in most prostate cancers and many gliomas and thyroid cancers, as well as other tumors. The gene product suppresses tumor cell growth by antagonizing tyrosine kinases and may also regulate invasion and metastasis through interactions at focal adhesions. Germ line mutations in *PTEN* are responsible for **Cowden syndrome**, a disorder that includes multiple hamartomas and an increased risk of cancers of the breast, thyroid, and endometrium.

Tumor Suppressor Genes and Oncogenic DNA Viruses

Unlike RNA tumor viruses, whose oncogenes have normal cellular counterparts, the transforming genes of DNA viruses are not homologous with any cellular genes. *The gene products of oncogenic DNA viruses lead to the inactivation of tumor suppressor proteins.* This phenomenon is analogous to the ability of mutant tumor suppressor proteins to inhibit their normal counterparts. Furthermore, the binding of a HPV protein to p53 accelerates the degradation of this suppressor protein. The transforming

proteins of polyomaviruses (including SV40), adenoviruses, HPVs, and human herpes virus (HHV)-8 also bind and inactivate Rb protein. The interaction of specific viral proteins with Rb releases the E2F family of transcription factors (see Fig. 5-22), which promotes unconstrained cell growth. In addition to activating genes necessary for progression through cell cycle, E2F activates p53 by upregulating the expression of *ARF*, which might provide a defense against aberrant growth. However, DNA tumor viruses have also evolved mechanisms to bind and degrade p53, thereby further “releasing the brake” on cell growth. These observations indicate that oncogenic DNA viruses use common mechanisms for altering growth regulation and, thereby, transforming cells.

DNA Methylation Is an Epigenetic Factor in Cancer

Epigenetics is the alteration in gene expression potential that is unrelated to gene nucleotide sequence. **DNA methylation** represents an important epigenetic regulatory layer for gene transcription. The principal mechanism is the methylation of cytosines within CpG dinucleotides, which occur five times more frequently in so-called CpG islands. DNA methylation patterns are established early in embryogenesis and are finely controlled during development. These regions span the promoter and the first few exons of more than half of all genes. DNA methylation is controlled by a group of enzymes known as the DNA methyltransferases (DNMT) and demethylases. Methylation of these sequences suppresses gene transcription or maintains prior gene silencing by blocking the binding of transcription factors. Another mode of transcriptional repression involves direct binding of specific transcriptional repressors to methylated DNA. DNA methylation can also affect histone modifications and chromatin structure, which, in turn, can alter gene expression. Normal methylation of CpG islands occurs in the case of imprinted genes, inactivated female X chromosomes, germ line genes, and tissue-specific genes. In addition, methylation in the human genome is thought to silence “parasite DNA” such as transposons and endogenous retroviruses, thereby preventing chromosomal instability.

Hypermethylation of many tumor suppressor and DNA repair genes has been demonstrated in human tumors, including the p53 pathway, the APC/ β catenin/*E-cadherin* signaling network, and a number of mismatched DNA repair genes. The pathways controlled by these genes are, therefore, suppressed. For example, about half of human cancers retain unaltered p53. However, the p53 pathway can be inactivated by hypermethylation of *ARF*, thereby preventing inhibition of the MDM2 oncogenic protein and the enhancement of p53 degradation. In this context, aberrant methylation of tumor suppressor genes may be an epigenetic mechanism for a “second hit,” thereby leading to LOH. The analysis of gene hypermethylation has promise as a prognostic tool for specific tumors.

The genome of cancer cells also may undergo conspicuous global **hypomethylation**, which may be reflected in up to 60% less DNA methylation than in the normal cell. Hypomethylation usually involves repeated DNA sequences such as long interspersed nuclear elements. Gene hypomethylation may lead to chromosomal instability, derepression of growth regulatory genes, and overexpression of antiapoptotic genes. Unlike genetic changes in cancer, epigenetic changes are potentially reversible, and a search for drugs that influence DNA methylation is under way.

Histone acetylation and deacetylation play important roles in transcriptional regulation by modifying chromatin

structure. A high degree of histone acetylation is associated with enhanced transcriptional activity, whereas deacetylation is linked to gene silencing. The yin and yang of acetylation and deacetylation of chromatin are fundamental to the process of cell growth. Experimentally, inhibitors of histone deacetylases arrest tumor growth and prevent the progression of metastases. They also promote apoptosis in animal models of leukemia. Although the precise mechanism of action is not clear, such inhibitors have found a role in the treatment of human acute promyelocytic leukemia and lymphoproliferative diseases.

DNA Repair Genes Protect the Integrity of the Genome

The third class of genes in which mutations contribute to the pathogenesis of cancer are genes involved in DNA mismatch repair, so-called **mutator genes** or **caretaker genes**. The human genome contains roughly 3×10^9 base pairs, distributed evenly among some 10^{14} cells in the body. Considering that DNA is continuously assaulted by mutagenic agents such as radiation, oxidative stress, and chemicals, and that the fidelity of DNA replication is not perfect, it is indeed remarkable that cancer arises in only about one third of the population. In general, the normal versions of the DNA repair genes exercise surveillance over the integrity of genetic information by participating in the cellular response to DNA damage. In this respect, DNA repair genes may be considered “**caretaker genes**.” The loss of these gene functions renders the DNA susceptible to the progressive accumulation of mutations; when these affect protooncogenes or tumor suppressor genes, cancer may result.

HEREDITARY NONPOLYPOSIS COLON CANCER (HNPCC): Also known as **Lynch syndrome**, HNPCC is a familial predisposition to the development of colorectal cancers in persons who do not suffer from APC (see Chapter 13). It is estimated that some 5% of all colorectal cancers fall into this category. Patients with HNPCC display heterozygous germ line mutations in at least one of five genes involved in the DNA mismatch repair system, whereas the tumors have lost the function of both alleles in the affected gene. After DNA replication is complete, this system leads to the excision and replacement of mismatched nucleotides. Mutations in these error correction genes are associated with up to a 1000-fold general increase in the rate of mutation. Replication errors, termed **microsatellite instability**, are present in the tumor DNA of patients with HNPCC, which arises from uncorrected mispairing of nucleotides and the resulting misalignment of DNA strands. The incidence of cancers of the stomach and small bowel is also increased in patients with HNPCC, and women with this syndrome display an increased risk for endometrial and ovarian cancers.

ATAXIA TELANGIECTASIA: Ataxia telangiectasia (AT) is a rare hereditary syndrome that features cerebellar degeneration; immunologic abnormalities; oculocutaneous telangiectasia; and a predisposition to cancer, including lymphomas, leukemias, stomach cancer, and breast cancer. About 15% of patients with this syndrome eventually die from a malignant disease. The gene responsible for AT (AT mutated [ATM]), located on chromosome 11q22-q23, codes for a nuclear phosphoprotein that participates in multiple responses to DNA damage, including control of checkpoints in the cell cycle, activation of DNA repair enzymes, and regulation of apoptosis. There is evidence that heterozygous mutations in ATM increase the risk of breast cancer in women. In view of a carrier rate of 1% in the general population, it has been suggested that ATM mutations may contribute to a significant number of sporadic breast cancers.

XERODERMA PIGMENTOSUM: Xeroderma pigmentosum is an autosomal recessive disease in which increased sensitivity to sunlight is accompanied by a high incidence of skin cancers, including basal cell carcinoma, squamous cell carcinoma, and malignant melanoma. Several xeroderma pigmentosum genes have been identified that are involved in nucleotide excision of ultraviolet (UV)-damaged DNA.

BLOOM SYNDROME: Bloom syndrome (BS) is an autosomal recessive disorder associated with small size, sun sensitivity, immunodeficiency, and a predisposition to an array of cancers. Cells from patients with BS show a high mutation frequency. The BS gene encodes a protein that has helicase activity involved in repair of DNA damage.

Telomerase Is Activated in Most Cancers

As cells in tissue culture continue to divide, the tips of the chromosomes, termed **telomeres**, progressively shorten (see Chapter 1). These structures are thought to protect the integrity of the DNA at the ends of the chromosomes, possibly by preventing exonuclease attack on these regions. Somatic cells do not normally express telomerase, an enzyme that recognizes the end of a chromosome and adds repetitive telomeric sequences to maintain telomere length. Thus, with each round of cell replication, the telomere progressively shortens. It has been proposed that the length of the telomeres acts as a molecular clock that governs the life span of replicating cells. Given that cancer cells have been found to express telomerase, the reactivation of this enzyme is said to be necessary for the immortalization of cancer cells.

Most human cancers show activation of the gene for the catalytic subunit of telomerase: human telomerase reverse transcriptase (hTERT). Although deregulated expression of telomerase might be linked to an increased risk of cancer, telomerase is not classified as an oncogene because it does not lead to growth deregulation. Many immortalized cell lines that express telomerase show no evidence of neoplastic capacity. Thus, despite extensive research in the field, the role of telomerase in oncogenesis remains controversial.

Inherited Cancer Syndromes Encompass a Wide Variety of Tumors

Heritable cancer syndromes attributed to germ line mutations make up only 1% of all cancers. These mutations principally involve tumor suppressor genes and DNA repair genes. As previously discussed for *Rb*, the transmission of a single mutated allele of a tumor suppressor gene results in a heterozygous offspring. Since such persons are at a high risk for LOH (i.e., inactivation of the normal allele), they suffer a conspicuous susceptibility to various types of cancer. Thus, inheritance of cancer susceptibility in such cases is said to be dominant. However, in the tumor cells, both tumor suppressor alleles are inactivated. By contrast, a number of inherited cancer syndromes, mostly involving DNA repair genes, display classical recessive inheritance.

The hereditary tumors can be arbitrarily divided into three categories:

1. Inherited malignant tumors (e.g., *Rb*, WT, and 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 and ataxia telangiectasia).

TABLE 5-2
Selected Hereditary Conditions Associated with an Increased Risk of Cancer

Syndrome	Gene	Predominant Malignancies	Gene Function	Inheritance*
<i>Chromosomal Instability Syndromes</i>				
Bloom syndrome	<i>BLM</i>	Many sites	DNA repair	R
Fanconi anemia	?	Acute myelogenous leukemia	DNA repair	R
<i>Hereditary Skin Cancer</i>				
Familial melanoma	<i>CDKN2 (p16)</i>	Malignant melanoma	Cell cycle regulation	D
Xeroderma pigmentosum	<i>XP group</i>	Squamous cell carcinoma of skin; malignant melanoma	DNA repair	R
<i>Endocrine System</i>				
Hereditary paraganglioma and pheochromocytoma	<i>SDHD</i>	Paraganglioma; pheochromocytoma	Oxygen sensing and signaling	D
Multiple endocrine neoplasia (MEN) type 1	<i>MEN1</i>	Pancreatic islet cell tumors	Transcriptional regulation	D
MEN type 2	<i>RET</i>	Thyroid medullary carcinoma; Pheochromocytoma (MEN type 2A)	Receptor tyrosine kinase; cell cycle regulation	D
<i>Breast Cancer</i>				
Breast/ovary cancer syndrome	<i>BRCA1</i>	Carcinomas of ovary, breast, and prostate	DNA repair	D
Site-specific breast cancer	<i>BRCA2</i>	Female and male breast carcinoma; carcinomas of prostate, pancreas and ovary	DNA repair	D
<i>Nervous System</i>				
Retinoblastoma	<i>Rb</i>	Retinoblastoma	Cell cycle regulation	D
<i>Phacomatoses</i>				
Neurofibromatosis type 1	<i>NF1</i>	Neurofibrosarcomas; astrocytomas; malignant melanomas	Regulator of ras-mediated signaling	D
Neurofibromatosis type 2	<i>NF2</i>	Meningiomas; schwannomas	Regulator of cytoskeleton	D
Tuberous sclerosis	<i>TSC1</i>	Renal cell carcinoma; astrocytoma	Regulator of cytoskeleton	D
<i>Gastrointestinal System</i>				
Familial adenomatous polyposis	<i>APC</i>	Colorectal carcinoma	Cell cycle regulation; migration and adhesion	D
Hereditary nonpolyposis colorectal carcinoma (HNPCC; Lynch syndrome)	<i>hMSH2, hMSH6, hMLH1, hPMS1, hPMS2</i>	Carcinomas of colon, endometrium, ovary, and bladder; malignant melanoma	DNA repair	D
Juvenile polyposis coli	<i>DPC4/SMAD4</i>	Colorectal carcinoma; endometrial carcinoma	TGF- β signaling	D
Peutz-Jeghers syndrome	<i>LKB1/STK11</i>	Stomach, small bowel and colon carcinomas	Serine threonine kinase	D
<i>Kidney</i>				
Hereditary papillary renal cell carcinoma	<i>MET</i>	Papillary renal cell carcinoma	Receptor tyrosine kinase; cell cycle regulation	D
Wilms tumor	<i>WT</i>	Wilms tumor	Transcriptional regulation	D
Von Hippel-Lindau	<i>VHL</i>	Renal cell carcinoma	Regulator of adhesion	D
<i>Multiple Sites</i>				
Carney complex	<i>PRKARIA</i>	Testicular neoplasms; thyroid carcinoma	cAMP signaling	D
Cowden syndrome	<i>PTEN</i>	Colorectal, breast, and thyroid carcinomas	Protein tyrosine phosphatase	D
Li-Fraumeni syndrome	<i>TP53</i>	Breast carcinoma; soft tissue sarcomas; brain tumors; leukemia	Transcriptional regulation	D
Werner syndrome	<i>WRN</i>	Soft tissue sarcomas	DNA repair	R
Ataxia-telangiectasia	<i>ATM</i>	Lymphoma; leukemia	Cell signaling and DNA repair	R

* D, autosomal dominant; R, autosomal recessive.

ATM = mutated AT (gene); cAMP = cyclic adenosine monophosphate; PTEN = phosphatase and tensin homologue.

Most of these are discussed in detail in the chapters dealing with specific organs, and selected examples are given in Table 5-2. In many cases, the underlying genetic defect responsible for the tumor development has been identified. Some disorders that are difficult to classify, called **phacomatoses** (e.g., tuberous sclerosis,

neurofibromatosis), have both developmental and neoplastic features. The tumors associated with these syndromes mostly involve the nervous system.

Although only a small proportion of all cancers show a mendelian pattern of inheritance, certain cancers exhibit an

undeniable tendency to run in families. It is estimated that in the case of many tumors, other members of the family of an affected person have a twofold to threefold increase in the risk of developing the same cancer. This predisposition is particularly marked for cancer of the breast and colon. The interplay of heredity and environment is exemplified by the case of lung cancer. Smokers who are closely related to a person with lung cancer have a higher risk of developing lung cancer themselves than smokers without this familial background.

Viruses and Human Cancer

Despite the existence of viral oncogenes, the number of human cancers definitely associated with viral infections is limited. Nevertheless, it is estimated that viral infections are responsible for 15% of all human cancers. The strongest associations between the presence of viruses and the development of cancer in humans are

- Human T-cell leukemia virus type I (HTLV-I) (**RNA retrovirus**) and T-cell leukemia/ lymphoma
- HPV (**DNA**) and carcinoma of the cervix
- Hepatitis B virus (HBV) (**DNA**) and hepatitis C virus (HCV) (**RNA**) and primary hepatocellular carcinoma
- Epstein-Barr virus (EBV) and certain forms of lymphoma and nasopharyngeal carcinoma
- HHV 8 (**DNA**) and Kaposi sarcoma.

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

Human T-Cell Leukemia Virus-I (HTLV-I) Is a Lymphotropic Agent

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 in other parts of the world. The etiological 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 less than 5% of persons infected with HTLV-I and exhibits a latency period on the order of 40 years for its development. 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 within the host genome. Oncogenic stimulation by HTLV-I is mediated principally by the viral transcriptional activation protein tax. Tax protein not only increases the transcription from its own viral genome, but also promotes the activity of other genes involved in cell proliferation. These include genes that code for interleukin (IL)-2 and its receptor, granulocyte macrophage colony-stimulating factor (GM-CSF), and the protooncogenes *c-fos* and *c-sis*. 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 appearance of the complete malignant phenotype.

DNA Viruses Encode Proteins That Bind Regulatory Proteins

Four DNA viruses (HPV, EBV, HBV, and HHV 8) are incriminated in the development of human cancers. The transforming

genes of oncogenic DNA viruses exhibit virtually no homology with cellular genes, whereas those of RNA retroviruses (oncogenes) are derived from, and are homologous with, their cellular counterparts (protooncogenes). As discussed above, oncogenic DNA viruses have genes that encode protein products that bind to, and inactivate, specific host proteins (the products of tumor suppressor genes, e.g., *Rb*, *p53*) involved in the regulation of cell proliferation and apoptosis.

Human Papillomaviruses

HPVs induce lesions in humans that progress to squamous cell carcinoma. Papillomaviruses manifest a pronounced tropism for epithelial tissues, and their full productive life cycle occurs only in squamous cells. More than 80 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 termed **epidermodysplasia verruciformis**, HPV produces flat warts that commonly progress to squamous carcinoma. At least 20 HPV types are associated with cancer of the uterine cervix, especially HPV 16 and 18 (see Chapter 18). A newly available vaccine protects against infection with most oncogenic HPV types and is expected to reduce the incidence of cervical cancer.

The major oncoproteins encoded by HPV are E6 and E7. E6 binds to p53 and targets it for degradation. E7 binds to Rb, thereby releasing its inhibitory effect on cell cycle progression. During the last half century, a cell line derived from cervical cancer, termed *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 with an indefinite lifespan. In a small proportion of primary infections with EBV, this lymphoblastoid transformation is manifested as infectious mononucleosis (see Chapter 9), a short-lived lymphoproliferative disease. However, EBV is also intimately associated with the development of certain human cancers.

When B lymphocytes are infected with EBV, they acquire the ability to proliferate indefinitely in vitro. A number of EBV genes are implicated in this lymphocyte immortalization, including Epstein-Barr nuclear antigens (EBNAs) and latent-infection-associated membrane proteins (LMPs). The EBNAs maintain the EBV genome in its episomal state and activate the transcription of viral and cellular genes. LMP1 interacts with cellular proteins that normally transduce signals from the TNF receptor, a critical pathway in lymphocyte activation and proliferation. *Both EBNAs and LMPs can be demonstrated in most EBV-associated cancers.*

BURKITT LYMPHOMA: EBV was the first virus to be unequivocally linked to the development of a human tumor. In 1958, 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.

African Burkitt lymphoma (BL) is a B cell tumor, in which the neoplastic lymphocytes invariably contain EBV in their DNA and manifest EBV-related antigens (see Chapter 20). The tumor has also been recognized in non-African populations, but in those cases, only about 20% contain the EBV genome. The localization of Burkitt lymphoma to equatorial Africa is not understood, but it has been suggested that prolonged stimulation of the immune system by endemic malaria may be important. Under normal circumstances, the EBV-stimulated B-lymphocyte 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, thereby providing the background for further genetic events that lead to the development of lymphoma. As discussed above, one of these is known to be a chromosomal translocation, in which the *c-myc* protooncogene is deregulated by being brought into proximity with an immunoglobulin promoter region. A postulated sequence in the multistep pathogenesis of African Burkitt lymphoma is as follows:

1. Infection and polyclonal lymphoblastoid transformation of B lymphocytes by EBV
2. Proliferation of B cells and inhibition of suppressor T cells induced by malaria
3. Deregulation of the *c-myc* protooncogene by chromosomal translocation in a single transformed B lymphocyte
4. Uncontrolled proliferation of a malignant clone of B lymphocytes

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 may be clinically and pathologically indistinguishable from true malignant lymphomas, but they differ in that most of them are polyclonal. The incidence of lymphoid neoplasia in immunosuppressed renal transplant recipients is 30 to 50 times that of the general population. In virtually all cases of lymphoproliferations associated with organ transplantation, EBNA or EBV genomic material is present in the neoplastic tissue. Similar B cell lymphoproliferative disorders are seen in a number of other acquired immunodeficiencies, notably, acquired immunodeficiency syndrome (AIDS). Occasionally, a true monoclonal lymphoma may develop in the background of an EBV-induced lymphoproliferative disorder. As in the case of Burkitt lymphoma, the deficiency of T cells directed against EBV-infected B cells permits the survival of the latter.

Congenital immunodeficiency states, including X-linked lymphoproliferative syndrome (XLP), Wiskott-Aldrich syndrome, and AT, are associated with EBV infections and aggressive lymphoproliferations. In the familial disorder XLP, clinical immunodeficiency is commonly inapparent until the onset of a particularly severe, and often fatal, form of infectious mononucleosis. In many of these patients who survive infectious mononucleosis, lymphoproliferative disorders and lymphomas ensue. Patients with XLP lack EBV-specific immune responses, including the formation of cytotoxic T cells that normally eliminate EBV-infected B cells.

NASOPHARYNGEAL CARCINOMA: Nasopharyngeal carcinoma is a variant of squamous cell carcinoma that has a

worldwide distribution and is particularly common in certain parts of Africa and Asia. EBV DNA and EBNA are present in virtually all of these cancers. It is thought that epithelial cells are exposed to EBV by lysis of infected lymphocytes traveling through lymphoid-rich epithelium. The pathogenesis of nasopharyngeal carcinoma may be related to infection with EBV in early childhood, with reactivation at 40 to 50 years of age and the appearance of tumors 1 to 2 years thereafter. Fortunately, 70% of patients with this disease are cured by radiation therapy alone.

Hepatitis B and C Viruses

Epidemiologic studies have established a strong association between chronic infection with HBV and HCV (chronic hepatitis and cirrhosis) and the development of primary hepatocellular carcinoma (see Chapter 14). Two mechanisms have been invoked to explain the mechanism of carcinogenesis in virus-related liver cancer. One theory holds that the continued liver cell proliferation that accompanies chronic liver injury eventually leads to malignant transformation. However, a small subset of patients with HBV infection develop hepatocellular carcinoma 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 has been shown in vitro to upregulate a number of cellular genes. In addition, like other DNA viral oncoproteins, HBx binds to and inactivates p53. The underlying mechanisms in HBV-induced carcinogenesis are still controversial and require further investigation.

Human Herpesvirus 8 (HHV 8)

Kaposi sarcoma is a vascular neoplasm that was originally described in eastern European elderly men and later in central African blacks (see Chapter 10). Kaposi sarcoma is today the most common neoplasm associated with AIDS. The neoplastic cells contain sequences of a novel herpesvirus, HHV 8. Interestingly, HHV 8 has also been demonstrated in specimens of Kaposi sarcoma from HIV-negative patients. In addition to infecting the spindle cells of Kaposi sarcoma, HHV 8 is lymphotropic and has been implicated in two uncommon B-cell lymphoid malignancies, namely, **primary effusion lymphoma** and **multicentric Castleman disease**.

Like other DNA viruses, the viral genome encodes proteins that interfere with the p53 and RB tumor suppressor pathways. HHV 8 also encodes gene products that downregulate class I major histocompatibility complex (MHC) expression, a mechanism by which the infected cells may evade recognition by cytotoxic T lymphocytes.

Chemical Carcinogenesis

The field of chemical carcinogenesis originated some 2 centuries ago in descriptions of an occupational disease (this was not the first recognition of an occupation-related cancer, since a peculiar 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* species. 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 termed **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 hematological malignancies at a later time.

AFLATOXIN: In contrast to the polycyclic hydrocarbons, which are for the most part formed either by the combustion of organic material or synthetically, a heterocyclic hydrocarbon, aflatoxin B₁, is a natural product of the fungus *Aspergillus flavus*. Like the polycyclic aromatic hydrocarbons, aflatoxin B₁ is metabolized to an epoxide, which can bind covalently to DNA. Aflatoxin B₁ is among the most potent liver carcinogens recognized, producing tumors in fish, birds, rodents, and primates. Since *Aspergillus* species 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 B₁. 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 B₁, 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 (Ni²⁺), lead (Pb²⁺),

cadmium (Cd²⁺), cobalt (Co²⁺), and beryllium (Be²⁺), 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 Ni²⁺ 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 9).

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, whereas 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 9.

Ultraviolet Radiation Causes Skin Cancers

Among fair-skinned persons, 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 persons of the white race. The skin of persons 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 non-neoplastic 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 “serpentine” or “amphiboles.” Serpentine, 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% to 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 amosite fibers or flexible chrysotile fibers. However, the distinction between these fibers in the causation of human disease should not be taken as absolute, particularly since mixtures of these fibers are characteristically found in human lungs.

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

In 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 genomic mechanisms underlying the development of neoplasia are summarized in Figure 5-30.

Tumor Immunology

It has long been recognized that malignant tumors elicit a chronic inflammatory response that is unrelated to necrosis or infection of the tumor. This observation led early investigators to postulate a host immune reaction to the neoplastic cells, but a refined understanding awaited the development of modern immunology. The inflammatory reaction is correlated with a better prognosis in some tumors, such as medullary carcinoma of the breast and seminoma, but in general no clear correlation exists. Although the infiltrate is composed principally of T cells and macrophages, suggesting a cell-mediated immune response, the antigens to which the cells respond have not been identified. Despite the paucity of direct evidence in human cancers, it is clear from animal experiments that immune defenses against malignant tumors exist.

Immunologic Defenses against Cancer Have Been Demonstrated in Experimental Animals and Humans

To invoke a role for an immune defense against cancer, it is necessary to postulate that tumor cells express antigens that differ from those of normal cells and that are recognized as foreign by the host. Such a condition has been indirectly demonstrated in experiments with inbred mice. When cells from a chemically induced or virally induced tumor are transplanted into a syngeneic mouse, the cells form a tumor. When cells from this tumor are passed into a second mouse, they again form a tumor. *However, if the first transplanted tumor is removed before it metastasizes (i.e., the mouse is cured of its tumor), reinjection of the tumor cells back into the cured mouse will not produce a tumor.* The transplanted tumor is **rejected** because of immunity acquired as a result of the first tumor transplant. Moreover, irradiated tumor cells or preparations of tumor cell membranes, when injected experimentally, aug-

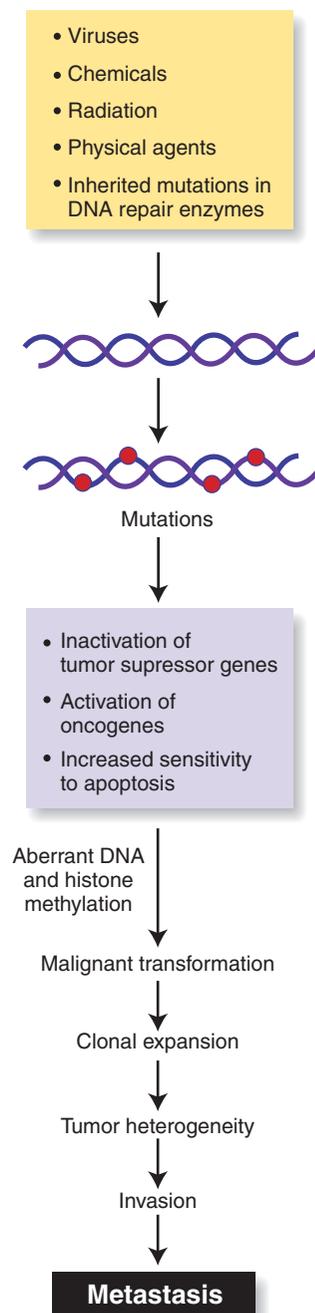


FIGURE 5-30. Summary of the genomic mechanisms of cancer.

ment resistance to tumor growth. Why the original tumor is not destroyed by the immunologic reaction remains unexplained.

An important observation is that tumors induced by the same chemical in different mice are antigenically distinct, whereas those induced by the same virus express the same virally determined antigens. Accordingly, mice sensitized to one chemically induced tumor do not reject a second tumor induced by the same chemical. By contrast, mice that have received a virus-induced tumor reject another similar tumor. These experiments provide compelling evidence that immunologic mechanisms can play a role in host defenses against tumors, at least against experimental tumors in animals.

Further evidence for the existence of immune mechanisms in the defense against cancer comes from studies in nude mice.

These animals are devoid of T-cell-mediated immunity and thus accept grafts from different species. Similarly, tumors from different species grow in an unrestrained fashion when transplanted into nude mice.

The effectiveness of immune mechanisms to limit the growth of malignant cells can be demonstrated by mixing mouse tumor cells with immune effector cells from a syngeneic mouse that has been sensitized to the tumor. The mixture is then injected into a normal (unsensitized) syngeneic recipient. In many instances, the growth of the tumor cells in the recipient is inhibited, compared with that of tumor cells mixed with unsensitized lymphoid cells. Similar approaches have been tested in cases of human melanoma. However, it has not proved possible to cure human melanomas by reinjecting tumor-sensitized lymphocytes into the patient.

Tumor Antigens

The immune response to experimental tumors must necessarily be directed against tumor antigens on the surface of malignant cells. Such antigens can be tumor-specific; that is, they are uniquely expressed by the cancer cells but not by their normal cellular counterparts. Alternatively, other tumor antigens represent proteins that are expressed by some normal cells, such as those in developing embryos. Such antigens are tumor-associated, rather than tumor-specific.

In experimental animals, tumors produced by chemicals and viruses display tumor-specific antigens. As noted above, each chemically induced cancer expresses unique tumor antigens; that is, no two tumors are antigenically alike. The precise nature of these antigens is obscure, although some may be altered histocompatibility antigens. By contrast, all tumors induced by the same virus express the same tumor-specific antigens, presumably because they are products encoded by the viral genome.

It is much more difficult to document the presence of tumor-specific antigens in human cancers, because patients cannot be subjected to an immunization challenge with tumor cells, as is used in experimental animals. Yet despite this experimental limitation, candidate human tumor-specific antigens have begun to emerge, for example, virally encoded antigens in tumors whose pathogenesis is linked to viruses (e.g., HPV). Neoantigens encoded by altered gene sequences have also been detected in malignant cells resulting from mutations or translocations. The tumor-specific antigens identified to date are peptides complexed to human leukocyte antigen (HLA) molecules on tumor cell surfaces.

There has been even more progress in identifying tumor-associated antigens for both human and experimental animal tumors. Early studies on melanoma showed that certain HLA-associated peptide antigens correspond to proteins that are present in small amounts in the adult but are abundant during development. Such tumor-associated oncodevelopmental antigens are not specific for a given patient's tumor per se but instead are shared by cancers in different persons and sometimes of varying histologic type. Although, there is no reason to believe that immune responses to these fetal antigens play any role in the host defense against cancer, their presence in the blood or the tumor (e.g., CEA, AFP) is useful in clinical diagnosis and treatment.

Inroads into the identification of tumor antigens have created new opportunities for developing immunotherapies against human cancers, at least in theory. Passive immunotherapies can draw upon tumor-infiltrating lymphocytes with specificity for

HLA-associated tumor peptide antigens and antibodies directed against various tumor surface proteins. Alternatively, active immunotherapeutic strategies can invoke tumor antigens as vaccines to elicit systemic antitumor immune responses.

Mechanisms of Immunologic Cytotoxicity

The contribution of any specific immunologic mechanism to tumor cell destruction *in vivo* has not been clearly defined. A number of possible mechanisms are recognized (Fig. 5-31):

- **T cell-mediated cytotoxicity:** The capacity of cytotoxic T cells to mediate the specific rejection of transplanted tumors is evidenced by the demonstration that lymphocytes from tumor-bearing hosts can transfer tumor immunity when injected into normal animals. Moreover, the transferred immunity is eliminated by the administration of antibodies directed against T-cell antigens. The mechanisms of T cell-mediated immunological cell killing are discussed in Chapter 4.
- **Natural killer cell-mediated cytotoxicity:** Another set of lymphocytes, the natural killer (NK) cells, have tumoricidal activity that does not depend on prior sensitization. These lymphocytes are generally more effective than untransformed cells in killing tumor cells. Tumor cells that are resistant to the action of NK cells may be lysed by NK cells that have been activated by IL-2. Such activated NK cells are referred to as **lymphokine-activated killer (LAK) cells**.
- **Macrophage-mediated cytotoxicity:** Macrophages are capable of killing tumor cells in a nonspecific manner. However, their role in the control of malignant tumors is far from clear, since under some circumstances *in vitro* factors

derived from macrophages can actually stimulate the proliferation of tumor cells.

- **Antibody-dependent cell-mediated cytotoxicity (ADCC):** Tumor-associated antigens can elicit a humoral antibody response, but these immunoglobulins by themselves do not kill tumor cells. However, as discussed in Chapter 4, such antibodies can participate in ADCC. The antibody binds both to the tumor antigen and to the Fc receptor of the effector cell, thereby bringing the effector cell into direct contact with its target. Depending on the conditions, the effector cells may be a lymphocyte killer cell (null cell), macrophage, or neutrophil.
- **Complement-mediated cytotoxicity:** Tumor cells that have been coated with specific antibodies may be lysed by the activation of complement.

Immune Surveillance

Considering the enormous number of chemical, viral, and physical agents that are carcinogenic, it seems remarkable that the incidence of cancer is not far greater than current statistics indicate. The theory of immune surveillance holds that mutant clones with neoplastic potential frequently arise but are recognized and expunged by cell-mediated immune responses. However, the evidence for this concept is highly controversial, and the subject deserves further study.

Immunologic Defenses against Cancer in Humans

Although some circumstantial evidence exists for the participation of immunologic defenses in the resistance to cancer in humans, conclusive proof that immunologic tumor surveillance is an ongoing process is lacking. Perhaps the strongest argument for immunologic tumor rejection in humans is the observation that immunodeficiency, whether acquired or congenital, is associated with an increased incidence of cancers, almost all of which are B-cell lymphomas. Three prominent examples are widely cited: patients with X-linked lymphoproliferative syndrome (XLP), patients with AIDS, and those who receive immunosuppressive therapy following organ transplantation. In XLP and AIDS, the enormously increased risk can be attributed to a polyclonal lymphoid hyperplasia induced by infection with EBV, coupled with a lack of cytotoxic T cells that normally limit the proliferation of virus-infected B cells. In immunosuppressed transplant patients, who manifest a 75-fold increased incidence of lymphomas, it remains unclear whether a direct effect of immunosuppressive agents on the regulation of lymphocyte proliferation and maturation or a nonspecific depression of immune defenses is responsible.

Additional arguments for the effectiveness of immunologic defenses against cancer in humans are also far from definitive. Rare instances of the regression of primary and metastatic tumors have been attributed to immunologic mechanisms, but many other factors may have been responsible (e.g., hormonal, nutritional, or vascular). Similarly, as noted above, the phenomenon of tumor dormancy may be related to comparable nonimmunologic circumstances. The presence of lymphoid cells and macrophages in the stroma of many cancers may represent a reaction to tumor antigens, but their effectiveness in limiting growth is problematic.

Tumor Cells May Be Able to Evade Immunologic Cytotoxicity

The fact that cancer is alive and well despite the presence of potential immunologic defenses implies that such mechanisms are either ineffective or that tumor cells can evade immunologic cy-

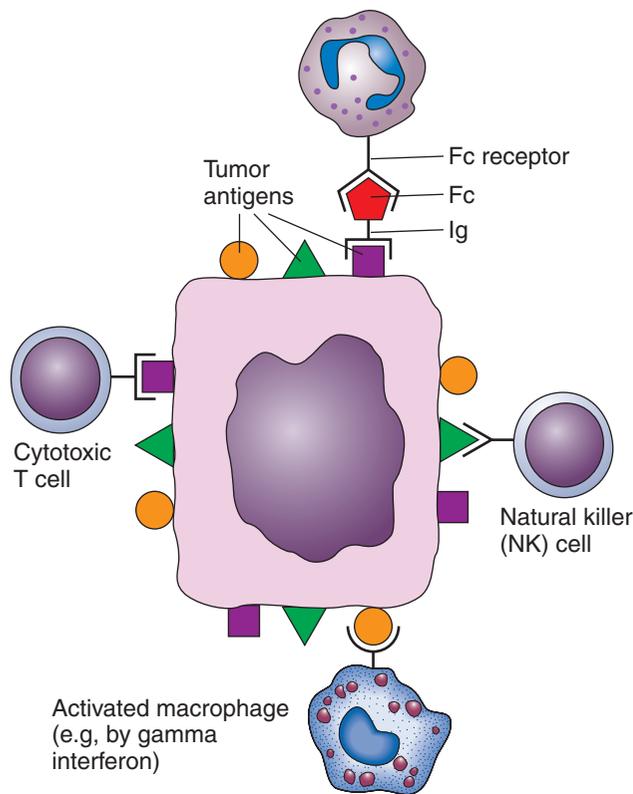


FIGURE 5-31. Possible mechanisms of immunological tumor cytotoxicity in animal studies. Ig = immunoglobulin.

totoxicity. A number of factors have been proposed to account for the failure of immune responses to limit tumor growth. These explanations remain theoretical and even controversial.

It is intuitively clear that an absence of tumor-specific antigens or a lack of immunogenicity by such antigens will permit unhampered growth of the neoplasm. *In this respect, tumor antigens are sometimes found to be expressed at low levels on human tumors, in conjunction with deficient HLA expression or antigenic peptide processing.* The concept of tumor heterogeneity predicts that even in strongly antigenic tumors, clones will arise that do not express tumor antigens or histocompatibility antigens and thus will be selected for survival. Besides antigenic variation, tumor cells tend to lack surface molecules such as co-stimulators that are needed for T cell activation. Additionally, malignant cells can express a variety of immunosuppressive factors that enable them to blunt anti-tumor immunologic responses. Defining and tackling these immune evasion mechanisms will be essential for developing effective immunotherapies for cancer.

Systemic Effects of Cancer on the Host

The symptoms of cancer are, for the most part, referable to the local effects of either 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, which are collectively termed **paraneoplastic syndromes**. Although such effects are rarely lethal, in some cases they dominate the clinical course. It is important to recognize these syndromes for several reasons. First, the signs and symptoms of the paraneoplastic syndrome may be the first clinical manifestation of a malignant tumor. When they are recognized, the cancer may be detected early enough to permit a cure. Second, the syndromes may be mistaken for those produced by advanced metastatic disease and may, therefore, lead to inappropriate therapy. Third, when the paraneoplastic syndrome itself is disabling, treatment directed toward alleviating 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.

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 have a decreased 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.

Endocrine Syndromes

Malignant tumors may produce a number of peptide hormones whose secretion is not under normal regulatory control. Most of these hormones are normally present in the brain, gastrointestinal tract, or endocrine organs. Their inappropriate secretion can cause a variety of effects.

CUSHING SYNDROME: Ectopic secretion of ACTH by a tumor leads to features of Cushing syndrome, including hypokalemia, hyperglycemia, hypertension, and muscle weakness (see Chapter 21). ACTH production is most commonly seen with cancers of the lung, particularly small cell carcinoma. It also complicates carcinoid tumors and other neuroendocrine tumors, such as pheochromocytoma, neuroblastoma, and medullary thyroid carcinoma.

INAPPROPRIATE ANTIDIURESIS: The production of arginine vasopressin (antidiuretic hormone [ADH]) by a tumor may cause sodium and water retention to such an extent that it is manifested as water intoxication, resulting in altered mental status, seizures, coma, and sometimes death. The tumor that most often produces this syndrome is small cell lung carcinoma. It is also reported with carcinomas of the prostate, gastrointestinal tract, and pancreas and with thymomas, lymphomas, and Hodgkin disease.

HYPERCALCEMIA: A paraneoplastic complication that afflicts 10% of all cancer patients, hypercalcemia, is usually caused by metastatic disease of bone. However, in about one tenth of cases it occurs in the absence of bony metastases. The most common cause of paraneoplastic hypercalcemia is the secretion of a parathormone-like peptide by an epithelial tumor, usually squamous cell lung carcinoma or breast adenocarcinoma. In multiple myeloma and lymphomas, hypercalcemia is attributed to the secretion of osteoclast activating factor. Other mechanisms of hypercalcemia involve the production of prostaglandins, active metabolites of vitamin D, TGF- α , and TGF- β .

HYPOCALCEMIA: Cancer-induced hypocalcemia is actually more common than hypercalcemia and complicates osteoblastic metastases from cancers of the lung, breast, and prostate. The cause of hypocalcemia is not known. Low calcium levels have been reported in association with calcitonin-secreting medullary carcinoma of the thyroid.

GONADOTROPIC SYNDROMES: Gonadotropins may be secreted by germ cell tumors, gestational trophoblastic tumors (choriocarcinoma, hydatidiform mole), and pituitary tumors. Less commonly, gonadotropin secretion is observed with hepatoblastomas in children and cancers of the lung, colon, breast, and pancreas in adults. High gonadotropin levels lead to precocious puberty in children, gynecomastia in men, and oligomenorrhea in premenopausal women.

HYPOLYCEMIA: The best-understood cause of hypoglycemia associated with tumors is excessive insulin production by pancreatic islet cell tumors. Other tumors, especially large mesotheliomas, fibrosarcomas, and primary hepatocellular carcinoma, are associated with hypoglycemia. The cause of hypoglycemia in nonendocrine tumors is not established, but the most likely candidate is production of somatomedins (IGFs), a

family of peptides normally produced by the liver under regulation by growth hormone.

Neurologic Syndromes

Neurologic disorders are common in cancer patients, usually resulting from metastases or from endocrine or electrolyte disturbances. Vascular, hemorrhagic, and infectious conditions affecting the nervous system are also common. However, there remains a small group of cancer patients who suffer from a variety of neurologic complaints without any demonstrable cause. Most of these cases reflect an autoimmune etiology mediated by circulating antibodies directed against neural antigens or by reactive T cells. Cerebral complications include dementia, subacute cerebellar degeneration, limbic encephalitis, and optic neuritis.

Spinal Cord

Subacute motor neuropathy, a disorder of the spinal cord, is characterized by slowly developing lower motor neuron weakness without sensory changes. It is so strongly associated with cancer that an intensive search for an occult neoplasm, often a lymphoma, should be made in patients who present with these symptoms.

Amyotrophic lateral sclerosis is well described among cancer patients. Conversely, as many as 10% of patients with this neurologic disease are found to have cancer.

Peripheral Nerves

Sensorimotor peripheral neuropathy, characterized by distal weakness and wasting and sensory loss, is common in cancer patients and when not associated with an overt neoplasm suggests the possibility of an occult tumor. Interestingly, the removal of the primary tumor usually does not reverse the neuropathy.

Purely sensory neuropathy, resulting from degenerative changes in the dorsal root ganglia, may also develop in persons with cancer.

Autonomic and gastrointestinal neuropathies, manifested as orthostatic hypotension, neurogenic bladder, and intestinal pseudoobstruction, are associated with small cell carcinoma of the lung.

Skeletal Muscle Syndromes

Patients with dermatomyositis or polymyositis have an incidence of cancer five to seven times higher than that in the general population. The association is most conspicuous in affected men older than 50 years; in this group more than 70% have cancer. In most cases, the muscle disorder and cancer present within a year of each other.

Eaton-Lambert syndrome is an uncommon myasthenic disorder that is strongly associated with small cell lung carcinoma. Although the symptoms superficially resemble those of true myasthenia gravis, muscle strength improves with exercise, and there is a poor response to an anticholinesterase. Thymoma has a well-recognized association with **myasthenia gravis**, but a wide variety of other tumors have on occasion been linked to this disorder of the neuromuscular junction.

Hematologic Syndromes

The most common hematologic complications of neoplastic diseases result either from direct infiltration of the marrow or from treatment. However, hematologic paraneoplastic syndromes,

which antedate the modern era of chemotherapy and radiation therapy, are well described.

Erythrocytosis

Cancer-associated erythrocytosis (polycythemia) is a complication of some tumors, particularly renal cell carcinoma, hepatocellular carcinoma, and cerebellar hemangioblastoma. Interestingly, benign kidney disease, such as cystic disease or hydronephrosis, and uterine myomas can lead to erythrocytosis. Elevated erythropoietin levels are found in the tumor and in the serum in about half of patients with erythrocytosis.

Anemia

One of the most common findings in patients with cancer is anemia, but the mechanism underlying this disorder is not clear. The anemia is usually normocytic and normochromic, although **iron deficiency anemia** is common in cancers that bleed into the gastrointestinal tract, such as colorectal cancers. **Pure red cell aplasia**, often associated with thymomas, and megaloblastic anemia are sometimes encountered.

Autoimmune hemolytic anemia may be associated with B cell neoplasms and with solid tumors, particularly in the elderly. In fact, autoimmune hemolytic anemia in an older person suggests the possibility of an underlying neoplasm. **Microangiopathic hemolytic anemia** is occasionally seen, often in association with disseminated intravascular coagulation and thrombotic thrombocytopenic purpura.

Leukocytes and Platelets

Paraneoplastic granulocytosis, characterized by a peripheral granulocyte count over $20,000/\mu\text{L}$, is a finding that may lead to an erroneous diagnosis of leukemia. This condition is usually caused by the secretion of a colony-stimulating factor by the tumor.

Eosinophilia is occasionally noted in association with cancer, particularly in Hodgkin disease, in which it may occur in one fifth of cases.

Thrombocytosis, with platelet counts above $400,000/\mu\text{L}$, occurs in one third of cancer patients. The platelet count usually returns to normal with successful treatment of the malignant disease.

The Hypercoagulable State

The association between cancer and venous thrombosis was noted more than a century ago. Since then, other abnormalities resulting from a hypercoagulable state (e.g., disseminated intravascular coagulation and nonbacterial thrombotic endocarditis) have been recognized. The cause of this hypercoagulable state is still debated.

VENOUS THROMBOSIS: This condition is most distinctly associated with carcinoma of the pancreas, in which there is a 50-fold increased incidence of this complication. Venous thrombosis, commonly in the deep veins of the legs, is also particularly frequent in association with other mucin-secreting adenocarcinomas of the gastrointestinal tract and with lung cancer. Tumors of the breast, ovary, prostate, and other organs are occasionally complicated by venous thrombosis.

DISSEMINATED INTRAVASCULAR COAGULATION: The widespread appearance of thrombi in small vessels in association with cancer may come to attention because of the chronic occurrence of thrombotic phenomena or an acute hemorrhagic diathesis. Sometimes a coagulation disorder is detected by laboratory tests alone. This complication is most commonly found with acute promyelocytic leukemia and adenocarcinomas.

NONBACTERIAL THROMBOTIC ENDOCARDITIS: The presence of noninfected verrucous deposits of fibrin and platelets on the left-sided heart valves occurs in cancer patients, particularly in debilitated persons (see Chapter 11). Although the effects on the heart are not of clinical importance, emboli to the brain and rarely the coronary arteries present a great danger. Paraneoplastic endocarditis may develop early in the course of a cancer and signal its presence long before the tumor would otherwise become symptomatic. This cardiac complication is most common with solid tumors but may occasionally be noted with leukemias and lymphomas.

Gastrointestinal Syndromes

Malabsorption of a variety of dietary components is an occasional paraneoplastic symptom, and half of cancer patients develop some histologic abnormalities of the small intestine, even though the tumor may not directly involve the bowel.

Hypoalbuminemia may result from a paraneoplastic depression of albumin synthesis by the liver or, in rare cases, a protein-losing enteropathy.

Nephrotic Syndrome

Nephrotic syndrome, as a consequence of renal vein thrombosis or amyloidosis, is a well-known complication of cancer. The nephrotic syndrome may also represent a paraneoplastic complication in the form of minimal-change disease (lipoid nephrosis) or glomerulonephritis produced by the deposition of immune complexes.

Cutaneous Syndromes

Pigmented lesions and keratoses are well-recognized paraneoplastic effects.

- **Acanthosis nigricans** is a cutaneous disorder marked by hyperkeratosis and pigmentation of the axilla, neck, flexures, and anogenital region. *It is of particular interest because more than half of patients with acanthosis nigricans have cancer.* The development of the disease may precede, accompany, or follow, the detection of the cancer. Over 90% of cases occur in association with gastrointestinal carcinomas, with tumors of the stomach accounting for one half to two thirds.
- **Exfoliative dermatitis** occasionally complicates certain lymphomas and Hodgkin disease, without any cutaneous involvement by tumor.
- **Erythema gyratum repens** is an unusual skin disorder, which presents with scaling and itching and is seen almost exclusively in cancer patients.

Amyloidosis

About 15% of cases of amyloidosis occur in association with cancers, particularly with multiple myeloma and renal cell carcinoma but also with other solid tumors and lymphomas (see Chapter 23). The presence of amyloidosis implies a poor prognosis; in patients with myeloma, amyloidosis is associated with a median survival of 14 months or less.

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 cardiovascular diseases and stroke. For most cancers, death rates in the United States have largely remained flat for more than half a century, with some notable exceptions (Fig. 5-32). 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 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 uterus corpus and cervix, possibly explained by better screening, diagnostic techniques, and therapeutic methods. Overall, after decades of steady increases, the age-adjusted mortality due to 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 5-3.

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. 5-33).

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

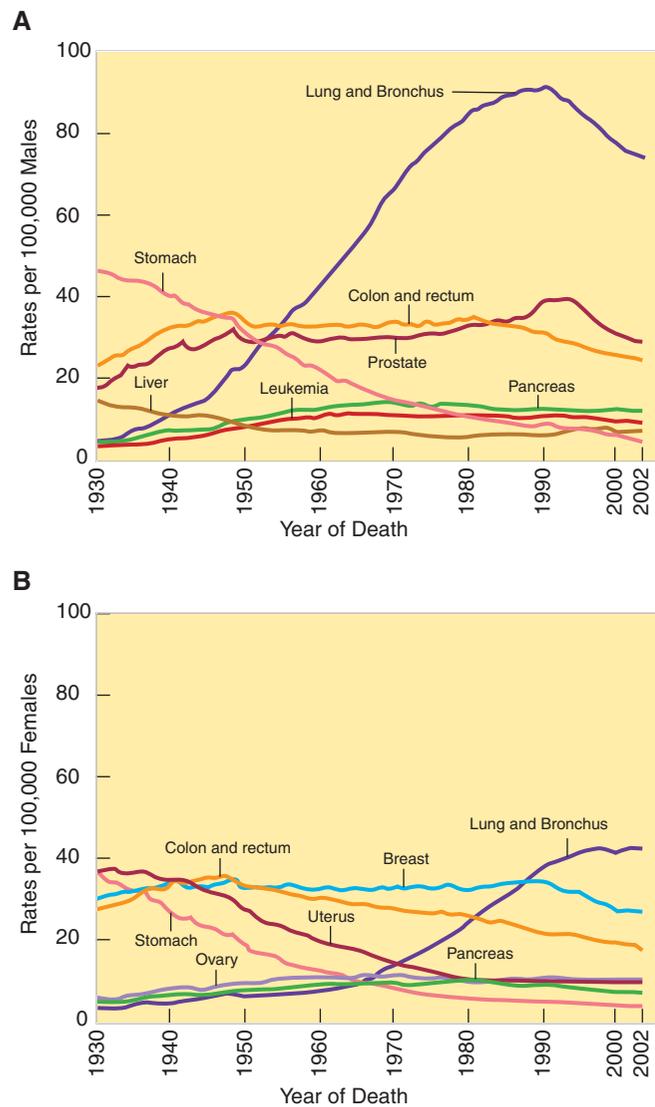


FIGURE 5-32. Cancer death rates in the United States, 1930 to 2002, among men (A) and women (B).

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

TABLE 5-3

Most Common Tumor Types in Men and Women

Tumor Type	%	Tumor Type	%
Men		Women	
Prostate	33	Breast	32
Lung and bronchus	14	Lung and bronchus	12
Colon and rectum	11	Colon and rectum	11
Urinary bladder	6	Uterine corpus	6
Melanoma	4	Ovary	4
Non-Hodgkin lymphoma	4	Non-Hodgkin lymphoma	4
Kidney	3	Melanoma	3
Oral cavity	3	Thyroid	3
Leukemia	3	Pancreas	2
Pancreas	2	Urinary bladder	2
All other sites	17	All other sites	20

times more common than in Japan, India, Africa, and Latin America. It has 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, although this concept has been seriously questioned.

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 B₁ 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 persons 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 blacks.

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. Epidemiological studies have contributed little to our understanding of the etiology of breast cancer. Although hormonal factors are clearly involved, except for a good correlation with age at first pregnancy, few confirmed hormonal correlations have surfaced. The role of dietary fat in the pathogenesis of breast cancer is still debated.

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

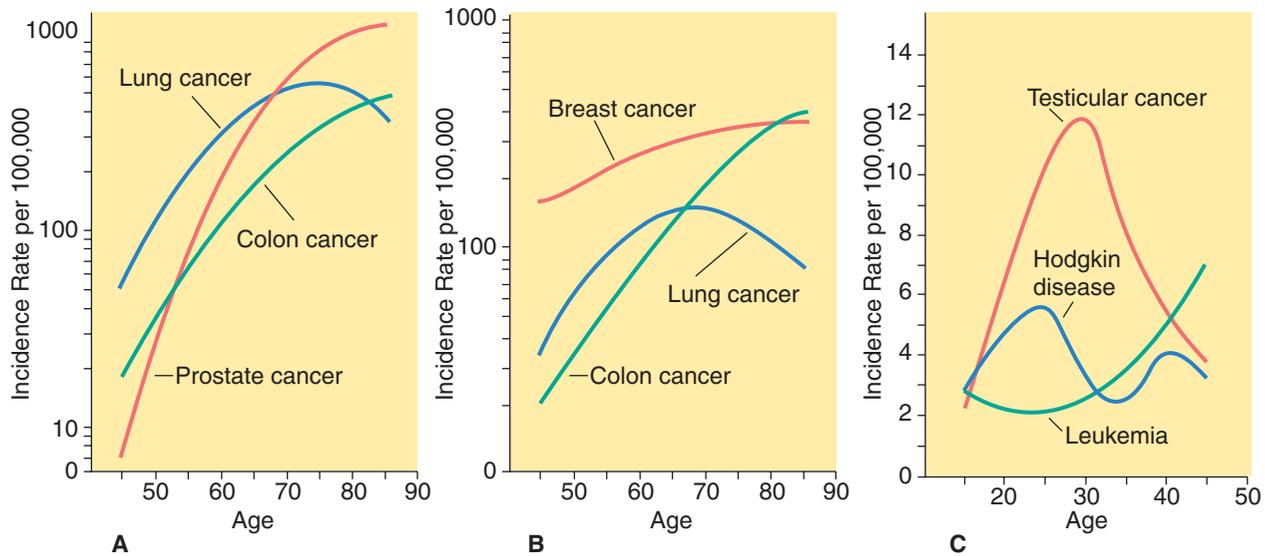


FIGURE 5-33. Incidence of specific cancers as a function of age. (A) Men. (B) Women. (C) Testicular cancer in men and Hodgkin disease and leukemia in both sexes. The incidence of these cancers in C peaks at younger ages than do those in A and B.

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 present 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, whereas 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 persons 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 persons 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.

CANCER OF THE STOMACH: A study of Japanese residents of Hawaii found that emigrants from Japanese regions with the highest risk of stomach cancer continued to exhibit an excess risk in Hawaii. By contrast, their offspring who were born in Hawaii had the same incidence of this cancer as American whites. Although dietary factors, such as pickled vegetables and salted fish, have been postulated to account for the higher incidence in Japan and the lower incidence in Hawaii, no firm evidence has been adduced to support this contention. More recently it has been shown in Japan that the population in regions at high risk for stomach cancer also display a high prevalence of chronic atrophic gastritis with intestinal metaplasia, lesions that are considered precursors of gastric cancer. Interestingly, when persons from these regions move to low-risk areas, they carry the high prevalence of intestinal metaplasia with them. Thus, the envi-

ronmental factors associated with stomach cancer may not be directly carcinogenic but rather may be related to atrophic gastritis and intestinal metaplasia.

COLORECTAL, BREAST, ENDOMETRIAL, OVARIAN, AND PROSTATIC CANCERS: Emigrant studies of the incidence of colorectal cancer show opposite trends to those of stomach cancer. Emigrants from low-risk areas in Europe and Japan 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. Such a pattern is characteristic of certain viral infections, although there is no evidence for an infectious etiology of Hodgkin disease. An exception to this generalization is noted in Japan, a developed country where young adult disease is distinctly uncommon. Further evidence for an environmental influence is the higher incidence of Hodgkin disease in Americans of Japanese descent than that in Japan.