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CHAPTER 7

Neoplasia

CHAPTER CONTENTS

Cancer is the second leading cause of death in the United States; only cardiovascular diseases exact a higher toll. Even more agonizing than the mortality rate is the emotional and physical suffering inflicted by cancers. Patients and the public often ask, "When will there be a cure for this scourge?" The answer to this simple question is difficult because cancer is not one disease but many disorders with widely different pathogeneses, natural histories, and responses to treatments. Some cancers, such as Hodgkin lymphoma, are curable, whereas others, such as pancreatic adenocarcinoma, are virtually always fatal. The only hope for controlling cancer lies in learning more about its causes and pathogenesis. Fortunately, great strides have been made in understanding its molecular basis, and some good news has emerged: cancer mortality for both men and women in the United States declined during the last decade of the 20th century and has continued its downward course in the 21st century.

In this chapter, we describe the vocabulary of tumor biology and pathology and then review the morphologic characteristics that define neoplasia and allow benign and malignant tumors to be identified and distinguished. Also reviewed is the epidemiology of cancer, which provides a measure of the impact of cancer on human populations as well as clues to its environmental causes, insights that have led to effective prevention campaigns against certain cancers. Building on this foundation, we then discuss the biologic properties of tumors and the molecular basis of carcinogenesis, emphasizing the critical role that genetic alterations play in the development of neoplasia. Finally, we turn to cancer diagnosis, focusing on new technologies that are helping to direct the use of cancer drugs that are targeted at particular molecular lesions. Throughout, we give examples of new analytic methods and therapies that are not only changing our approach to cancer treatment but also providing new insights into cancer pathophysiology.

NOMENCLATURE

Neoplasia means "new growth," and the collection of cells and stroma composing new growths are referred to as *neoplasms*. *Tumor* originally described swelling caused by inflammation, but is now equated with neoplasm. *Oncology* (Greek *oncos* = tumor) is the study of tumors or neoplasms. Although physicians know what they mean when they use the term *neoplasm,* it has been difficult to develop a precise definition. In the modern era, a neoplasm is defined as a genetic disorder of cell growth that is triggered by acquired or less commonly inherited mutations affecting a single cell and its clonal progeny. As discussed later, these causative mutations alter the function of particular genes and give the neoplastic cells a survival and growth advantage, resulting in excessive proliferation that is independent of physiologic growth signals and controls.

All tumors are composed of two components: (1) neoplastic cells that constitute the tumor parenchyma and (2) reactive stroma made up of connective tissue, blood vessels, and cells of the adaptive and innate immune system. The classification of tumors and their biologic behavior are based primarily on the parenchymal component, but their growth and spread are critically dependent on their stroma. In some tumors, connective tissue is scant, and the neoplasm is soft and fleshy. In others, parenchymal cells stimulate the formation of abundant collagenous stroma, referred to as *desmoplasia.* Some desmoplastic tumors—for example, some cancers of the female breast—are stony hard or *scirrhous.*

Benign Tumors

Benign tumors remain localized at their site of origin and are generally amenable to surgical removal. Predictably, the patient generally survives. Exceptions arise, however, when benign tumors occur in vulnerable locations such as the brain; here, even "benign" tumors may cause significant morbidity and are sometimes even fatal.

Naming of benign tumors of mesenchymal cells is relatively simple; in general, the suffix "-oma" is attached to the name of the cell type from which the tumor arises. Thus a benign tumor of fibroblast-like cells is called a *fibroma,* a benign cartilaginous tumor is a *chondroma,* and so on. The nomenclature of benign epithelial tumors is more complex; some are classified based on their cell of origin, others on their microscopic appearance, and still others on their macroscopic architecture. *Adenoma* is applied to benign epithelial neoplasms derived from glandular tissues even if the tumor cells fail to form glandular structures. Thus, a benign epithelial neoplasm that arises from renal tubular cells and forms tightly clustered glands and a mass of adrenal cortical cells growing as a solid sheet are both referred to as adenomas. Benign epithelial neoplasms producing fingerlike or warty projections from epithelial surfaces are called *papillomas,* whereas those that form large cystic masses, such as in the ovary, are referred to as *cystadenomas.* Some tumors produce papillary projections that protrude into cystic spaces and are called papillary cystadenomas. When a neoplasm—benign or malignant—produces a grossly visible projection above a mucosal surface, for example, into the gastric or colonic lumen, it is termed a *polyp*. If the polyp has glandular tissue, it is called an adenomatous polyp (Fig. 7.1).

Malignant Tumors

Malignant tumors can invade and destroy adjacent structures and spread to distant sites (metastasize). Malignant tumors are collectively referred to as *cancers,* derived from the Latin word for crab, because they tend to adhere to any part that they seize on in an obstinate manner. Not all cancers pursue a deadly course; some are discovered at early stages

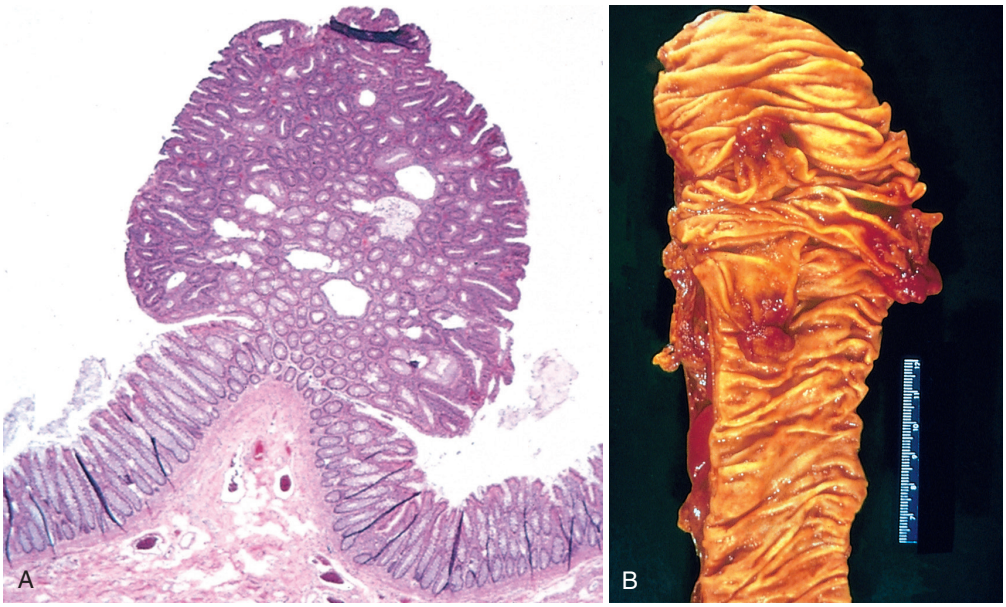

Figure 7.1 Colonic polyp. (A) An adenomatous (glandular) polyp is projecting into the colonic lumen and is attached to the mucosa by a distinct stalk. (B) Gross appearance of several colonic polyps.

that allow for surgical excision, and others are cured with systemically administered drugs or therapeutic antibodies. Nevertheless, the designation "malignant" always raises a red flag.

The nomenclature of malignant tumors follows essentially the same schema used for benign neoplasms, with certain additions. Malignant tumors arising in solid mesenchymal tissues are usually called *sarcomas* (Greek *sar* = fleshy; e.g., fibrosarcoma and chondrosarcoma), whereas those arising from blood-forming cells are designated *leukemias* (literally, white blood) or *lymphomas* (tumors of lymphocytes or their precursors). Malignant neoplasms of epithelial cell origin are called *carcinomas.* Carcinomas may be further qualified. In *squamous cell carcinoma* the tumor cells resemble stratified squamous epithelium, whereas in *adenocarcinoma* the neoplastic epithelial cells grow in a glandular pattern. Sometimes the tissue or organ of origin can be identified and is added as a descriptor, as in renal cell adenocarcinoma or bronchogenic squamous cell carcinoma. In approximately 2% of cases, cancers are composed of cells of unknown origin and must be designated merely as undifferentiated malignant tumors.

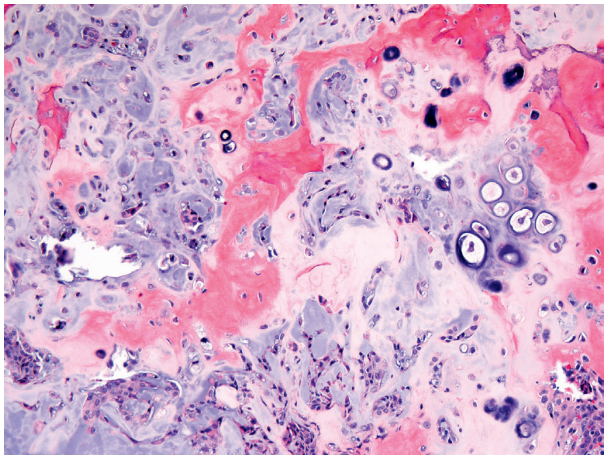

Figure 7.2 Mixed tumor of the parotid gland. Small nests of epithelial cells and myxoid stroma forming cartilage and bone (an unusual feature) are present in this field. (Courtesy Dr. Vicky Jo, Department of Pathology, Brigham and Women's Hospital, Boston, Mass.)

Mixed Tumors

In most neoplasms, all parenchymal cells closely resemble one another, but in some types of tumors more than one line of differentiation is evident, creating distinct subpopulations of cells. A classic example is the mixed tumor of the salivary gland, which contains epithelial components scattered within a myxoid stroma that may contain islands of cartilage or bone (Fig. 7.2). All of these elements arise from a single neoplastic clone capable of producing both epithelial and mesenchymal cells; thus the preferred designation of this neoplasm is *pleomorphic adenoma.* The great majority of neoplasms, including mixed tumors, are composed of cells from a single germ layer (mesoderm, endoderm, or ectoderm). An exception is a tumor called a *teratoma,* which contains recognizable mature or immature cells or tissues belonging to more than one germ cell layer (and sometimes all three). Teratoma originates from totipotential germ cells that are normally present in the ovary and testis and sometimes also found in abnormal midline embryonic rests. Such cells can differentiate into any cell type found in the body and so, not surprisingly, may give rise to neoplasms that contain, in a helter-skelter fashion, bone, epithelium, muscle, fat, nerve, and other tissues. A particularly common pattern is seen in the *ovarian cystic teratoma (dermoid cyst),* which differentiates principally along ectodermal lines to create a cystic tumor lined by squamous epithelium that is replete with hair, sebaceous glands, and tooth structures (Fig. 7.3).

The nomenclature of the more common types of tumors is presented in Table 7.1. Included in this list are some inappropriate but deeply entrenched names. For instance, the benign-sounding designations lymphoma, melanoma, mesothelioma, and seminoma are used for malignant neoplasms. Alternatively, ominous-sounding terms are applied to some trivial lesions. *Hamartomas* are disorganized masses composed of cells indigenous to the involved tissue. Once thought to be a developmental malformation unworthy

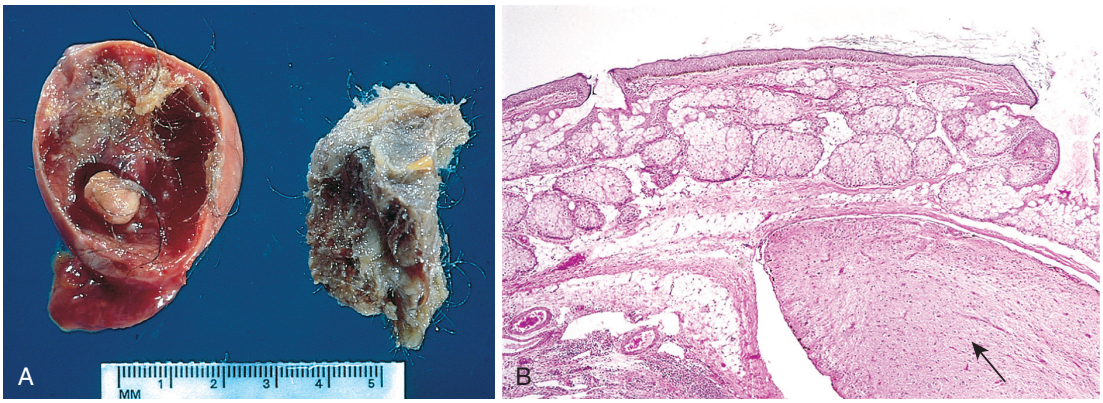

Figure 7.3 (A) Gross appearance of an opened cystic teratoma of the ovary. Note the presence of hair, sebaceous material, and a tooth. (B) Microscopic view of a similar tumor shows skin, sebaceous glands, fat cells, and a tract of neural tissue *(arrow).*

Table 7.1 Nomenclature of Tumors

Tissue of Origin	Benign	Malignant
Composed of One Parenchymal Cell Type		
Tumors of Mesenchymal Origin		
Connective tissue and derivatives	Fibroma Lipoma Chondroma Osteoma	Fibrosarcoma Liposarcoma Chondrosarcoma Osteogenic sarcoma
Vessels and Surface Coverings		
Blood vessels	Hemangioma	Angiosarcoma
Lymph vessels	Lymphangioma	Lymphangiosarcoma
Mesothelium	Benign fibrous tumor	Mesothelioma
Brain coverings	Meningioma	Invasive meningioma
Blood Cells and Related Cell Types		
Hematopoietic cells		Leukemias
Lymphoid tissue		Lymphomas
Muscle		
Smooth	Leiomyoma	Leiomyosarcoma
Striated	Rhabdomyoma	Rhabdomyosarcoma
Tumors of Epithelial Origin		
Stratified squamous	Squamous cell papilloma	Squamous cell carcinoma
Basal cells of skin or adnexa		Basal cell carcinoma
Melanocytes	Nevus	Malignant melanoma
Epithelial lining of glands or ducts	Adenoma Papilloma Cystadenoma	Adenocarcinoma Papillary carcinomas Cystadenocarcinoma
Respiratory passages	Bronchial adenoma	Bronchogenic carcinoma
Renal epithelium	Renal tubular adenoma	Renal cell carcinoma
Liver cells	Hepatic adenoma	Hepatocellular carcinoma
Urinary tract epithelium (transitional epithelium)	Transitional cell papilloma	Transitional cell carcinoma
Placenta epithelium	Hydatidiform mole	Choriocarcinoma
Testicular epithelium (germ cells)		Seminoma Embryonal carcinoma
More Than One Neoplastic Cell Type—Mixed Tumors, Usually Derived From One Germ Cell Layer		
Salivary glands	Pleomorphic adenoma (mixed tumor of salivary origin)	Malignant mixed tumor of salivary gland origin
Renal anlage		Wilms tumor
More Than One Neoplastic Cell Type Derived From More Than One Germ Cell Layer—Teratogenous		
Totipotential cells in gonads or in embryonic rests	Mature teratoma, dermoid cyst	Immature teratoma, teratocarcinoma

of the "-oma" designation, most hamartomas have clonal chromosomal aberrations that are acquired through somatic mutation and on this basis are now considered benign neoplasms. *Choristoma* is the term applied to a heterotopic (misplaced) rest of cells. For example, a small nodule of well-developed, normally organized pancreatic tissue may be found in the submucosa of the stomach or small intestine. The term *choristoma,* suggesting a neoplasm, imparts a gravity to these lesions that far exceeds their actual significance.

CHARACTERISTICS OF BENIGN AND MALIGNANT NEOPLASMS

The differentiation between benign and malignant tumors is one of the most important distinctions a pathologist can make, as nothing is more important to an individual with a tumor than being told, "It is benign." Although an innocent face may mask an ugly nature, benign and malignant tumors usually can be distinguished on the basis of various histologic and anatomic features (described later). Malignant tumors also tend to grow more rapidly than benign tumors, but there are so many exceptions that growth rate is not a reliable discriminator between benignity and malignancy. In fact, even cancers exhibit remarkably varied growth rates, from slow-growing tumors associated with survival for many years, often without treatment, to rapidly growing tumors that may be lethal within months or weeks.

Differentiation and Anaplasia

Differentiation refers to the extent to which neoplastic parenchymal cells resemble the corresponding normal parenchymal cells, both morphologically and functionally; lack of differentiation is called *anaplasia*. In general, benign tumors are well differentiated (Figs. 7.4 and 7.5). The neoplastic cells of a lipoma, a proliferation of benign adipocytes, may so closely resemble normal adipocytes as to be unrecognizable as a tumor by microscopic examination. Only the growth of these cells into a discrete mass discloses

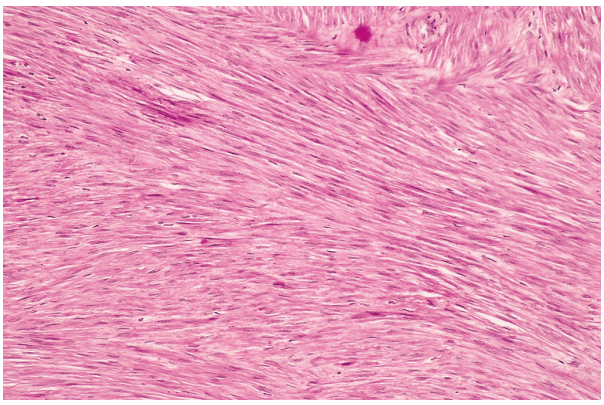

Figure 7.4 Leiomyoma of the uterus. This benign, well-differentiated tumor contains interlacing bundles of neoplastic smooth muscle cells that are virtually identical in appearance to normal smooth muscle cells in the myometrium.

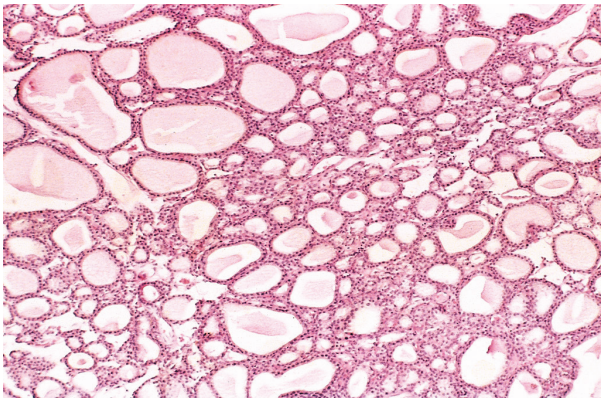

Figure 7.5 Benign tumor (adenoma) of the thyroid. Note the normal-looking (well-differentiated), colloid-filled thyroid follicles. (Courtesy Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, Tex.)

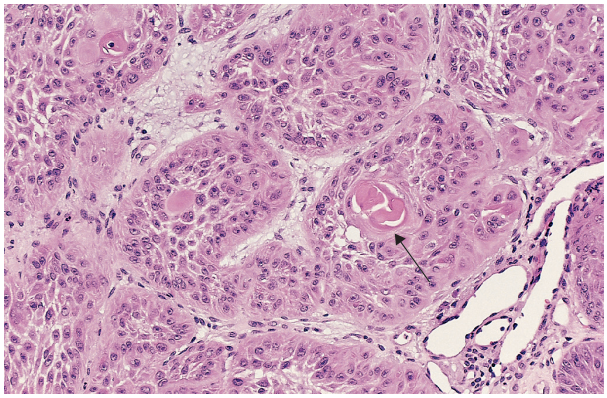

Figure 7.6 Well-differentiated squamous cell carcinoma of the skin. The tumor cells are strikingly similar to normal squamous epithelial cells, with intercellular bridges and nests of keratin pearls *(arrow)*. (Courtesy Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, Tex.)

their neoplastic nature. One may get so close to the tree that one loses sight of the forest. In well-differentiated benign tumors, mitoses are usually rare and are of normal configuration.

By contrast, **most malignant neoplasms exhibit morphologic alterations that betray their potential for aggressive behavior**. In well-differentiated tumors, these features may be quite subtle. Well-differentiated adenocarcinomas of the thyroid, for example, form normal-appearing follicles, and some squamous cell carcinomas contain cells that appear identical to normal squamous epithelial cells (Fig. 7.6). The malignant nature of such tumors is revealed by invasion of adjacent tissues and their ability to metastasize. At the other end of the spectrum lie highly anaplastic, poorly differentiated tumors exhibiting little or no evidence of differentiation (Fig. 7.7), a morphologic appearance that is highly predictive of malignant behavior. In between these two extremes lie tumors that are loosely referred to as moderately well differentiated (Fig. 7.8).

In addition to anaplasia, cancer cells often exhibit other telltale morphologic changes:

- *Pleomorphism.* Pleomorphism refers to variation in cell size and shape. Thus, cells within the same tumor are not uniform, but range from small cells with an undifferentiated appearance to *tumor giant cells* many times larger than their neighbors. Some tumor giant cells possess only a single huge polymorphic nucleus, while others may have two or more large, hyperchromatic nuclei (Fig. 7.9). These giant cells are not to be confused with inflammatory Langhans or foreign body giant cells, which are derived from macrophages and contain many small, normal-appearing nuclei.
- *Abnormal nuclear morphology.* Characteristically, cancer cells have nuclei that are disproportionately large, with a nuclear-to-cytoplasm ratio that may approach 1:1 instead of the normal 1:4 to 1:6. The nuclear shape is variable and often irregular, and the chromatin is often coarsely clumped and distributed along the nuclear

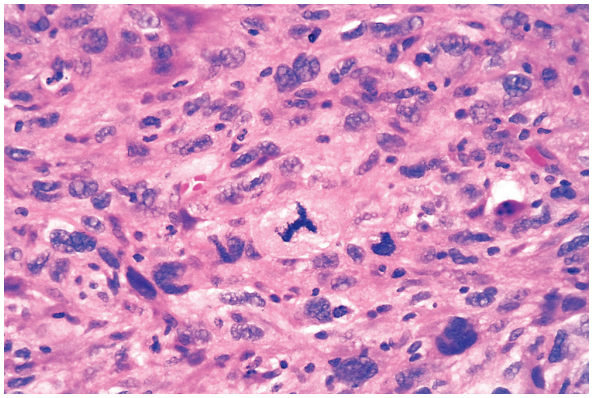

Figure 7.7 Anaplastic tumor showing cellular and nuclear variation in size and shape. The prominent cell in the center field has an abnormal tripolar spindle.

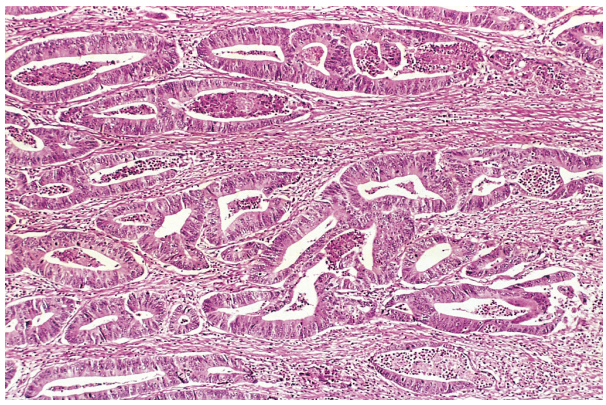

Figure 7.8 Malignant tumor (adenocarcinoma) of the colon. Note that compared with the well-formed and normal-looking glands characteristic of a benign tumor, the cancerous glands are irregular in shape and size and do not resemble the normal colonic glands. This tumor is considered moderately well differentiated because gland formation is seen. The malignant glands have invaded the muscular layer of the colon. (Courtesy Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, Tex.)

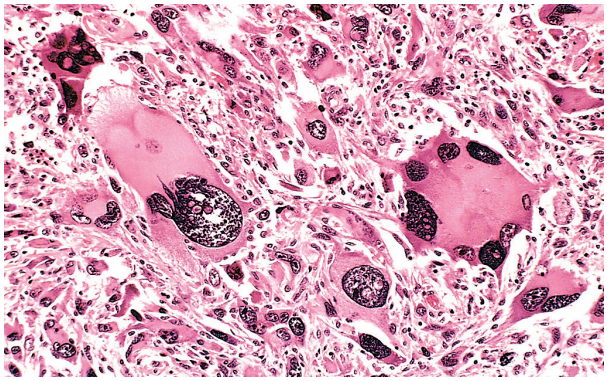

Figure 7.9 Pleomorphic tumor of the skeletal muscle (rhabdomyosarcoma). Note the marked cellular and nuclear pleomorphism, hyperchromatic nuclei, and tumor giant cells. (Courtesy Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, Tex.)

membrane or more darkly stained than normal *(hyperchromatic)*. Abnormally large nucleoli are also commonly seen.

- *Mitoses.* Unlike benign tumors and some well-differentiated malignant neoplasms, undifferentiated cancers often contain many cells in mitosis, reflecting their high rate of proliferation. The presence of mitoses, however, does not equate with malignancy. For example, cells in mitosis are often seen in normal tissues exhibiting rapid turnover, such as the epithelial lining of the gut and nonneoplastic proliferations such as hyperplasias. More important as a morphologic feature of malignancy are *atypical, bizarre mitotic figures* (see Fig. 7.8).
- *Loss of polarity.* In addition to cytologic abnormalities, the orientation of anaplastic cells with respect to each other or to supporting structures like basement membranes is markedly disturbed. Sheets or large masses of tumor cells grow in a disorganized fashion.
- *Other changes.* While growing tumor cells must have a blood supply, the vascular stroma is often insufficient; as a result, many rapidly growing cancers develop areas of ischemic necrosis.

As one might surmise, well-differentiated transformed cells have a greater likelihood of retaining the functional capabilities of their normal counterparts. Benign tumors are almost always well differentiated and often retain normal functions, as do many well-differentiated cancers. Thus, well-differentiated tumors of endocrine glands frequently secrete hormones characteristic of their origin into the blood, where they can be detected and quantified to diagnose and follow the response of such tumors to treatment. Similarly, well-differentiated squamous cell carcinomas synthesize keratin, and well-differentiated hepatocellular carcinomas elaborate bile. By contrast, highly anaplastic undifferentiated tumors typically lose the specialized functional activities of their tissue of origin, but sometimes acquire new and unanticipated functions. Thus some malignant tumors express fetal proteins that are not produced by their normal adult counterparts, while others express proteins that are normally found only in other types of cells. For example, bronchogenic carcinomas may produce corticotropin, parathyroid-like hormone, insulin, glucagon, and other hormones, giving rise to paraneoplastic syndromes (described later).

Metaplasia, Dysplasia, and Carcinoma In Situ

These terms describe morphologically recognizable changes in differentiation that variously represent an adaptation to chronic injury (metaplasia), a premalignant change (dysplasia), or a cancer that has yet to invade (carcinoma in situ).

- *Metaplasia* is defined as the replacement of one type of cell with another type (Chapter 2). Metaplasia is nearly always found in association with tissue damage, repair, and regeneration. Often the replacing cell type is better suited to some alteration in the local environment. For example, in Barrett esophagus, gastroesophageal reflux damages the squamous epithelium of the esophagus, leading to its replacement by glandular (gastric or intestinal) epithelium better suited to an acidic environment. Unfortunately, the metaplastic epithelium is prone to malignant transformation. The same is true of squamous metaplasia of the bronchial epithelium in chronic smokers, often a prelude to the development of lung cancer.
- *Dysplasia* is a term that literally means "disordered growth." It is encountered principally in epithelial cells and is recognized on the basis of several morphologic changes. Dysplastic cells may exhibit considerable pleomorphism and often contain large hyperchromatic nuclei with a high nuclear-to-cytoplasmic ratio. Dysplastic epithelial surfaces also typically show architectural disarray and a loss of orderly differentiation. For example, in dysplastic squamous epithelium the normal progressive maturation of tall cells in the basal layer to flattened squames on the surface may fail in part or entirely, leading to replacement of the epithelium by basal-like cells with hyperchromatic nuclei. In addition, mitotic figures are more abundant than in the normal squamous epithelium and may be seen throughout dysplastic epithelium, rather than being confined to the basal layer, as is the normal case.
- *Carcinoma in situ.* When dysplasia is severe and involves the full thickness of the epithelium but the lesion does not penetrate the basement membrane, it is referred to as *carcinoma in situ* (Fig. 7.10). Carcinoma in situ is often seen in the skin, breast, bladder, and uterine cervix. In situ epithelial cancers display all of the cytologic features of malignancy and unless treated have high probability of progression to invasive cancers.

Dysplastic changes are often found adjacent to foci of invasive carcinoma, and in some situations, such as in the cervix, severe epithelial dysplasia or carcinoma in situ frequently antedates the appearance of cancer. Moreover, some mutations associated with full-blown cancer (described later) may be present in even "mild" dysplasias. Nevertheless, **although dysplasia may be a precursor to malignant transformation, it does not always progress to cancer.** With removal of inciting causes, even moderately severe dysplasias may be completely reversible. Even carcinoma in situ may persist for years before it becomes invasive. As discussed later, cancers arise by accumulation of mutations, and the time interval for evolution of full-blown cancers from in situ lesions relates most likely to the time required for

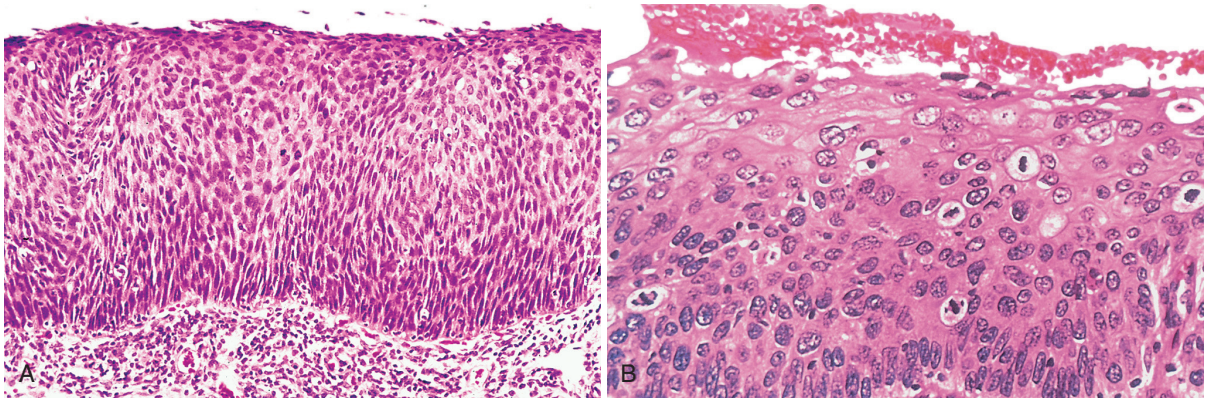

Figure 7.10 (A) Carcinoma in situ. Low-power view shows that the epithelium is entirely replaced by atypical dysplastic cells. There is no orderly differentiation of squamous cells. The basement membrane is intact, and there is no tumor in the subepithelial stroma. (B) High-power view of another region shows failure of normal differentiation, marked nuclear and cellular pleomorphism, and numerous mitotic figures extending toward the surface.

accumulation of all the mutations that are needed to induce a fully malignant phenotype. Finally, it should be noted that while dysplasia often occurs in metaplastic epithelium, not all metaplastic epithelium is dysplastic.

Local Invasion

The growth of cancers is accompanied by progressive invasion, destruction of surrounding tissue, and eventually systemic spread, whereas nearly all benign tumors grow as cohesive, expansile masses that remain localized to their site of origin and lack the capacity to invade or metastasize to distant sites. Because benign tumors grow and expand slowly, they usually develop a rim of compressed fibrous tissue called a capsule that separates them from the surrounding normal tissue. The tumor capsule consists of extracellular matrix (ECM) deposited by stromal cells such as fibroblasts, which are activated by hypoxic damage resulting from the pressure of the expanding tumor. Such encapsulation creates a tissue plane that makes the tumor discrete, readily palpable, movable (nonfixed), and easily excisable by surgical enucleation (Fig. 7.11). There are a few exceptions to this rule, however. For example, hemangiomas (benign neoplasms composed of tangled blood vessels) are often unencapsulated and permeate the site in which they arise (e.g., the dermis of the skin and the liver); when such lesions are extensive, they may be unresectable.

In contrast, malignant tumors are, in general, poorly demarcated from the surrounding normal tissue and lack well-defined cleavage planes (Fig. 7.12). Slowly expanding malignant tumors may develop an apparently enclosing fibrous capsule and push along a broad front into adjacent normal structures. However, histologic examination of such "pseudoencapsulated" masses almost always shows rows of tumor cells penetrating the margin and infiltrating adjacent structures, a crablike pattern of growth that fits the popular image of cancer.

Next to the development of metastases, invasiveness is the most reliable discriminator of malignant and benign tumors. Most malignant tumors do not recognize normal anatomic boundaries. Given time, they will penetrate the wall of the colon or uterus, for example, or fungate through the surface of the skin. This invasiveness makes their complete surgical resection difficult or impossible, and even if the tumor appears well circumscribed it is necessary to remove a large margin of adjacent, apparently normal tissue to ensure complete local excision.

Metastasis

Metastasis is defined as the spread of a tumor to sites that are physically discontinuous with the primary tumor, an

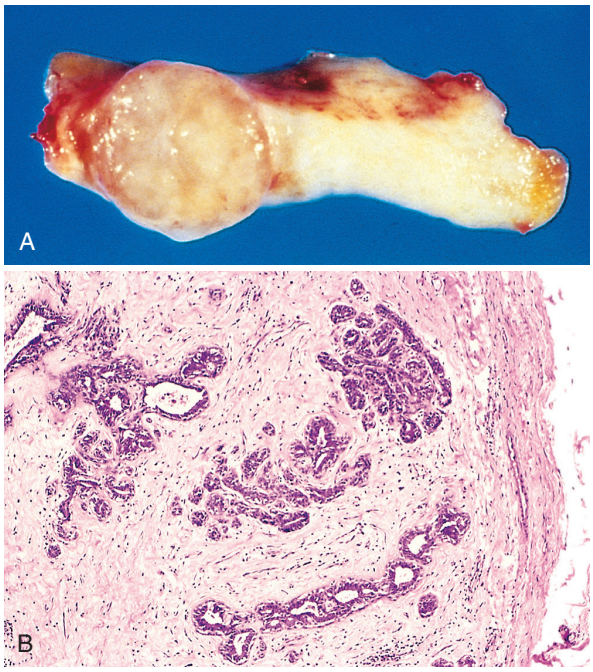

Figure 7.11 Fibroadenoma of the breast. (A) The tan-colored, encapsulated small tumor is sharply demarcated from the whiter breast tissue. (B) Microscopic view shows that the fibrous capsule *(right)* delimits the tumor from the surrounding tissue. (B, Courtesy Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, Tex.)

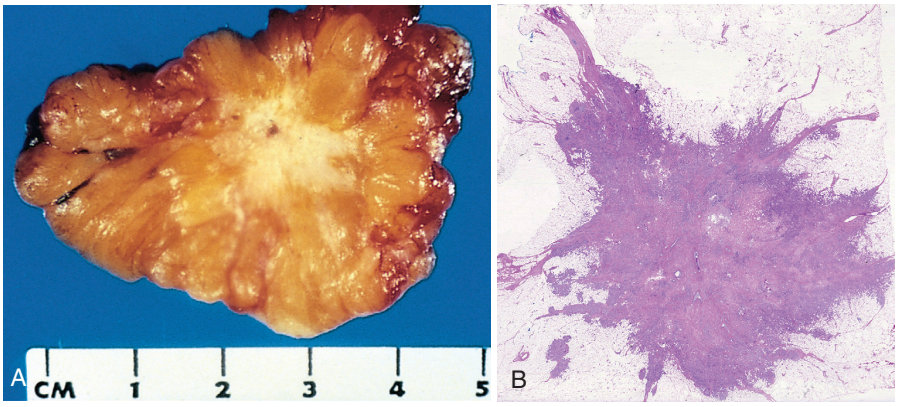

Figure 7.12 Invasive ductal carcinoma of the breast. (A) On cut section, the lesion is retracted and infiltrates the surrounding breast substance and would be stony hard on palpation. (B) Low-power microscopic view shows irregular infiltrative borders without a well-defined capsule and intense stromal reaction. (A, Courtesy Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, Tex.; B, Courtesy Dr. Susan Lester, Brigham and Women's Hospital, Boston, Mass.)

event that unequivocally marks a tumor as malignant. The invasiveness of cancers permits them to penetrate blood vessels, lymphatics, and body cavities, providing the opportunity for spread. All malignant tumors can metastasize, but some do so very infrequently. Examples include malignant neoplasms of the glial cells in the central nervous system, called *gliomas,* and basal cell carcinomas of the skin, both of which invade early in their course but rarely metastasize. It is evident then that the properties of invasiveness and metastasis are separable. Blood cancers (leukemias and lymphomas) are a special case. These tumors are derived from cells that normally have the capacity to enter the bloodstream and travel to distant sites. As a result, leukemias and lymphomas (sometimes referred to as "liquid tumors") are often disseminated at diagnosis and are always taken to be malignant, unlike all other tumors (so-called "solid" tumors), which are derived from cells that do not normally circulate in the bloodstream.

Overall, approximately 30% of solid tumors (excluding skin cancers other than melanomas) present as metastatic disease. In general, the likelihood of metastasis of a solid tumor correlates with other features of malignancy, including lack of differentiation, aggressive local invasion, rapid growth, and large size. There are innumerable exceptions, however. Small, well-differentiated, slow-growing lesions sometimes metastasize widely; conversely, some rapidly growing, large lesions remain localized for years. Metastasis is thus a complex and unpredictable process that involves many factors relating to both invader and host (discussed later). Metastatic spread strongly reduces the possibility of cure; hence, short of prevention of cancer, no achievement would be of greater benefit to patients than an effective means to block metastasis, with the important caveat that many tumors that kill the patient have already spread by the time of initial diagnosis.

Pathways of Spread

Dissemination of cancers occurs through three pathways: (1) direct seeding of body cavities or surfaces, (2) lymphatic spread, and (3) hematogenous spread. Although iatrogenic spread of tumor cells on surgical instruments may occur—it is the reason, for example, why biopsies of testicular masses are never done—it is rare and not discussed further.

Seeding of Body Cavities and Surfaces. Seeding of body cavities and surfaces occurs when a malignant neoplasm penetrates into a natural "open field" lacking physical barriers. Most often involved is the peritoneal cavity (Fig. 7.13), but any body cavity—pleural, pericardial, subarachnoid, and joint spaces—may be affected. Such seeding is particularly characteristic of carcinomas arising in the ovaries, which often spread to peritoneal surfaces, producing a heavy cancerous coating. Remarkably, the tumor cells may remain confined to the surface of the abdominal viscera without penetrating into the substance. Sometimes, mucus-secreting

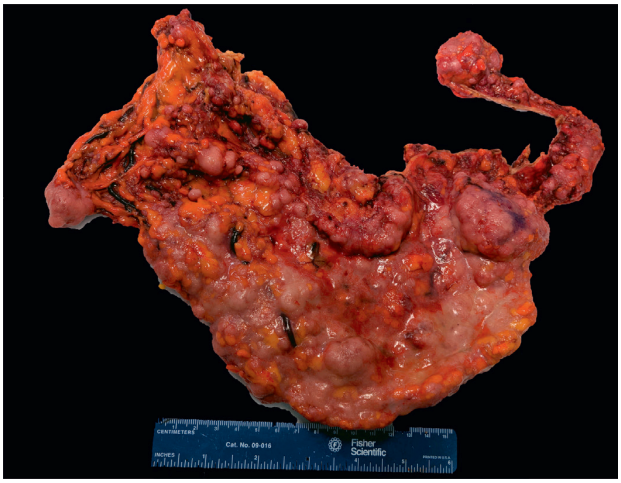

Figure 7.13 Involvement of omentum by metastatic ovarian carcinoma. Innumerable nodules and more subtle "glazing" are evident due to seeding by carcinoma cells via the peritoneal cavity. (Courtesy Dr. Sarah Hill, Brigham and Women's Hospital, Boston, Mass.)

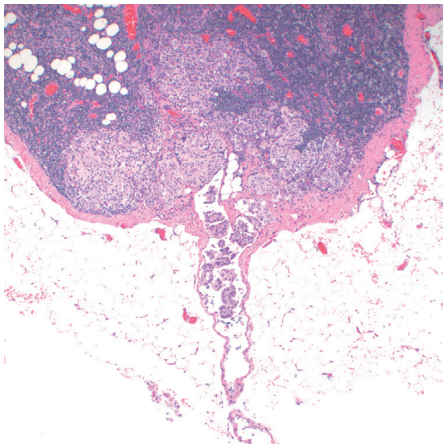

Figure 7.14 Axillary lymph node with metastatic breast carcinoma. Note the aggregates of tumor cells within the substance of the node and the dilated lymphatic channel. (Courtesy Dr. Susan Lester, Brigham and Women's Hospital, Boston, Mass.)

appendiceal carcinomas or ovarian carcinomas fill the peritoneal cavity with a gelatinous neoplastic mass referred to as *pseudomyxoma peritonei*.

Lymphatic Spread. **Transport through lymphatic vessels is the most common pathway for the initial dissemination of carcinomas** (Fig. 7.14). Sarcomas also sometimes use this route. Tumors do not contain functional lymphatic vessels, but lymphatic vessels located at the margins of invading cancers are apparently sufficient for the lymphatic spread of tumor cells. The pattern of spread follows the natural routes of lymphatic drainage. For example, because carcinomas of the breast usually arise in the upper outer quadrants, they generally disseminate first to the axillary lymph nodes and then to infraclavicular and supraclavicular lymph nodes. Carcinomas of the lung arising in the major respiratory passages metastasize first to perihilar tracheobronchial and mediastinal lymph nodes. Local lymph nodes, however, may be bypassed—so-called skip metastasis—possibly because microscopic metastases are missed or because of variation in normal patterns of lymphatic drainage.

In breast cancer, axillary lymph node examination is used to determine the prognosis and select the most suitable therapeutic options. To avoid the surgical morbidity associated with a full axillary lymph node dissection, biopsy of sentinel nodes is often used to assess the presence or absence of metastatic lesions. **A sentinel lymph node is defined as "the first node in a regional lymphatic basin that receives lymph flow from the primary tumor."** Sentinel node mapping can be done by injection of radiolabeled tracers or colored dyes, and examination of frozen sections of the sentinel lymph node performed during surgery can guide the surgeon to the appropriate therapy. Sentinel node examination has also been used to assess the spread of melanomas, colon cancers, and other tumors.

In many cases the regional nodes serve as effective barriers against further dissemination of the tumor, at least for a while. Conceivably, after arrest within the node the cells may be destroyed by a tumor-specific immune response. The immune response to tumor cells or antigens in draining lymph nodes may lead to enlargement (hyperplasia) of the nodes. Thus, enlarged lymph nodes do not always harbor metastases, which can be assessed definitively only by microscopic examination.

Hematogenous Spread. **Hematogenous spread is typical of sarcomas but is also seen with carcinomas**. In general, histologic evidence of penetration of small vessels at the site of the primary neoplasm is an ominous feature associated with hematogenous metastasis. The involved vessels are usually small veins, as arteries, with their thicker walls, are more resistant to penetration. Arterial spread may occur, however, when tumor cells pass through pulmonary capillary beds or pulmonary arteriovenous shunts or when cancers in the lung (primary or metastatic) give rise to tumor emboli.

Several factors influence the patterns of vascular metastasis. With venous invasion, the blood-borne tumor cells often come to rest in the first capillary bed they encounter. Understandably, the liver and the lungs (Fig. 7.15) are most frequently involved by hematogenous dissemination because all portal area drainage flows to the liver and all caval blood

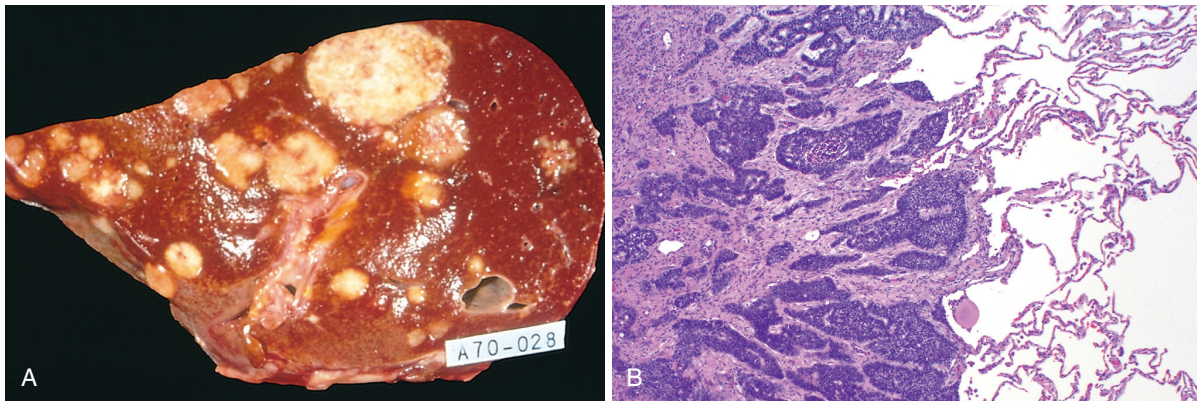

Figure 7.15 Cancer metastasis. (A) Liver studded with metastatic cancer. (B) Microscopic view of lung metastasis. A colonic adenocarcinoma has formed a metastatic nodule in the lung. (B, Courtesy Dr. Shuji Ogino, Dana Farber Cancer Institute, Boston, Mass.)

Table 7.2 Comparisons Between Benign and Malignant Tumors

Characteristics	Benign	Malignant
Differentiation/ anaplasia	Well differentiated; structure sometimes typical of tissue of origin	Some lack of differentiation (anaplasia); structure often atypical
Rate of growth	Usually progressive and slow; may come to a standstill or regress; mitotic figures rare and normal	Erratic, may be slow to rapid; mitotic figures may be numerous and abnormal
Local invasion	Usually cohesive, expansile, well-demarcated masses that do not invade or infiltrate surrounding normal tissues	Locally invasive, infiltrating surrounding tissue; sometimes may be misleadingly cohesive and expansile
Metastasis	Absent	Frequent; more likely with large undifferentiated primary tumors

flows to the lungs. Cancers arising in close proximity to the vertebral column often embolize through the paravertebral plexus; this pathway produces frequent vertebral metastases from carcinomas of the thyroid and prostate. Nonetheless, many observations suggest that the location of the primary tumor and its natural pathways of venous drainage do not wholly explain the observed patterns of metastatic spread, which are often cancer-specific. Unfortunately, most cancers have not read pathology textbooks! The basis of tissue-specific patterns of metastasis is discussed later.

Certain cancers have a curious propensity for growth within large veins. Renal cell carcinoma often invades the branches of the renal vein and then the renal vein itself, growing in a snakelike fashion up the inferior vena cava until it sometimes reaches the right side of the heart. Similarly, hepatocellular carcinomas often penetrate portal and hepatic radicles and then grow into the main venous channels. Remarkably, such intravenous growth may not be accompanied by widespread metastasis.

The distinguishing features of benign and malignant tumors are summarized in Table 7.2 and Fig. 7.16. Having completed our overview of the morphology and behavior of neoplasms, we now discuss the pathogenesis of neoplasia, starting with clues gleaned from studies of the epidemiology of cancer.

KEY CONCEPTS

CHARACTERISTICS OF BENIGN AND MALIGNANT NEOPLASMS

- Benign and malignant tumors can be distinguished from one another based on the degree of differentiation, local invasiveness, and distant spread.
- Benign tumors resemble the tissue of origin and are well differentiated; malignant tumors are less well differentiated or completely undifferentiated (anaplastic).

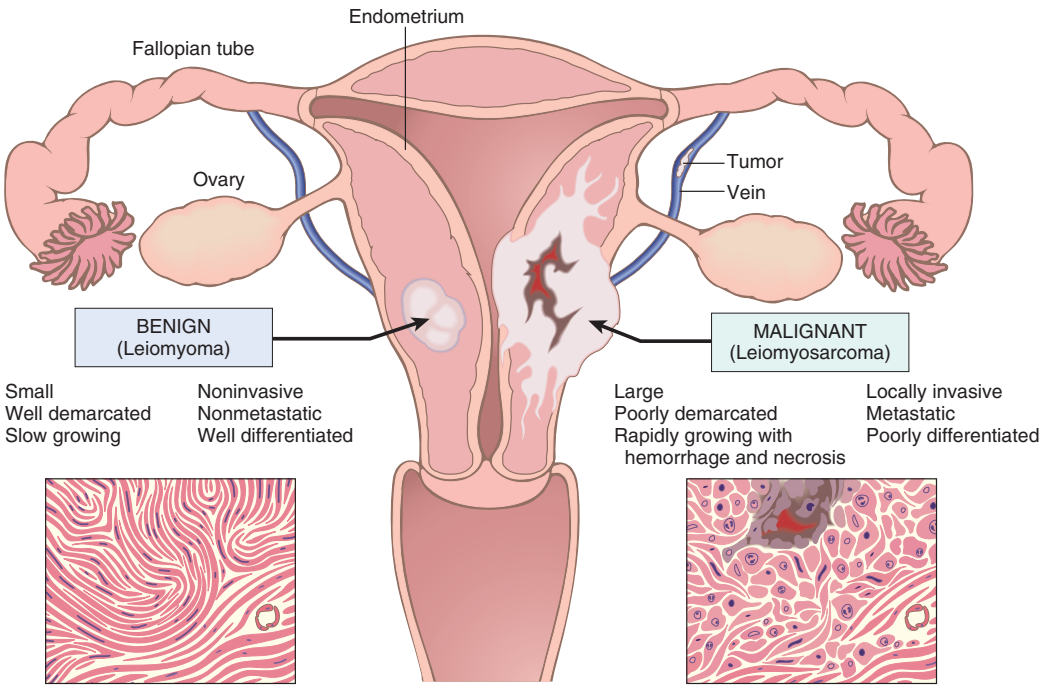

Figure 7.16 Comparison between a benign tumor of the myometrium (leiomyoma) and a malignant tumor of the same origin (leiomyosarcoma).

- Benign tumors are more likely to retain functions of their cells of origin, whereas malignant tumors sometimes acquire unexpected functions due to derangements in differentiation.
- Benign tumors are slow growing, while malignant tumors generally grow faster.
- Benign tumors are circumscribed and have a capsule; malignant tumors are poorly circumscribed and invade surrounding normal tissues.
- Benign tumors remain localized at the site of origin; malignant tumors metastasize to distant sites. Carcinomas tend to spread via lymphatics, whereas sarcomas prefer the hematogenous route.

EPIDEMIOLOGY OF CANCER

Study of cancer in defined populations has contributed substantially to knowledge about its origins. Epidemiologic studies have established the causative link between smoking and lung cancer, and comparison of diet and cancer rates in different regions of the world has linked diets high in fat and low in fiber to colon cancer. It is hoped that additional insights into the causes of cancer will be obtained by studies that relate particular environmental, racial (possibly hereditary), and cultural influences to specific neoplasms. The strong association of certain inflammatory and other diseases with cancer also provides clues to its pathogenesis. In the following sections, we discuss the overall incidence of cancer and then review environmental and host factors that influence the predisposition to cancer.

The Global Impact of Cancer

In 2018 it was estimated that there were over 9.5 million deaths caused by cancer worldwide, representing nearly 1 in 6 of all deaths. Moreover, due to increasing population size and age, cancer cases and cancer-related deaths worldwide are projected to increase to 21.4 million and 13.2 million, respectively by the year 2030. The major organ sites affected and the estimated frequency of cancer deaths in the United States are shown in Fig. 7.17. The most common tumors in men arise in the prostate, lung, and colon/rectum. In women, cancers of the breast, lung, and colon/rectum are the most frequent. Cancers of the lung, female breast, prostate, and colon/rectum constitute more than 50% of cancer diagnoses and cancer deaths in the United States.

Most longitudinal data pertaining to cancer incidence come from higher income countries, where age-adjusted death rates (deaths per 100,000 population) for many cancers have changed significantly over the years. In the last 50 years of the 20th century, the age-adjusted cancer death rate increased significantly in both men and women. However, since 1995 the cancer incidence rate in men has been stable, and since 1990 the cancer death rate has decreased by approximately 20%. Similarly, the cancer incidence rate also stabilized in women in 1995, and the cancer death rate has fallen by approximately 10% since 1991. Among men, nearly 80% of the decrease is accounted for by lower death rates from lung, prostate, and colorectal cancers; among women, nearly 60% of the decrease is due to reductions in death rates from breast and colorectal cancers. Decreased use of tobacco products is responsible for the reduction in lung cancer deaths, while improved detection and treatment are responsible for the decrease in death rates for colorectal, female breast, and prostate cancer.

The last half-century has also seen a sharp decline in deaths caused by cervical cancer in the United States. This decrease is largely attributable to the Papanicolaou (Pap) smear test, which enables detection of "precursor lesions" (discussed later) and early, curable cancers. By contrast,

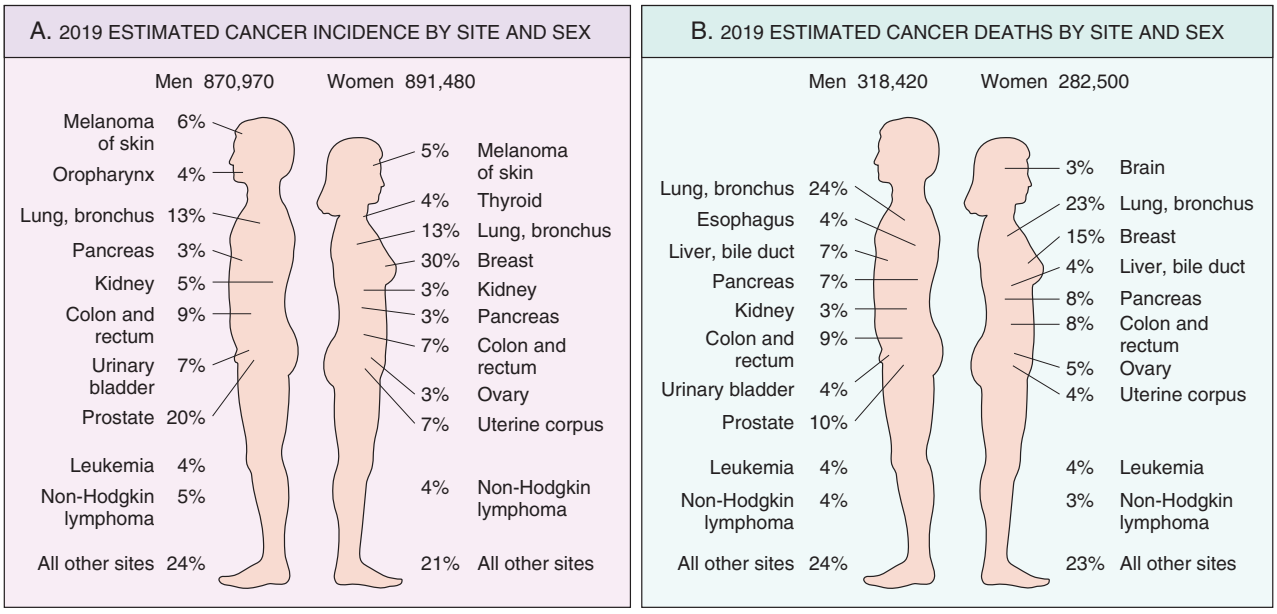

Figure 7.17 Cancer incidence (A) and mortality (B) by site and sex. Excludes basal cell and squamous cell skin cancers and in situ carcinomas except urinary bladder. (Modified from Siegel RL, Miller KD, Jemal A: Cancer statistics, 2017, *CA Cancer J Clin* 67:7–30, 2017.)

between 1990–1991 and 2004, lung cancer death rates in women and liver and intrahepatic bile duct cancer death rates in men increased substantially, offsetting some of the improvement in survival from other cancers. Indeed, although carcinomas of the breast occur about 2.5 times more frequently than those of the lung in women, lung cancer now causes more deaths in women.

Race is not a discrete biologic variable, but it can define groups at risk for certain cancers. The disparity in cancer mortality rates between Americans who are Caucasian or of African descent persists, but African Americans had the largest decline in cancer mortality during the past decade. People identifying as Hispanic living in the United States have a lower frequency of the most common cancers affecting the Caucasian non-Hispanic population and a higher incidence of cancers of the stomach, liver, uterine cervix, and gallbladder as well as certain leukemias.

Environmental Factors

Although both genetic and environmental factors contribute, environmental influences are the dominant risk factors for most cancers. One line of evidence supporting this idea comes from longitudinal changes in cancer incidence in the United States. Examples include the tight tracking of lung cancer incidence with changes in smoking habits over time; the sharp drop in stomach cancer incidence during the 20th century, believed to stem from decreased exposure to unknown environmental carcinogens; and a recent increase in the incidence of liver cancer, which is likely due to rising rates of chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection and obesity. Other evidence is found in the wide geographic variation that exists in the incidence of specific cancers (Fig. 7.18). For example, the most common cancer of men in the United States and in most other higher income countries is prostate cancer, but in other countries or regions, cancers of the liver, stomach, esophagus, bladder, lung, oropharynx, and the immune system rise to the top of the list. Similarly, the incidence of breast cancer is generally much higher in women living in higher income countries than in lower income countries. Although racial predisposition may factor in, it is believed that environmental influences—some known, some not—underlie most of these differences in cancer incidence.

Among the best-established environmental factors affecting cancer risk are the following.

- *Infectious agents.* About 15% of all cancers worldwide are caused directly or indirectly by infectious agents, with the burden of cancers linked to infections being roughly three times higher in the developing world than in the developed world. For example, *human papillomavirus (HPV),* an agent spread through sexual contact, has a causative role in the majority of cervical carcinomas and an increasing fraction of head and neck cancers. Specific infectious agents and their associated cancers are discussed later in this chapter.
- *Smoking.* Cigarette smoking is the single most important environmental factor contributing to premature death in the United States. Smoking, particularly of cigarettes, is implicated in cancer of the mouth, pharynx, larynx, esophagus, pancreas, and bladder and, most significantly, in about 90% of lung cancers (Chapter 9).
- *Alcohol consumption.* Alcohol abuse increases the risk of carcinoma of the oropharynx (excluding lip), larynx, and esophagus and, by the development of alcoholic cirrhosis, hepatocellular carcinoma. Moreover, the risk of cancers in the upper airways and digestive tract imposed by alcohol is increased synergistically when combined with tobacco use.
- *Diet.* Although the precise dietary factors that affect cancer risk remain a matter of debate, wide geographic variation in the incidences of colorectal carcinoma, prostate carcinoma, and breast carcinoma has been ascribed to differences in diet.
- *Obesity.* Given that the obesity epidemic in the United States is spreading to other parts of the world (Chapter 9), it is concerning that obesity is associated with increased cancer risk. The most overweight individuals in the U.S. population have 52% (men) to 62% (women) higher death rates from cancer than do their slimmer counterparts; it follows that approximately 14% of cancer deaths in men and 20% in women are associated with obesity.
- *Reproductive history.* Lifelong cumulative exposure to estrogen stimulation, particularly if unopposed by progesterone, increases the risk of cancers of the breast and endometrium, tissues that are responsive to these hormones. It is likely that some of the geographic variation in breast cancer incidence is related to differing cultural mores that influence the timing and number of pregnancies a woman has during her lifetime.
- *Environmental carcinogens.* There is no paucity of well-characterized environmental carcinogens: they lurk in the ambient environment, in the workplace (Table 7.3), in food, and in personal practices. Individuals may be exposed to carcinogenic factors when they go outside (e.g., ultraviolet [UV] rays, smog), drink well water (e.g., arsenic, particularly in Bangladesh), take certain medications (e.g., methotrexate), go to work (e.g., asbestos), or lounge at home (e.g., grilled meat, high-fat diet, alcohol).

It appears that almost everything one does to earn a livelihood or for pleasure is fattening, immoral, illegal, or, even worse, carcinogenic!

Age

Age has an important influence on the risk of cancer. Most carcinomas occur in adults older than 55 years of age. Cancer is the leading cause of death among women aged 40 to 79 and among men aged 60 to 79; the decline in cancer deaths after age 80 is due to the lower number of individuals who reach this age. The rising incidence of cancer with age is likely explained by the accumulation of somatic mutations that accompanies the aging of cells (discussed later). A decline in immune competence in older individuals may also be a factor.

Tragically, children are not spared; cancer accounts for approximately 10% of all deaths in children younger than age 15 in the United States, second only to accidents. However, the types of cancers that predominate in children are different from those seen in adults; in part, this is because pediatric cancers are more likely to be caused by inherited mutations (particularly in tumor suppressor genes, described later) and much less likely to stem from exposure to

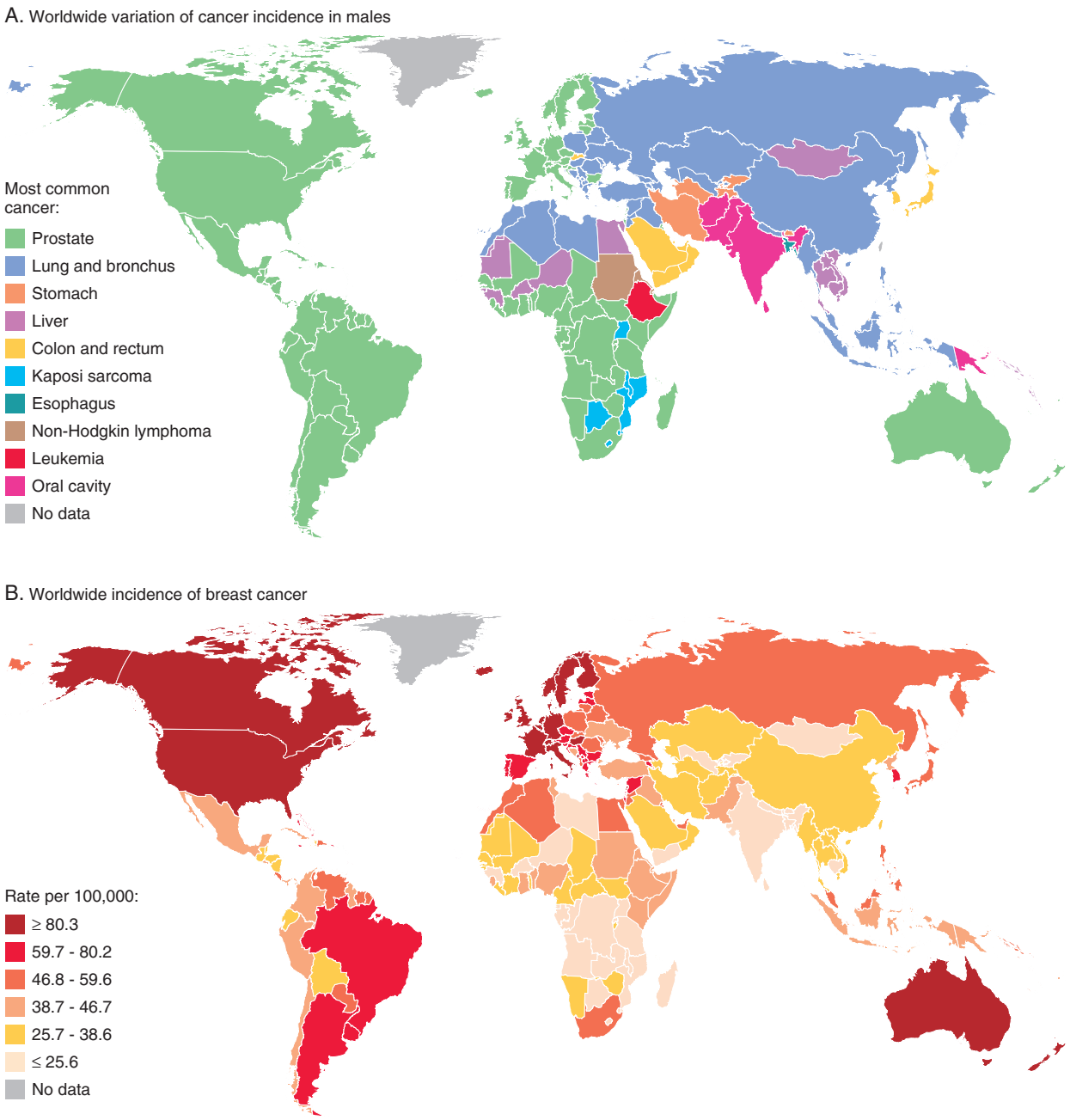

Figure 7.18 Geographic variation in cancer incidence. (A) Most common cancers in men by country. (B) Variation in breast cancer incidence in women by country. (Modified from American Cancer Society: *Global Cancer Facts & Figures*, ed 3, Atlanta, 2015, American Cancer Society.)

environmental carcinogens (e.g., cigarette smoking). This difference explains why carcinomas, which are frequently caused by carcinogens and are the most common general type of tumor in adults, are very rare in children. Instead, acute leukemia and distinctive neoplasms of the central nervous system cause approximately 60% of childhood cancer deaths. The common neoplasms of infancy and childhood include the so-called small round blue cell tumors such as neuroblastoma, Wilms tumor, retinoblastoma, acute lymphoblastic leukemia, and rhabdomyosarcoma. These are discussed in Chapter 10 and elsewhere in the text.

Acquired Predisposing Conditions

Acquired conditions that predispose to cancer can be divided into chronic inflammatory disorders, precursor

Table 7.3 Occupational Cancers

Agents or Groups of Agents	Human Cancers for Which Reasonable Evidence Is Available	Typical Use or Occurrence
Arsenic and arsenic compounds	Lung carcinoma, skin carcinoma	By-product of metal smelting; component of alloys, electrical and semiconductor devices, medications and herbicides, fungicides, and animal dips
Asbestos	Lung, esophageal, gastric, and colon carcinoma; mesothelioma	Formerly used for many applications because of fire, heat, and friction resistance; still found in existing construction as well as fire-resistant textiles, friction materials (i.e., brake linings), underlayment and roofing papers, and floor tiles
Benzene	Acute myeloid leukemia	Principal component of light oil; despite known risk, many applications exist in printing and lithography, paint, rubber, dry cleaning, adhesives and coatings, and detergents; formerly widely used as solvent and fumigant
Beryllium and beryllium compounds	Lung carcinoma	Missile fuel and space vehicles; hardener for lightweight metal alloys, particularly in aerospace applications and nuclear reactors
Cadmium and cadmium compounds	Prostate carcinoma	Uses include yellow pigments and phosphors; found in solders; used in batteries and as alloy and in metal platings and coatings
Chromium compounds	Lung carcinoma	Component of metal alloys, paints, pigments, and preservatives
Nickel compounds	Lung and oropharyngeal carcinoma	Nickel plating; component of ferrous alloys, ceramics, and batteries; by-product of stainless-steel arc welding
Radon and its decay products	Lung carcinoma	From decay of minerals containing uranium; potentially serious hazard in quarries and underground mines
Vinyl chloride	Hepatic angiosarcoma	Refrigerant; monomer for vinyl polymers; adhesive for plastics; formerly inert aerosol propellant in pressurized containers

Modified from Stellman JM, Stellman SD: Cancer and workplace, *CA Cancer J Clin* 46:70, 1996.

lesions, and immunodeficiency states. Chronic inflammatory disorders and precursor lesions span a diverse set of conditions that are all associated with increased cellular replication, which appears to create a "fertile" soil for the development of malignant tumors. Indeed, repeated rounds of cell division may be required for neoplastic transformation, as proliferating cells are most at risk for somatic mutations that lead to carcinogenesis. Immunodeficiency states predispose to virus-induced cancers. Each of these acquired predisposing conditions is described next.

- *Chronic inflammation.* Virchow first proposed a cause-and-effect relationship between chronic inflammation and cancer in 1863. The scope of this association is now clear; cancer risk is increased in individuals affected by a wide variety of chronic inflammatory diseases, both infectious and noninfectious (Table 7.4). Tumors arising in the context of chronic inflammation are mostly carcinomas, but also include mesothelioma and several kinds of lymphoma. As with any cause of tissue injury, these disorders are accompanied by a compensatory proliferation of cells that serves to repair the damage. In some cases, chronic inflammation may increase the pool of tissue stem cells, which may be particularly susceptible to transformation. Additionally, activated immune cells produce reactive oxygen species that may damage DNA and inflammatory mediators that may promote cell survival, even in the face of genomic damage. Whatever the precise mechanism, the link between chronic inflammation and cancer has practical implications. For instance, diagnosis and effective treatment of *Helicobacter pylori* gastritis with antibiotics can quell a chronic inflammatory condition that might otherwise lead to the development of a gastric cancer.
- *Precursor lesions.* Precursor lesions are defined by localized morphologic changes that identify a field of epithelium that is at increased risk for malignant transformation. These changes may take the form of hyperplasia, metaplasia, or dysplasia. The link between epithelial dysplasia and metaplasia with various forms of carcinoma has already been mentioned. Precursor lesions consisting of hyperplasias often stem from chronic exposure to trophic factors. One of the most common precursors of this type is *endometrial hyperplasia,* which is caused by sustained estrogenic stimulation of the endometrium. Other "at risk" lesions consist of benign neoplasms. A classic lesion of this type is the colonic *villous adenoma,* which progresses to cancer in about 50% of cases if left untreated. It should be emphasized, however, that most benign tumors transform rarely (e.g., uterine leiomyomas, pleomorphic adenoma) and others not at all (e.g., lipomas). Why most benign tumors have a negligible risk of malignant transformation is an unsettled question; one possibility is that benign tumors at high risk for malignant transformation possess the cancer-enabling property of genomic instability (discussed later), whereas other benign tumors do not.
- *Immunodeficiency and cancer.* Patients who are immunodeficient, particularly those with deficits in T-cell immunity, are at increased risk for cancer, especially types caused by oncogenic viruses, presumably because

Table 7.4 Chronic Inflammatory States and Cancer

Pathologic Condition	Associated Neoplasm(s)	Etiologic Agent(s)
Asbestosis, silicosis	Mesothelioma, lung carcinoma	Asbestos fibers, silica particles
Inflammatory bowel disease	Colorectal carcinoma	
Lichen sclerosis	Vulvar squamous cell carcinoma	
Pancreatitis	Pancreatic carcinoma	Alcoholism, germline mutations (e.g., in the trypsinogen gene)
Chronic cholecystitis	Gallbladder cancer	Bile acids, bacteria, gallbladder stones
Reflux esophagitis, Barrett esophagus	Esophageal carcinoma	Gastric acid
Sjögren syndrome, Hashimoto thyroiditis	MALT lymphoma	
Opisthorchis, cholangitis	Cholangiocarcinoma, colon carcinoma	Liver flukes (*Opisthorchis viverrini*)
Gastritis/ulcers	Gastric adenocarcinoma, MALT lymphoma	*Helicobacter pylori*
Hepatitis	Hepatocellular carcinoma	Hepatitis B and/or C virus
Osteomyelitis	Carcinoma in draining sinuses	Bacterial infection
Chronic cervicitis	Cervical carcinoma	Human papillomavirus
Chronic cystitis	Bladder carcinoma	Schistosomiasis

MALT, Mucosa-associated lymphoid tissue.

Modified from TIsty TD, Coussens LM: Tumor stroma and regulation of cancer development, *Ann Rev Pathol Mech Dis* 1:119, 2006.

these individuals have a higher than normal incidence of chronic infection with viruses. These virus-associated tumors include lymphomas, certain carcinomas, and some sarcomas and sarcoma-like proliferations. The relationship between infections, immunity, and cancer is discussed later in this chapter.

Genetic Predisposition and Interactions Between Environmental and Inherited Factors

In some families, cancer is an inherited trait, usually due to germline mutations in a tumor suppressor gene (described later). What then can be said about the influence of heredity on sporadic malignant neoplasms, which constitute roughly 95% of cancers in the United States? While the evidence suggests that these cancers are largely attributable to environmental factors or acquired predisposing conditions, lack of family history does not preclude an inherited component. It is generally difficult to sort out hereditary and nonhereditary contributions because their interactions are often complex, particularly when tumor development depends on the action of multiple genes. Even in cancers with a well-defined inherited component, the risk of cancer development may be greatly influenced by nongenetic factors. For instance, breast cancer risk in females who inherit mutated copies of the *BRCA1* or *BRCA2* tumor suppressor genes (discussed later) has been observed to be almost threefold higher for women born after 1940 than for women born before that year, perhaps because of changes in reproductive history. Conversely, genetic factors can alter the likelihood of cancers that are primarily induced by environmental carcinogens. This is because genetic variation (polymorphisms) in certain enzymes, such as the cytochrome P-450 system, influences the conversion of procarcinogens to active carcinogens. A cardinal example, discussed later, is a polymorphism in one of the P-450 genes that confers susceptibility to smoking-induced lung cancer.

KEY CONCEPTS

EPIDEMIOLOGY OF CANCER

- The incidence of cancer varies with geography, age, race, and genetic background. Cancers are most common in adults older than 55 years of age, but occur in adults at all ages and in children and infants. The geographic variation is thought to mainly stem from different environmental exposures.
- Important environmental factors implicated in carcinogenesis include infectious agents, smoking, alcohol, diet, obesity, reproductive history, and exposure to environmental carcinogens.
- The risk of cancer is increased by reparative proliferations caused by chronic inflammation or tissue injury, certain forms of hyperplasia, and immunodeficiency.
- Interactions between environmental factors and genetic factors may be important determinants of cancer risk.

MOLECULAR BASIS OF CANCER: ROLE OF GENETIC AND EPIGENETIC ALTERATIONS

Evidence for the genetic origins of cancer has been building for decades. However, a full accounting of the extent of these genetic aberrations is only now nearing completion, made possible by technologic advances in DNA sequencing and other methods that permit genome-wide analysis of cancer cells. The complexity of these data is daunting, and the messages hidden within them have yet to be fully decoded, but certain "genomic themes" have emerged that are likely relevant to every cancer.

- *Nonlethal genetic damage lies at the heart of carcinogenesis.* The initial damage (or mutation) may be caused by environmental exposures, may be inherited in the germline, or may be spontaneous and random, falling into the category of "bad luck." The term environmental, used in this context, refers to any acquired mutation caused by exogenous agents such as viruses or environmental chemicals or by endogenous products of cellular metabolism that have the potential to damage DNA (such as reactive oxygen species) or alter gene expression through epigenetic mechanisms (e.g., so-called oncometabolites, described later).

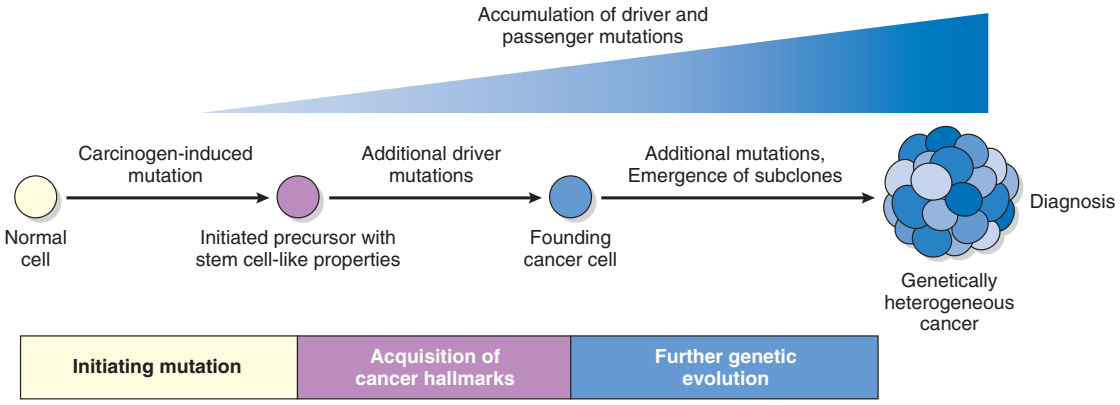

Figure 7.19 Development of a cancer through stepwise acquisition of complementary mutations. The order in which various driver mutations occur in initiated precursor cells is not known and may vary from tumor to tumor. See text for details.

- *A tumor is formed by the clonal expansion of a single precursor cell that has incurred genetic damage (i.e., tumors are clonal).* Alterations in DNA are heritable, being passed to daughter cells, and thus all cells within an individual tumor share the same set of mutations that were present at the moment of transformation. Such tumor-specific mutations are most often identified by DNA sequencing (e.g., point mutations) or by chromosomal analyses (e.g., chromosomal translocations and copy number changes, discussed later).
- *Four classes of genes—growth-promoting proto-oncogenes, growth-inhibiting tumor suppressor genes, genes that regulate programmed cell death (apoptosis), and genes that are responsible for DNA repair—are the principal targets of cancer-causing mutations.* Mutations that activate proto-oncogenes may either cause an increase in one or more normal functions of the encoded gene product that promote tumorigenesis or the appearance of a completely new function that is oncogenic. Because these mutations cause a "gain of function," they can transform cells despite the presence of a normal copy of the same gene. Thus, in genetic parlance, oncogenes are dominant over their normal counterparts. Mutations that affect tumor suppressor genes generally cause a "loss of function," and in most instances both alleles must be damaged before transformation can occur. As a result, mutated tumor suppressor genes usually behave in a recessive fashion. However, there are exceptions: sometimes loss of only a single tumor suppressor gene allele (a state termed haploinsufficiency) reduces the quantity of the encoded protein enough to release the brakes on cell proliferation and survival. Such a finding indicates that two "doses" of the gene are essential for normal function. Apoptosis-regulating genes may acquire abnormalities that result in less cell death and therefore enhanced survival. These abnormalities include gain-of-function mutations in genes whose products suppress apoptosis and loss-of-function mutations in genes whose products promote cell death. Loss-of-function mutations affecting DNA repair genes contribute to carcinogenesis indirectly by impairing the ability of the cell to recognize and repair nonlethal genetic damage in other genes. As a result, affected cells acquire mutations at an accelerated rate, a state referred to as a *mutator phenotype* that is marked by *genomic instability.*
- *Carcinogenesis results from the accumulation of complementary mutations in a stepwise fashion over time* (Fig. 7.19). Malignant neoplasms have several phenotypic attributes referred to as *cancer hallmarks* (discussed in detail later), such as excessive growth, local invasiveness, and the ability to form distant metastases, which stem from genomic alterations that change the expression and function of key genes and thereby impart a malignant phenotype.
  - *Mutations that contribute to the acquisition of cancer hallmarks are referred to as driver mutations.* The first driver mutation that starts a cell on the path to malignancy is the *initiating mutation,* which is typically maintained in all the cells of the subsequent cancer. However, because no single mutation appears to be fully transforming, development of a cancer requires that the "initiated" cell acquire a number of additional driver mutations, each of which also contributes to the development of the cancer. The time over which this occurs is unknown in most cancers, but appears to be lengthy; even in aggressive cancers that clinically seem to appear "out of the blue," such as childhood acute lymphoblastic leukemia, cells bearing initiating mutations may be found in blood samples taken as long as a decade before diagnosis. The persistence of initiated cells during this long preclinical prodrome is consistent with the idea that cancers arise from cells with stem cell–like properties, so-called *cancer stem cells,* that have a capacity for self-renewal and long-term persistence.
  - *Loss-of-function mutations in genes that maintain genomic integrity appear to be a common early step on the road to malignancy, particularly in solid tumors.* Mutations that lead to genomic instability not only increase the likelihood of acquiring driver mutations, but also greatly increase the frequency of mutations that have no phenotypic consequence, so-called *passenger mutations,* which are much more common than driver mutations. As a result, by the time a cell acquires all of the driver mutations that are needed for malignant behavior, it may bear hundreds or even thousands of passenger mutations.

- Mutations in many other genes contribute to tumorigenesis by interfering with host immune responses or altering interactions with the stroma, or by other mechanisms. By convention, these are not classified under driver and passenger mutations, since the terms are largely restricted to genes that influence the behavior of the cells in a cell-intrinsic manner.

Once established, tumors evolve genetically during their outgrowth and progression under the pressure of Darwinian selection (survival of the fittest). Early on, all the cells in a tumor are genetically identical, being the progeny of a single founding transformed cell. However, by the time a tumor comes to clinical attention (generally when it attains a mass of about 1 g, or about 10^9 cells), it has gone through a minimum of 30 cell doublings (this number is actually a substantial underestimation because a fraction of cells in all tumors dies by apoptosis during preclinical stages of tumor development). During the expansion process, individual tumor cells acquire additional mutations at random; this is particularly true in tumors with driver mutations conferring a mutator phenotype. As a result of this tumor evolution, even though cancers are clonal in origin, by the time they become clinically evident their constituent cells are often extremely heterogeneous genetically (see Fig. 7.19). These diverse tumor subclones compete for access to nutrients and microenvironmental niches, and those that are most fit "win" this Darwinian struggle and come to dominate the tumor mass. This pernicious tendency of tumors to become more aggressive over time is referred to as *tumor progression*.

A skeptical student might well ask, "How do we know that genetically distinct subclones really exist in any particular cancer?" Supportive data have emerged from studies of solid cancers such as renal cell carcinoma, in which multiple regions of the primary tumor and metastatic deposits from the same patient have been subjected to DNA sequencing (Fig. 7.20). As predicted, two types of mutations were identified in these studies: (1) mutations that are present in all tumor sites tested, which were presumably present in the founding cell at the moment of transformation, and (2) mutations that are unique to a subset of tumor sites, which were likely acquired after transformation during the outgrowth and spread of the tumor. This second type of mutation can be used to create tumor "family trees" showing the genetic relationships of various subclones. Remarkably, subclones within tumors appear to diverge genetically in a fashion that is very similar to the manner in which new species are thought to emerge in complex ecosystems; a cardinal example of the latter are the finches on the Galapagos Islands that inspired Darwin, in part, to propose evolution as the origin of the species. In the case of species, this genetic divergence occurs over a period of many millennia, whereas in tumors, subclones may arise and diverge on a timescale of years, months, or even weeks.

Selection of the fittest cells can explain not only the natural history of cancer, but also changes in tumor behavior following therapy. One of the most profound selective pressures that cancer cells face is effective therapy given by treating physicians. Tumors that recur after therapy are almost always found to be resistant if the same treatment is given again, presumably because therapy selects for subclones that, by chance, have a genotype that allows them to survive.

In addition to DNA mutations, epigenetic aberrations also contribute to the malignant properties of cancer cells. Epigenetic modifications include DNA methylation, which tends to silence gene expression, and modifications of histones, the proteins that package DNA into chromatin, which depending on their nature may either enhance or dampen gene expression. The epigenetic state of the cell dictates which genes are expressed, which in turn determines the lineage commitment and differentiation state of both normal and neoplastic cells. Epigenetic modifications are

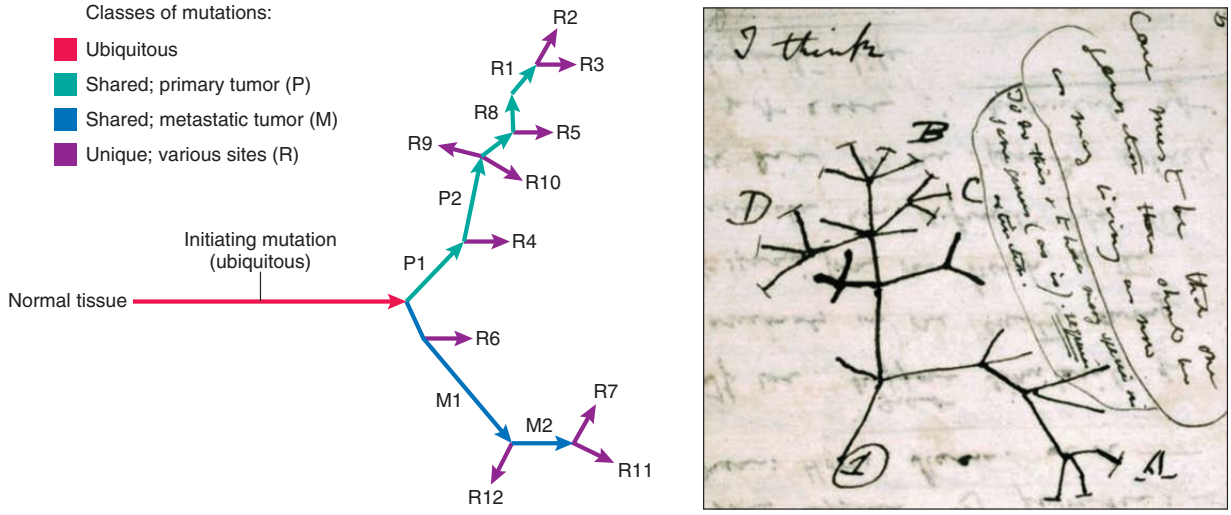

Figure 7.20 Tumor evolution. Evolution of a renal cell carcinoma *(left panel)* and Darwin's finches *(right panel)*. The renal cell carcinoma evolutionary tree is based on genetic comparisons drawn from sequencing of DNA obtained from different tumor sites; the finch evolutionary tree was surmised by Darwin based on morphologic comparisons of different species of finches on the Galapagos Islands. (*right panel*, from Darwin CR: Notebook B: Transmutation of species, 1837–1838, p 26.)

usually passed on faithfully to daughter cells, but on occasion (just as with DNA mutations) alterations may occur that result in changes in gene expression. Aberrant DNA methylation in cancer cells is responsible for the silencing of some tumor suppressor genes, while tumor-specific changes in histone modifications may have far-ranging effects on gene expression (see later). The increasing awareness of the role of epigenetic alterations in cancer has revealed a new path forward for cancer treatment; unlike DNA mutations, epigenetic changes are potentially reversible by drugs that inhibit DNA-modifying or histone-modifying factors. Thus, there is great interest in treating cancers with drugs that correct epigenetic abnormalities.

We will come back to these themes throughout the subsequent discussion, which next turns to the cellular and molecular properties that underlie the malignant behavior of cancer cells.

Cellular and Molecular Hallmarks of Cancer

Over the past several decades, hundreds of genes that are mutated in cancer have been discovered. Traditionally, the functional consequences of these alterations were described one gene at a time. However, the blizzard of mutated genes emerging from the sequencing of cancer genomes has blanketed the landscape and revealed the limitations of trying to grasp the fundamental properties of cancer gene by gene. For example, compilation of a partially complete catalog of recurrent genetic alterations in breast carcinoma required whole genomic sequencing of thousands of tumors and led to the identification of hundreds of distinct driver mutations—and this is just one of hundreds of different kinds of cancer, some of which are substantially more genetically complex than breast carcinoma.

A more tractable and conceptually satisfying way to think about the biology of cancer is to consider the common biologic properties that are imparted to cancer cells by their diverse genomic and epigenomic alterations. It appears that **all cancers display eight fundamental changes in cell physiology, which are considered the hallmarks of cancer.** These changes are illustrated in Fig. 7.21 and consist of the following:

- *Self-sufficiency in growth signals.* Tumors have the capacity to proliferate without external stimuli, usually as a consequence of oncogene activation.
- *Insensitivity to growth-inhibitory signals.* Tumors may not respond to molecules that inhibit the proliferation of normal cells, usually because of inactivation of tumor suppressor genes that encode components of growth inhibitory pathways.
- *Altered cellular metabolism.* Tumor cells undergo a metabolic switch to aerobic glycolysis (called the *Warburg effect*), which enables the synthesis of the macromolecules and organelles that are needed for rapid cell growth.
- *Evasion of apoptosis.* Tumors are resistant to programmed cell death.
- *Limitless replicative potential (immortality).* Tumors have unrestricted proliferative capacity, a stem cell–like property that permits tumor cells to avoid cellular senescence and mitotic catastrophe.
- *Sustained angiogenesis.* Tumor cells, like normal cells, are not able to grow without a vascular supply to bring nutrients and oxygen and remove waste products. Hence, tumors must induce angiogenesis.
- *Ability to invade and metastasize.* Tumor metastases are the cause of the vast majority of cancer deaths and arise from the interplay of processes that are intrinsic to tumor cells and signals that are initiated by the tissue environment.
- *Ability to evade the host immune response.* You will recall that the cells of the innate and adaptive immune system can recognize and eliminate cells displaying abnormal antigens (e.g., a mutated oncoprotein). Cancer cells exhibit a number of alterations that allow them to evade the host immune response.

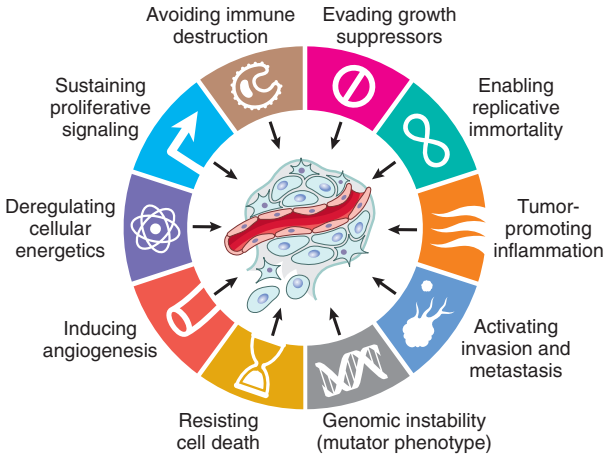

Figure 7.21 Hallmarks of cancer. (Modified from Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation, *Cell* 144:646, 2011.)

The acquisition of the genetic and epigenetic alterations that confer these hallmarks may be accelerated by *genomic instability* and by *cancer-promoting inflammation*. These are considered enabling characteristics because they promote cellular transformation and subsequent tumor progression.

In the following sections, each of the hallmarks and enabling characteristics of cancer cells is discussed, focusing on the most important contributing genes and cellular pathways. The discussion of cancer pathophysiology ends with a review of the roles that epigenetic changes and noncoding RNAs play in the disease.

Self-Sufficiency in Growth Signals: Oncogenes

Oncogenes are mutated genes that cause excessive cell growth, even in the absence of growth factors and other growth-promoting external cues. A major discovery in cancer was that oncogenes are mutated or overexpressed versions of normal cellular genes, which are called proto-oncogenes. Through a variety of mechanisms, discussed later, these mutations increase or alter the function of oncoproteins, which are constitutively active and resistant to control by external signals. Cells expressing oncoproteins are thus freed from normal checkpoints and proliferate excessively.

To aid in the understanding of the nature and functions of oncoproteins and their role in cancer, it is necessary to briefly describe how normal cells respond to growth factors.

Physiologic growth factor–induced signaling can be resolved into the following steps:

- The binding of a growth factor to its specific receptor
- Transient and limited activation of the growth factor receptor, which in turn activates several cytoplasmic signal-transducing proteins
- Transmission of the transduced signal to the nucleus via additional cytoplasmic effector proteins and second messengers or by a cascade of signal transduction molecules
- Induction and activation of transcription factors and epigenetic alterations that initiate and sustain DNA transcription
- Expression of genes and encoded factors that promote entry and progression of the cell into the cell cycle, ultimately resulting in cell division
- In parallel, changes in the expression of other genes that support cell survival and metabolic alterations that are needed for optimal growth

Aberrations in multiple signaling pathways have been identified in neoplasms, and many components of these pathways act as oncoproteins when mutated (Table 7.5). Conversely, many tumor suppressors act by inhibiting one or more components of these same pro-growth pathways (discussed later). In Chapter 1, the major signaling pathways

Table 7.5 Selected Oncogenes, Their Mode of Activation, and Associated Human Tumors

Category	Proto-Oncogene	Mode of Activation in Tumor	Associated Human Tumor
Growth Factors			
PDGF-β	*PDGFB*	Overexpression	Astrocytoma
Fibroblast growth factors	*HST1* *FGF3*	Overexpression Amplification	Osteosarcoma Stomach cancer Bladder cancer Breast cancer Melanoma
TGF-α	*TGFA*	Overexpression	Astrocytomas
HGF	*HGF*	Overexpression	Hepatocellular carcinomas Thyroid cancer
Growth Factor Receptors			
EGF-receptor family	*ERBB1 (EGFR)* *ERBB2 (HER)*	Mutation Amplification	Adenocarcinoma of lung Breast carcinoma
FMS-like tyrosine kinase 3	*FLT3*	Point mutation or small duplications	Leukemia
Receptor for neurotrophic factors	*RET*	Point mutation	Multiple endocrine neoplasia 2A and B, familial medullary thyroid carcinomas
PDGF receptor	*PDGFRB*	Amplification, translocation	Gliomas, leukemias
Receptor for KIT ligand	*KIT*	Point mutation	Gastrointestinal stromal tumors, seminomas, leukemias
ALK receptor	*ALK*	Translocation Point mutation	Adenocarcinoma of lung, certain lymphomas Neuroblastoma
Proteins Involved in Signal Transduction			
GTP-binding (G) proteins	*KRAS* *HRAS* *NRAS* *GNAQ* *GNAS*	Point mutation Point mutation Point mutation Point mutation Point mutation	Colon, lung, and pancreatic tumors Bladder and kidney tumors Melanomas, hematologic malignancies Uveal melanoma Pituitary adenoma, other endocrine tumors
Nonreceptor tyrosine kinase	*ABL*	Translocation	Chronic myelogenous leukemia Acute lymphoblastic leukemia
RAS signal transduction	*BRAF*	Point mutation	Melanomas, leukemias, colon carcinoma, others
Notch signal transduction	*NOTCH1*	Point mutation, translocation	Leukemias, lymphomas, breast carcinoma
JAK/STAT signal transduction	*JAK2*	Point mutation, translocation	Myeloproliferative disorders Acute lymphoblastic leukemia
Nuclear Regulatory Proteins			
Transcriptional activators	*MYC* *NMYC*	Translocation Amplification	Burkitt lymphoma Neuroblastoma
Cell Cycle Regulators			
Cyclins	*CCND1* (cyclin D1)	Translocation Amplification	Mantle cell lymphoma, multiple myeloma Breast and esophageal cancers
Cyclin-dependent kinase	*CDK4*	Amplification or point mutation	Glioblastoma, melanoma, sarcoma

that regulate cellular behavior are laid out, including the receptor tyrosine kinase pathway, the G protein–coupled receptor pathway, the JAK/STAT pathway, the WNT pathway, the Notch pathway, the Hedgehog pathway, the TGF-β/SMAD pathway, and the NF-κB pathway. Abnormalities in each of these pathways are implicated in the development and progression of various cancers.

Traditionally, discussion of oncoproteins and tumor suppressors has centered on their ability to accelerate or inhibit, respectively, DNA replication and cell cycle progression. This view has merit, and we will follow it in our initial description of their activities. However, the proliferation of cells requires not only DNA replication but also sufficient biosynthesis of membrane, protein, and various macromolecules and organelles to enable a "mother" cell to divide and produce two complete daughter cells. Cell growth pathways implicated in oncogenesis also initiate signals that promote and coordinate the biosynthesis of all essential cellular components (discussed later). This insight has generated interest in therapeutic targeting of many aspects of oncogenic pro-growth signaling including the altered cellular metabolism that is characteristic of cancer cells.

Building on this framework, we next discuss some of the most important oncoproteins and the mechanisms by which they contribute to the autonomous growth of cancer cells.

Oncoproteins and Cell Growth

Oncogenes have multiple roles, but virtually all encode constitutively active oncoproteins that participate in signaling pathways that drive the proliferation of cells. Thus proto-oncogenes, the normal regulated versions of oncogenes, may encode growth factors, growth factor receptors, signal transducers, transcription factors, or cell cycle components. In most instances, the corresponding oncogenes encode oncoproteins that serve functions similar to their normal counterparts, with the important difference that they are usually constitutively active and thereby relieve cells of their normal dependency on growth factors.

In the following sections, we "walk down" a prototypical growth factor signaling pathway from the membrane to the nucleus (Fig. 7.22), discussing at each step along the way some of the genes and factors that are most commonly dysregulated (and therefore most important) in cancer.

Growth Factors. Most growth factors are made by one cell type and act on a neighboring cell of a differing type expressing the appropriate growth factor receptor (paracrine action). Some cancer cells, however, synthesize the same growth factor to which they are responsive, creating an autocrine loop. For example, brain tumors called *glioblastomas* (Chapter 28) often express both platelet-derived growth factor (PDGF) and PDGF receptor (PDGFR), and many sarcomas overexpress transforming growth factor α (TGF-α) and its receptor, epidermal growth factor receptor (EGFR).

Growth Factor Receptors. A large number of oncogenes encode growth factor receptors, of which receptor tyrosine kinases are arguably the most important in cancer. Recall that receptor tyrosine kinases are transmembrane proteins with an extracellular growth factor–binding domain and a cytoplasmic tyrosine kinase domain (Chapter 1). Normally the receptor is activated transiently by binding of a specific growth factor, an event that induces a rapid change in receptor conformation to an active dimeric state. The activated receptor then autophosphorylates tyrosine residues in its own intracellular tail, and these modified residues serve as sites for recruitment of other signaling molecules including RAS and PI3K, key players in receptor tyrosine kinase signaling (described later). The oncogenic versions of these receptors are associated with mutations that lead to constitutive, growth factor–independent tyrosine kinase activity. Hence, the mutant receptors deliver mitogenic signals to the cell continuously, even in the absence of growth factor in the environment.

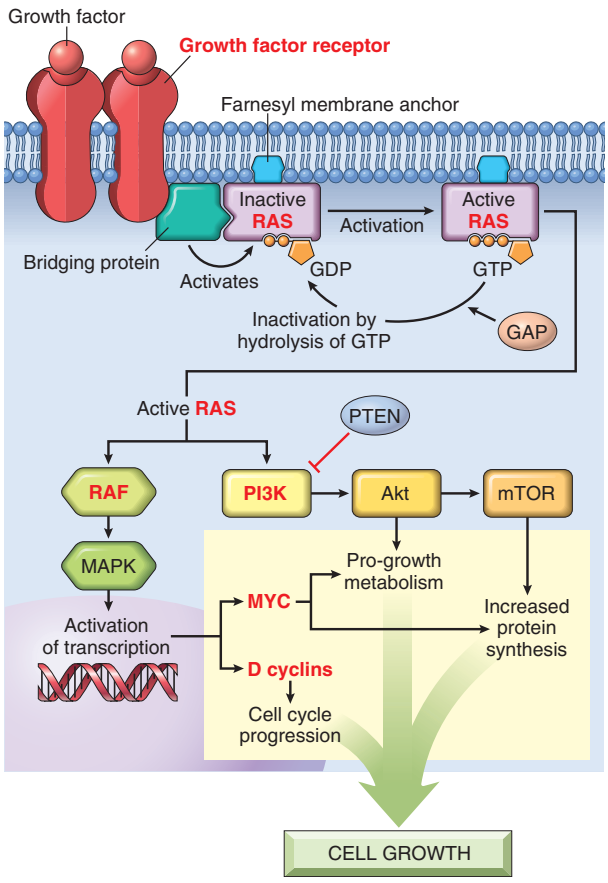

Figure 7.22 Growth factor signaling pathways in cancer. Growth factor receptors RAS, PI3K, MYC, and D cyclins are oncoproteins that are activated by mutations in various cancers. GTPase-activating proteins *(GAPs)* apply brakes to RAS activation, and phosphatase and tensin homologue *(PTEN)* serves the same function for PI3K. *GDP,* Guanosine diphosphate; *GTP,* guanosine triphosphate.

Receptor tyrosine kinases are constitutively activated in tumors by multiple mechanisms including point mutations, gene rearrangements, and gene amplifications. A few of the best-characterized oncogenic mutations involving growth factor receptors are listed in Table 7.5; the following are salient examples of particular clinical importance.

- *ERBB1* encodes EGFR. Several different *ERBB1* point mutations found in a subset of lung adenocarcinomas produce constitutive activation of the EGFR tyrosine kinase.
- *ERBB2* encodes a different member of the receptor tyrosine kinase family, HER2. Rather than being activated by point mutations, the *ERBB2* gene is amplified in certain breast carcinomas, leading to overexpression of the HER2 receptor and constitutive tyrosine kinase activity.
- *ALK* is a receptor tyrosine kinase that may be produced in a constitutively active form as a result of a gene rearrangement. For example, in a subset of lung adenocarcinomas, a deletion on chromosome 5 fuses part of the *ALK* gene with part of another gene called *EML4*. The resulting *EML4-ALK* fusion gene encodes a chimeric EML4-ALK protein with constitutive tyrosine kinase activity.

The importance of these mutated receptor tyrosine kinases has been proven in no small part by the therapeutic effectiveness of agents that inhibit their enzymatic activities. Breast cancers with *ERBB2* amplification and overexpression of HER2 respond well to antibodies or drugs that inhibit HER2 activity. These inhibitors not only block tumor growth but also induce apoptosis and tumor regression, reflecting the ability of receptor tyrosine kinase signaling to augment cell survival as well as proliferation. Inhibitors of EGFR and ALK produce similar therapeutic responses in patients with lung adenocarcinomas harboring *ERBB1* mutations or *EML4-ALK* fusion genes, respectively.

Unfortunately, these targeted therapies are usually not curative in advanced cancers. The tumor cells that withstand therapy typically are found to have other acquired mutations that sidestep the effects of the drug. For example, lung cancers that develop resistance to EGFR inhibitors often have mutations in EGFR that prevent inhibitors from binding, or amplifications in a gene called *MET,* which encodes yet another receptor tyrosine kinase. This experience highlights one of the most daunting clinical problems in the treatment of advanced cancers—the presence of subclones within the genetically heterogeneous tumor cell population that afford resistance to targeted therapies.

Downstream Components of the Receptor Tyrosine Kinase Signaling Pathway. As mentioned, receptor tyrosine kinase activation stimulates RAS and two major downstream signaling "arms," the MAPK cascade and the PI3K/AKT pathway. In line with the importance of these pathways in mediating cell growth, RAS, PI3K, and other components of these pathways are frequently involved by gain-of-function mutations in different types of cancer. Of interest, when *RAS* mutations are present in a tumor, activating mutations in receptor tyrosine kinases are almost always absent, at least within the dominant tumor clone, implying that in such tumors activated RAS can completely substitute for tyrosine kinase activity. Thus, lung adenocarcinomas fall into mutually exclusive molecular subtypes that are associated with mutations involving *RAS* or various tyrosine kinase genes, an insight that has important implications for targeted therapies in this type of cancer.

Point mutations of *RAS* family genes constitute the most common type of abnormality involving proto-oncogenes in human tumors. The RAS genes, of which there are three in humans (*HRAS*, *KRAS*, and *NRAS*), were discovered initially within the genomes of transforming retroviruses. Approximately 15% to 20% of all human tumors have RAS mutations, but in some types of cancers the frequency of RAS mutations is much higher. For example, 90% of pancreatic adenocarcinomas contain RAS mutations, as do about 50% of colon, endometrial, and thyroid cancers and 30% of lung adenocarcinomas and myeloid leukemias.

Recall that RAS proteins are members of a family of membrane-associated small G proteins that bind guanosine nucleotides (guanosine triphosphate [GTP] and guanosine diphosphate [GDP]), similar to the larger trimolecular G proteins. RAS normally flips back and forth between an excited signal-transmitting state in which it is bound to GTP and a quiescent state in which it is bound to GDP. Stimulation of receptor tyrosine kinases by growth factors leads to exchange of GDP for GTP and subsequent conformational changes that generate active RAS. Activation of RAS is transient because RAS has an intrinsic GTPase activity that is accelerated by GTPase-activating proteins (GAPs), which bind to active RAS and augment its GTPase activity by more than 1000-fold, thereby terminating signal transduction. Thus, GAPs prevent uncontrolled RAS activity.

Several distinct *RAS* point mutations have been identified in cancer cells that markedly reduce the GTPase activity of the RAS protein. These mutated forms of RAS are trapped in the activated GTP-bound form, and as a result the cell receives pro-growth signals continuously. It follows from this scenario that the consequences of gain-of-function mutations in RAS proteins should be mimicked by loss-of-function mutations in GAPs that normally restrain RAS activity. Indeed, disabling mutations of neurofibromin 1, a GAP encoded by the *NF1* gene, are associated with the inherited cancer syndrome *familial neurofibromatosis type 1* (Chapter 25). *NF1* is therefore an example of a tumor suppressor gene that acts through negative regulation of RAS signaling.

The MAPK and PI3K/AKT cascades both lie downstream of RAS and consist of a series of kinases, many of which are mutated in cancer cells. Components positioned close to the top of each cascade are frequently involved by oncogenic gain-of-function mutations in various cancers, as follows:

- *Mutations in BRAF,* a member of the RAF family of serine/threonine protein kinases, are found in close to 100% of hairy cell leukemias, 60% of melanomas, and a smaller percentage of a wide variety of other neoplasms including colon carcinomas. Like activating RAS mutations, activating mutations in BRAF stimulate downstream kinases and ultimately activate transcription factors. Mutations in other MAPK family members downstream of BRAF are less common in cancer, suggesting mutations affecting factors near the top of the cascade are most effective at producing pro-growth effects.
- *Mutations in kinases of the PI3K family* are also very common in certain cancers. For example, about 30% of breast carcinomas have PI3K gain-of-function mutations. In other instances, PI3K is "unbridled" by loss-of-function mutations in its negative regulator, called PTEN, a tumor suppressor that is commonly mutated in endometrial carcinoma. Under normal circumstances, following

receptor tyrosine kinase activation, PI3K is recruited to plasma membrane–associated protein complexes. Here, like BRAF, it activates a cascade of serine/threonine kinases, including AKT. AKT phosphorylates more than 150 proteins and constitutes a major signaling node. Its substrates include key regulators of protein synthesis (mTOR) and apoptosis (BAD, FOXO transcription factors, MDM2, and IAP, all described elsewhere).

Because RAS proteins are so frequently mutated in cancer, much effort has been spent trying to develop drugs that inhibit RAS. Unfortunately, none of these strategies has been successful, in large part because what is required is the restoration of a missing enzymatic activity (GTPase activity), an effect that is generally difficult to achieve with drugs. In contrast, treatment of patients with advanced melanomas with BRAF inhibitors has produced striking clinical responses. Such responses are strictly limited to tumors with *BRAF* mutations since these are dependent on BRAF signaling, whereas melanomas with wild-type *BRAF* genes do not respond. This phenomenon, termed *oncogene addiction* (described below), highlights the need for molecular analysis to guide appropriate therapy. Multiple drugs that inhibit various PI3K isoforms have also been developed, and some are now approved for treatment of particular cancers.

Nonreceptor Tyrosine Kinases. Oncogenic mutations also occur in several nonreceptor tyrosine kinases that normally localize to the cytoplasm or the nucleus. In many instances the mutations take the form of chromosomal translocations or rearrangements that create fusion genes encoding constitutively active tyrosine kinases. Despite their nonmembranous localization, these oncoproteins seem to activate the same signaling pathways as receptor tyrosine kinases. An important example of this oncogenic mechanism involves the ABL tyrosine kinase. In chronic myeloid leukemia (CML) and a subset of acute lymphoblastic leukemia, the *ABL* gene is translocated from its normal abode on chromosome 9 to chromosome 22 (Fig. 7.23), where it fuses with the *BCR* gene (see discussion of chromosomal translocations later in this chapter). The resultant fusion gene encodes a chimeric BCR-ABL protein with constitutive tyrosine kinase activity. The most important contribution of the BCR moiety is to promote self-association of BCR-ABL, which appears to be sufficient to unleash the tyrosine kinase activity of ABL. This represents a recurrent story in cancer, as many different oncogenic tyrosine kinases consist of chimeric proteins in which the non–tyrosine kinase partner drives self-association.

Treatment of CML has been revolutionized by the development of BCR-ABL kinase inhibitors, another example of rational drug design emerging from an understanding of the molecular basis of cancer. The remarkable therapeutic response of CML to BCR-ABL inhibitors is one of the first and best examples of *oncogene addiction*, in which tumor cells are highly dependent on the activity of one oncoprotein. Despite accumulation of mutations in other cancer-associated genes in CML cells, signaling through the BCR-ABL tyrosine kinase is required for most CML tumor cells to proliferate and survive; hence, inhibition of its activity is a highly effective therapy. The presence of a *BCR-ABL* fusion gene defines CML and must be the initiating event in this disease;

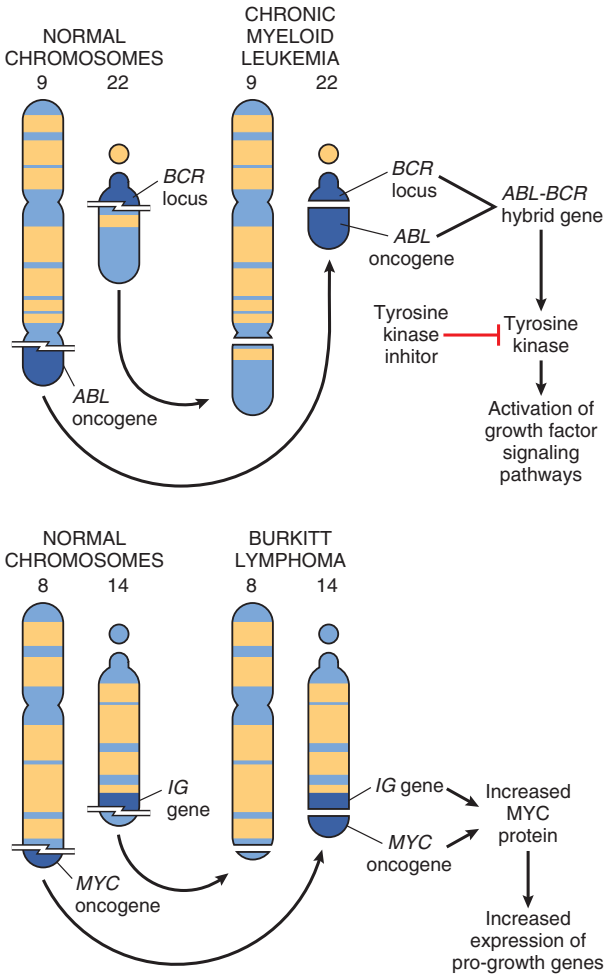

Figure 7.23 Chromosomal translocation and associated oncogenes in Burkitt lymphoma and chronic myeloid leukemia.

thus, additional mutations acquired by the founding clone are selected for their ability to complement the effects of incessant BCR-ABL signaling. BCR-ABL can be seen as the central lodgepole around which an oncogenic signaling "structure" is built. If the lodgepole is removed by treatment with BCR-ABL kinase inhibitors, the entire structure collapses. Unfortunately, treatment of this "addiction" to BCR-ABL does not lead to cure. Even though the proliferating component of the tumor is suppressed by BCR-ABL inhibitors and the patient seems completely well, rare CML "stem cells" harboring the *BCR-ABL* fusion gene persist, apparently because these cells do not require BCR-ABL signals for their survival. As a result, therapy with BCR-ABL inhibitors must be continued indefinitely; otherwise, the malignant stem cells spawn rapidly proliferating offspring, and the full-blown leukemia returns. This outcome highlights a second important concept that we will return to: the existence of "stem-like" cells in certain cancers that may be particularly resistant to therapeutic targeting.

In other instances, nonreceptor tyrosine kinases are activated by point mutations that abrogate the function of negative autoregulatory domains that normally hold enzyme

activity in check. An example of this type of mutation is found in the nonreceptor tyrosine kinase JAK2. JAK2 participates in the JAK/STAT signaling pathway, which transduces mitogenic signals from growth factor and cytokine receptors that lack tyrosine kinase activity (as described in Chapter 1). JAK/STAT activation alters the expression of target genes that bind STAT transcription factors. Several myeloid neoplasms are frequently associated with activating point mutations in JAK2 that relieve the tumor cells of their normal dependence on hematopoietic growth factors such as erythropoietin (Chapter 13). Recognition of this molecular lesion has led to the clinical development of JAK2 inhibitors and has stimulated searches for activating mutations in other nonreceptor tyrosine kinases.

Transcription Factors. Just as all roads lead to Rome, all signal transduction pathways converge on the nucleus, where the expression of target genes that orchestrate the cell's orderly advance through the cell cycle is activated. Indeed, the ultimate consequence of deregulated mitogenic signaling pathways is inappropriate and continuous stimulation of nuclear transcription factors that drive growth-promoting genes. Thus not surprisingly, growth autonomy may also occur as a consequence of mutations affecting transcription factors that regulate the expression of pro-growth genes and cyclins. Transcription factors of this class include the products of the *MYC, MYB, JUN, FOS,* and *REL* proto-oncogenes. Of these, *MYC* is most commonly affected in cancer, and hence a brief overview of its regulation and function follows.

MYC. The *MYC* proto-oncogene is expressed in virtually all eukaryotic cells and belongs to the immediate early response genes, which are rapidly and transiently induced by RAS/MAPK signaling following growth factor stimulation of quiescent cells. Under normal circumstances, MYC protein concentrations are tightly controlled at the level of transcription, translation, and protein stability, and virtually all pathways that regulate growth impinge on MYC through one or more of these mechanisms.

How MYC promotes normal and neoplastic cell growth is incompletely understood, but a multitude of studies have shown that **MYC has remarkably broad activities, several of which contribute not only to deregulated cell growth but also to several other hallmarks of cancer**.

- *MYC activates the expression of many genes that are involved in cell growth.*
  - Some MYC target genes, like D cyclins, are directly involved in cell cycle progression.
  - MYC also upregulates the expression of ribosomal RNA (rRNA) genes and rRNA processing, thereby enhancing the assembly of ribosomes needed for protein synthesis.
  - MYC upregulates a program of gene expression that leads to metabolic reprogramming and the Warburg effect, another cancer hallmark (discussed later). Among the genes involved in metabolism that are upregulated by MYC are multiple glycolytic enzymes and factors involved in glutamine metabolism, both of which contribute to the generation of metabolic intermediates that are needed for synthesis of macromolecules such as DNA, proteins, and lipids.
  - Based on these protean effects, MYC can be considered a master transcriptional regulator of cell growth. Indeed, the fastest growing human tumors, such as Burkitt lymphoma, which virtually always bears a chromosomal translocation involving *MYC* (see Fig. 7.23), are those with the highest levels of MYC.
- *In some contexts, MYC upregulates expression of telomerase.* As discussed later, telomerase is one of several factors that contribute to the endless replicative capacity (the immortalization) of cancer cells.
- *MYC is one of a handful of transcription factors that can act together to reprogram somatic cells into pluripotent stem cells* (Chapter 1). This capacity has led to suspicions that MYC also contributes to cancer cell "stemness," another important aspect of the immortality of cancers.

Given the importance of MYC in regulation of cell growth, it should come as no surprise that it is deregulated in cancer through a large variety of mechanisms. Sometimes deregulation involves genetic alterations of *MYC* itself. In Burkitt lymphoma and a subset of other B- and T-cell tumors, the *MYC* gene is translocated into an antigen receptor gene locus, which contains gene regulatory elements called enhancers that are highly active in lymphocytes. Rather than driving the expression of B- or T-cell receptors, these misplaced enhancers instead cause the deregulation and overexpression of MYC protein. Alternatively the *MYC* gene is amplified in cancers of the breast, colon, lung, and other tissues, again resulting in overexpression of MYC. The functionally identical *NMYC* and *LMYC* genes are also amplified in neuroblastomas (Fig. 7.24) and small cell cancers of the lung, respectively. In many other instances, oncogenic

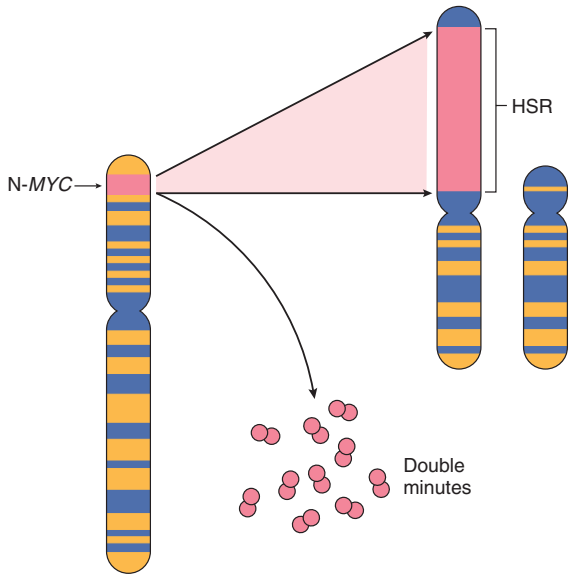

Figure 7.24 Amplification of the *NMYC* gene in human neuroblastomas. The *NMYC* gene, normally present on chromosome 2p, becomes amplified and is seen either as extra chromosomal double minutes or as a chromosomally integrated, homogeneous staining region *(HSR)*. The integration involves other autosomes such as 4, 9, or 13. (Modified from Brodeur GM: Molecular correlates of cytogenetic abnormalities in human cancer cells: implications for oncogene activation. In Brown EB, editor: *Progress in Hematology,* vol 14, Orlando, Fla, 1986, Grune & Stratton, pp 229–256.)

mutations involving components of upstream signaling pathways elevate MYC protein levels by increasing *MYC* transcription, enhancing *MYC* messenger RNA (mRNA) translation, and/or stabilizing MYC protein. Thus, constitutive RAS/MAPK signaling (many cancers), Notch signaling (several cancers), Wnt signaling (colon carcinoma), and Hedgehog signaling (medulloblastoma) all transform cells in part through upregulation of *MYC*. Finally, several single nucleotide polymorphisms (SNPs) associated with an inherited risk for cancers such as prostate and ovarian carcinoma and certain leukemias lie within enhancer elements that flank *MYC;* these variants appear to stimulate higher levels of *MYC* RNA expression in response to growth-promoting signals. Thus, there is seemingly no end to the ways in which MYC may be deregulated in cancer cells.

Cyclins and Cyclin-Dependent Kinases. As mentioned in Chapter 1, growth factors transduce signals that stimulate the orderly progression of cells through the various phases of the cell cycle, the process by which cells replicate their DNA in preparation for cell division. Progression of cells through the cell cycle is orchestrated by cyclin-dependent kinases (CDKs), which are activated by binding to cyclins, so called because of the cyclic nature of their production and degradation. The CDK-cyclin complexes phosphorylate crucial target proteins that drive cells forward through the cell cycle. While cyclins arouse the CDKs, CDK inhibitors, of which there are many, silence the CDKs and exert negative control over the cell cycle (Table 7.6). Expression of these inhibitors is downregulated by mitogenic signaling pathways, thus promoting the progression of the cell cycle.

There are two main cell cycle checkpoints, one at the G_1/S transition and the other at the G_2/M transition, both of which are tightly regulated by a balance of growth-promoting and growth-suppressing proteins, as well as by sensors of DNA damage (Chapter 1). If activated, these DNA-damage sensors transmit signals that arrest cell cycle progression and, if the damage cannot be repaired, initiate apoptosis. Understandably, defects in the G_1/S checkpoint are more important in cancer because these not only lead to dysregulated growth but may also impair DNA repair, creating a "mutator" phenotype that (as mentioned) enables cancer development and progression.

The major cancer-associated mutations that affect the G_1/S checkpoint can be broadly grouped into two classes.

- *Gain-of-function mutations in D cyclin genes and CDK4, which promote unregulated G_1/S progression and thus function as oncogenes.* There are three D cyclin genes, D1, D2, and D3, which are functionally interchangeable and often dysregulated by acquired mutations in cancer, including chromosomal translocations in lymphoid tumors and gene amplification in a variety of solid tumors. Amplification of the *CDK4* gene also occurs in melanomas, sarcomas, and glioblastomas. CDK4 inhibitors are effective in treatment of advanced breast cancers associated with excessive CDK4 activity. Mutations affecting other CDKs and cyclin E also occur in cancers, but they are infrequent, presumably because these factors are less important in control of the G_1/S transition, which has a preeminent role in regulating tumor growth rates.
- *Loss-of-function mutations in genes that inhibit G_1/S progression.* Examples of these tumor suppressor genes are those encoding CDK inhibitors, which inhibit cyclin D/CDK complexes and are frequently mutated or otherwise silenced in many human malignancies. For example, germline mutations of *p16 (CDKN2A)* are present in 25% of melanoma-prone kindreds, and somatically acquired deletion or inactivation of p16 is seen in 75% of pancreatic carcinomas, 40% to 70% of glioblastomas, 50% of esophageal cancers, 20% to 70% of acute lymphoblastic leukemias, and 20% of non–small cell lung carcinomas, soft tissue sarcomas, and bladder cancers. Furthermore, the two most important tumor suppressor genes, *RB* and *TP53,* both encode proteins that inhibit G_1/S progression.

Table 7.6 Cell Cycle Components and Inhibitors That Are Frequently Mutated in Cancer

Cell Cycle Component	Main Function
Cyclins and Cyclin-Dependent Kinases	
CDK4; D cyclins	Form a complex that phosphorylates RB, allowing the cell to progress through the G_1 restriction point
Cell Cycle Inhibitors	
CIP/KIP family: p21, p27 (CDKN1A–D)	Block the cell cycle by binding to cyclin-CDK complexes p21 is induced by tumor suppressor p53 p27 responds to growth suppressors such as TGF-β
INK4/ARF family (CDKN2A–C)	p16/INK4a binds to cyclin D–CDK4 and promotes the inhibitory effects of RB p14/ARF increases p53 levels by inhibiting MDM2 activity
Cell Cycle Checkpoint Components	
RB	Tumor suppressive "pocket" protein that binds E2F transcription factors in its hypophosphorylated state, preventing G_1/S transition Interacts with transcription factors that regulate differentiation
p53	Tumor suppressor altered in the majority of cancers Induced by DNA damage Causes cell cycle arrest by upregulating the CDK inhibitor p21 Induces apoptosis by upregulating BAX and other pro-apoptotic genes

KEY CONCEPTS

ONCOGENES, ONCOPROTEINS, AND UNREGULATED CELL PROLIFERATION

Proto-oncogenes: normal cellular genes whose products promote cell proliferation.

Oncogenes: mutated or overexpressed versions of proto-oncogenes that function autonomously, having lost dependence on normal growth-promoting signals.

Oncoprotein: a protein encoded by an oncogene that drives increased cancer cell proliferation, which may result from a variety of aberrations.

- Constitutive expression of growth factors and their cognate growth factor receptors, setting up an autocrine cell signaling loop.
- Mutations in growth factor receptors, nonreceptor tyrosine kinases, or downstream signaling molecules that lead to constitutive signaling, such as:
  - Activation of the EGF receptor tyrosine kinase by point mutations (lung cancer), activation of the HER2 receptor tyrosine kinase by gene amplification (breast cancer), and activation of the JAK2 tyrosine kinase by point mutations (myeloproliferative neoplasms).
  - Activation of the ABL nonreceptor tyrosine kinase by chromosomal translocation and creation of a *BCR-ABL* fusion gene (chronic myeloid leukemia, acute lymphoblastic leukemia).
  - Activation of RAS by point mutations (many cancers).
  - Activation of PI3K and BRAF serine/threonine kinases by point mutations (many cancers).
  - Increased expression of MYC, a master transcription factor that regulates genes needed for rapid cell growth by deregulation through chromosomal translocations (Burkitt lymphoma, other hematologic malignancies), gene amplication (neuroblastoma), and increased activity of upstream signaling pathways (many cancers).
  - Mutations that increase the activity of cyclin-dependent kinase 4 (CDK4)/D cyclin complexes, which promote cell cycle progression.

Insensitivity to Growth Inhibition: Tumor Suppressor Genes

Whereas oncogenes drive the proliferation of cells, the products of most tumor suppressor genes apply brakes to cell proliferation, and abnormalities in these genes lead to failure of growth inhibition, another fundamental hallmark of carcinogenesis. Tumor suppressor proteins control a series of checkpoints that prevent uncontrolled growth. Many tumor suppressors, such as RB and p53, are part of a regulatory network that recognizes genotoxic stress from any source and responds by shutting down proliferation. Indeed, expression of an oncogene in normal cells with intact tumor suppressor genes leads to quiescence or permanent cell cycle arrest (oncogene-induced senescence, discussed later), rather than uncontrolled proliferation. Ultimately, the growth inhibitory pathways may prod the cells into apoptosis. Another set of tumor suppressors seems to be involved in cell differentiation, causing cells to enter a postmitotic, differentiated pool without replicative potential. Similar to mitogenic signals, signals that induce growth inhibition and differentiation originate outside the cell and use receptors, signal transducers, and nuclear transcription regulators to accomplish their effects; tumor suppressors form a portion of these networks. Thus the protein products of tumor suppressor genes may function as transcription factors, cell cycle inhibitors, signal transduction molecules, cell surface receptors, and regulators of cellular responses to DNA damage.

In this section, we describe tumor suppressor genes, their products, and mechanisms by which loss of their function contributes to unregulated cell growth (Table 7.7). Many of our current concepts of tumor suppressors evolved from studies of the retinoblastoma (*RB*) gene, the first tumor suppressor gene discovered, which remains a prototype of genes of this type. Like many discoveries in medicine, *RB* was identified by studying a rare inherited disease, familial retinoblastoma. Approximately 40% of retinoblastomas are familial, with the predisposition to develop the tumor being transmitted as an autosomal dominant trait. Carriers of this trait have a 10,000-fold increased risk of developing retinoblastoma (often in both eyes) compared with the general population and are at greatly increased risk of developing osteosarcoma and other soft tissue sarcomas. The remaining 60% of retinoblastomas occur sporadically (virtually always in only one eye), and such patients are not at increased risk for other forms of cancer. To explain these two patterns of occurrence of retinoblastoma, Knudson proposed his now canonic "two-hit" hypothesis of oncogenesis. In molecular terms, Knudson's hypothesis can be stated as follows (Fig. 7.25):

- Two mutations (hits), involving both alleles of *RB* are required to produce retinoblastoma.
- In familial cases, children inherit one defective copy of *RB* (the first hit) and one normal copy of *RB* in the germline. Retinoblastoma develops when the normal *RB* allele is mutated in retinoblasts as a result of a spontaneous somatic mutation (the second hit). Because second hits seem to be virtually inevitable in a small fraction of retinoblasts, most individuals inheriting a germline defect in one *RB* allele develop unilateral or bilateral retinoblastoma, and the disease is inherited as an autosomal dominant trait.
- In sporadic cases both normal *RB* alleles must undergo somatic mutation in the same retinoblast (two hits). The probability of this event is low (explaining why retinoblastoma is uncommon in the general population), but the end result is the same: a retinal cell loses *RB* function and becomes cancerous.

A child carrying an inherited mutant *RB* allele in all somatic cells is perfectly normal (except for the increased risk of developing cancer); it follows that one defective *RB* gene does not have adverse effects on cell behavior. Thus, although the genetic trait (increased cancer risk) associated with germline *RB* mutations is inherited in an autosomal dominant fashion, at the level of individual cells the phenotype associated with *RB* loss of function behaves like a recessive trait.

Following the identification of *RB,* a large number of other tumor suppressor genes were discovered, often through study of other types of familial cancer. In general, the major themes that emerged from the study of familial retinoblastoma hold for other familial cancers: the risk of cancer is inherited as an autosomal dominant trait due to a germline mutation in a tumor suppressor gene; tumors have second "hits" in the sole normal tumor suppressor gene allele; and the same tumor suppressor gene is frequently mutated in sporadic tumors of the same type.

Some of the most important tumor suppressor genes, their associated familial syndromes, and their normal

Table 7.7 Selected Tumor Suppressor Genes and Associated Familial Syndromes and Cancers, Sorted by Cancer Hallmarks[a]

Gene	Protein	Function	Familial Syndromes	Sporadic Cancers
Inhibitors of Mitogenic Signaling Pathways				
APC	Adenomatous polyposis coli protein	Inhibitor of WNT signaling	Familial colonic polyps and carcinomas	Carcinomas of stomach, colon, pancreas; melanoma
NF1	Neurofibromin-1	Inhibitor of RAS/MAPK signaling	Neurofibromatosis type 1 (neurofibromas and malignant peripheral nerve sheath tumors)	Neuroblastoma, juvenile myeloid leukemia
NF2	Merlin	Cytoskeletal stability, Hippo pathway signaling	Neurofibromatosis type 2 (acoustic schwannoma and meningioma)	Schwannoma, meningioma
PTCH	Patched	Inhibitor of Hedgehog signaling	Gorlin syndrome (basal cell carcinoma, medulloblastoma, several benign tumors)	Basal cell carcinoma, medulloblastoma
PTEN	Phosphatase and tensin homologue	Inhibitor of PI3K/AKT signaling	Cowden syndrome (variety of benign skin, GI, and CNS growths; breast, endometrial, and thyroid carcinoma)	Diverse cancers, particularly carcinomas and lymphoid tumors
SMAD2, SMAD4	SMAD2, SMAD4	Component of the TGF-β signaling pathway, repressors of MYC and CDK4 expression, inducers of CDK inhibitor expression	Juvenile polyposis	Frequently mutated (along with other components of the TGF-β signaling pathway) in colonic and pancreatic carcinoma
Inhibitors of Cell Cycle Progression				
RB	Retinoblastoma (RB) protein	Inhibitor of G_1/S transition during cell cycle progression	Familial retinoblastoma syndrome (retinoblastoma, osteosarcoma, other sarcomas)	Retinoblastoma; osteosarcoma; carcinomas of breast, colon, lung
CDKN2A	p16/INK4a and p14/ARF	p16: Negative regulator of cyclin-dependent kinases; p14, indirect activator of p53	Familial melanoma	Pancreatic, breast, and esophageal carcinoma; melanoma; certain leukemias
Inhibitors of Pro-growth Programs of Metabolism and Angiogenesis				
VHL	von Hippel–Lindau (VHL) protein	Inhibitor of hypoxia-induced transcription factors (e.g., HIF1α)	von Hippel–Lindau syndrome (cerebellar hemangioblastoma, retinal angioma, renal cell carcinoma)	Renal cell carcinoma
STK11	Liver kinase B1 (LKB1) or STK11	Activator of AMPK family of kinases; suppresses cell growth when cell nutrient and energy levels are low	Peutz-Jeghers syndrome (GI polyps, GI cancers, pancreatic carcinoma, and other carcinomas)	Diverse carcinomas (5%–20% of cases, depending on type)
SDHB, SDHD	Succinate dehydrogenase complex subunits B and D	TCA cycle, oxidative phosphorylation	Familial paraganglioma, familial pheochromocytoma	Paraganglioma
Inhibitors of Invasion and Metastasis				
CDH1	E-cadherin	Cell adhesion, inhibition of cell motility	Familial gastric cancer	Gastric carcinoma, lobular breast carcinoma
Enablers of Genomic Stability				
TP53	p53 protein	Cell cycle arrest and apoptosis in response to DNA damage	Li-Fraumeni syndrome (diverse cancers)	Most human cancers
DNA Repair Factors				
BRCA1, BRCA2	Breast cancer-1 and breast cancer-2 (BRCA1 and BRCA2)	Repair of double-stranded breaks in DNA	Familial breast and ovarian carcinoma; carcinomas of male breast; chronic lymphocytic leukemia (BRCA2)	Rare
MSH2, MLH1, MSH6	MSH1, MLH1, MSH6	DNA mismatch repair	Hereditary nonpolyposis colon carcinoma	Colonic and endometrial carcinoma
Unknown Mechanisms				
WT1	Wilms tumor-1 (WT1)	Transcription factor	Familial Wilms tumor	Wilms tumor, certain leukemias
MEN1	Menin	Transcription factor	Multiple endocrine neoplasia-1 (MEN1) (pituitary, parathyroid, and pancreatic endocrine tumors)	Pituitary, parathyroid, and pancreatic endocrine tumors

[a]Some tumor suppressors impact multiple cancer phenotypes (e.g., p53 affects cell cycle progression, genomic stability, susceptibility to cell death, and cellular metabolism); only a subset of major effects are given for each tumor suppressor gene listed.

CNS, Central nervous system; *GI,* gastrointestinal; *TCA,* tricarboxylic acid.

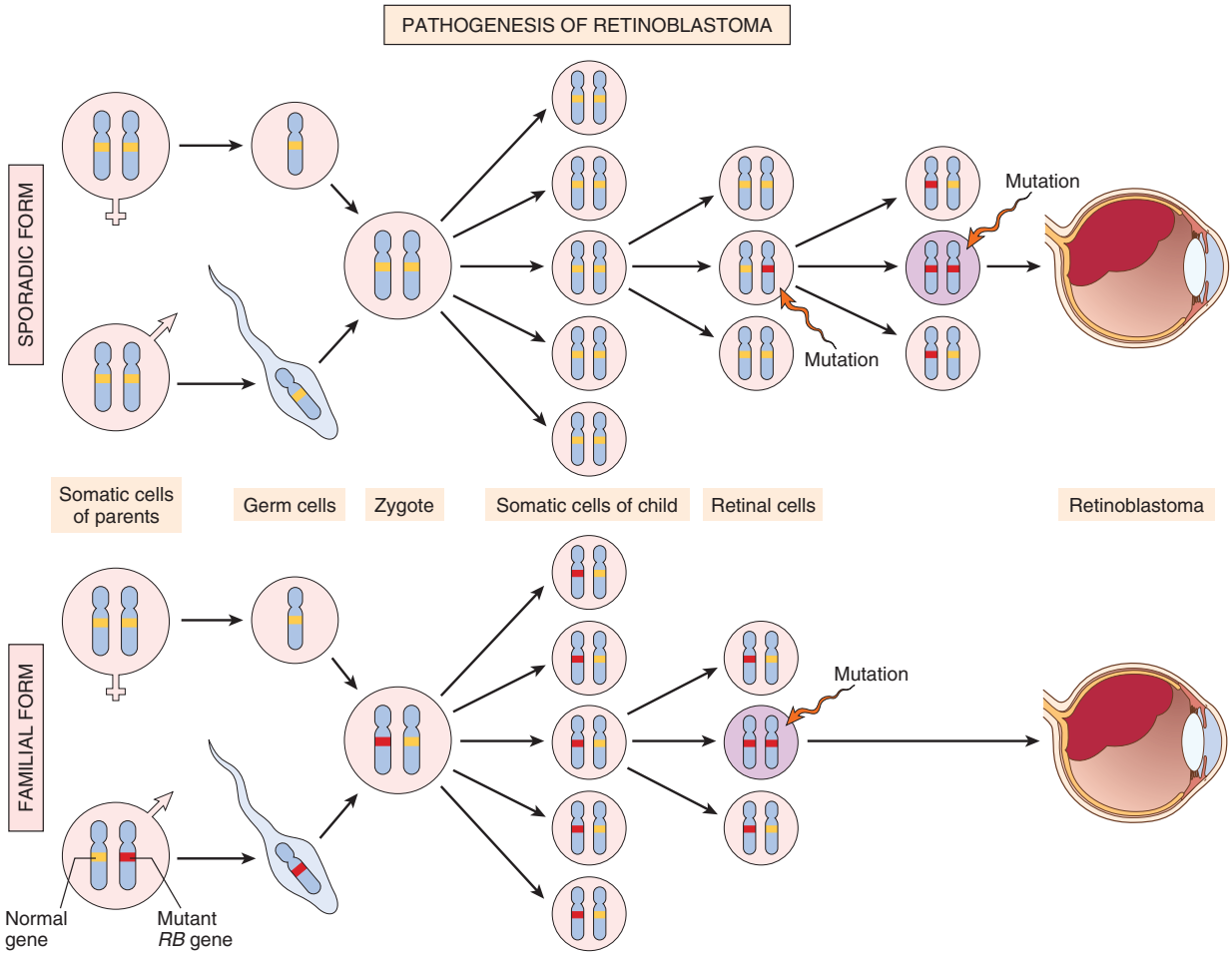

Figure 7.25 Pathogenesis of retinoblastoma. Two mutations of the *RB* locus on chromosome 13q14 lead to neoplastic proliferation of the retinal cells. In the sporadic form, both *RB* mutations in the tumor-founding retinal cell are acquired. In the familial form, all somatic cells inherit one mutated copy of *RB* gene from a carrier parent, and as a result only one additional *RB* mutation in a retinal cell is required for complete loss of RB function.

functions are listed in Table 7.7. Note that while tumor suppressors were initially thought of narrowly as proteins that put the brakes on cell cycle progression and DNA replication, it is now appreciated that some tumor suppressors prevent cellular transformation through other mechanisms such as by altering cell metabolism or by ensuring genomic stability. Thus, while most tumor suppressors have inhibitory effects on cell growth through one mechanism or another, a more inclusive definition of a tumor suppressor is simply a protein or gene that opposes any of the various hallmarks of cancer.

We next consider how specific tumor suppressors function, focusing on factors that are frequently mutated in cancer or that highlight pathogenically important molecular mechanisms.

RB: Governor of Proliferation. **RB, a key negative regulator of the G_1/S cell cycle transition, is directly or indirectly inactivated in most human cancers.** RB exists in an active hypophosphorylated state in quiescent cells and an inactive hyperphosphorylated state in cells passing through the G_1/S cell cycle transition (Chapter 1). Its function may be compromised in two different ways.

- Loss-of-function mutations involving both *RB* alleles.
- A shift from the active hypophosphorylated state to the inactive hyperphosphorylated state caused by gain-of-function mutations that upregulate CDK/cyclin D activity or by loss-of-function mutations that abrogate the activity of CDK inhibitors.

As discussed previously, the "decision" of a cell to progress from G_1 into S is of great importance, as once a cell enters S phase it is obligated to complete mitosis. High levels of CDK4/cyclin D, CDK6/cyclin D, and CDK2/cyclin E complexes lead to hyperphosphorylation and inhibition of RB, releasing E2F transcription factors that drive the expression of genes that are needed for progression to S phase (Fig. 7.26). Growth factor signaling pathways generally upregulate the activity of CDK/cyclin complexes and drive cells through the G_1/S transition, whereas growth inhibitors

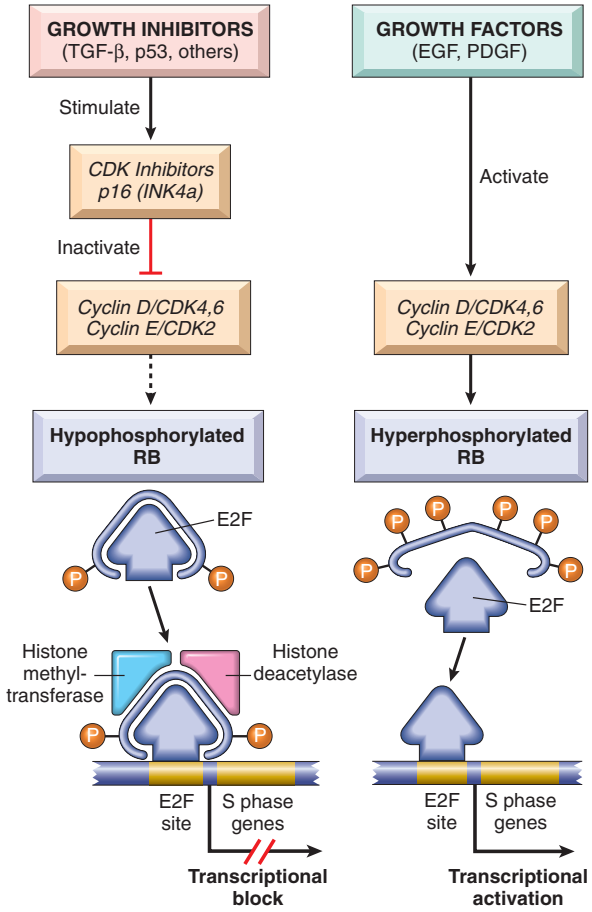

Figure 7.26 The role of RB in regulating the G_1-S checkpoint of the cell cycle. Hypophosphorylated RB in complex with the E2F transcription factors binds to DNA, recruits chromatin-modifying factors (histone deacetylases and histone methyltransferases), and inhibits transcription of genes whose products are required for the S phase of the cell cycle. When RB is phosphorylated by the cyclin D–CDK4, cyclin D–CDK6, and cyclin E–CDK2 complexes, it releases E2F. The latter then activates transcription of S-phase genes. Phosphorylation of RB is inhibited by cyclin-dependent kinase inhibitors because they inactivate cyclin–CDK complexes. In most cancers, the G_1-S checkpoint is defective as a result of mutation of one of four genes that regulate the phosphorylation of RB; these genes are *RB*, *CDK4*, the genes encoding cyclin D proteins, and *CDKN2A* (p16). *EGF,* Epidermal growth factor; PDGF, platelet-derived growth factor; *TGF-β,* transforming growth factor-β.

tip the balance the other way by upregulating CDK inhibitors. RB is the point of integration of these opposing signals, making it a key regulator of cell cycle progression.

It was mentioned previously that germline and somatic loss-of-function *RB* mutations are associated with retinoblastoma and osteosarcoma, and analyses of cancer cell genomes have identified similar somatic *RB* mutations in subsets of glioblastoma and lung, breast, and bladder carcinoma. However, given that RB is expressed in all cells, one may ask, why do patients with germline *RB* mutations preferentially develop retinoblastoma instead of other cancers? And, conversely, why are acquired mutations of *RB* not found in all kinds of cancer?

The reason why persons who inherit one defective allele of *RB* develop retinoblastoma and not other tumors is not understood, but a possible explanation is that other RB family members exist with RB-like activities; these proteins may fulfill the role of RB in cell types other than retinoblasts. With respect to the second question, the answer is much simpler: mutations in other genes that control RB phosphorylation can mimic the effect of *RB* loss, and such genes are mutated in many cancers that have normal *RB* genes. Thus, for example, mutational activation of cyclin D or CDK4 and mutational inactivation of CDK inhibitors favors cell proliferation by facilitating the hyperphosphorylation and inactivation of RB. The current paradigm is that **loss of normal cell cycle control is central to malignant transformation and that at least one of four key regulators of the cell cycle (p16/INK4a, cyclin D, CDK4, RB) is dysregulated in the vast majority of human cancers**. In cells that harbor mutations in any one of these genes or in upstream factors that regulate their expression and function (e.g., receptor tyrosine kinases, RAS), RB may be functionally inactivated even if the *RB* gene itself is not mutated.

The transforming proteins of several oncogenic animal and human DNA viruses also neutralize the growth inhibitory activities of RB. Of greatest relevance to human cancer, polyomavirus large T antigens and E7 proteins from high-risk types of HPV (such as HPV16) bind to hypophosphorylated RB through the same "pocket" that RB uses to bind and sequester E2F transcription factors. Binding of the viral proteins thus inactivates RB and releases E2F transcription factors, freeing them to cause cell cycle progression.

KEY CONCEPTS

RB, GOVERNOR OF THE CELL CYCLE

- When hypophosphorylated, RB exerts antiproliferative effects by binding and inhibiting E2F transcription factors that regulate genes required for cells to pass through the G_1/S phase cell cycle checkpoint. Normal growth factor signaling leads to RB hyperphosphorylation and inactivation, thus promoting cell cycle progression.
- The antiproliferative effect of RB is abrogated in cancers through a variety of mechanisms, including:
  - Loss-of-function *RB* mutations
  - Amplifications of the CDK4 and cyclin D genes
  - Loss-of-function mutations affecting cyclin-dependent kinase inhibitors (e.g., p16/INK4a)
  - Viral oncoproteins that bind and inhibit RB (E7 protein of HPV)

TP53: Guardian of the Genome. ***TP53,* a tumor suppressor gene that regulates cell cycle progression, DNA repair, cellular senescence, and apoptosis, is the most frequently mutated gene in human cancers.** Loss-of-function mutations in *TP53,* located on chromosome 17p13.1, are found in more than 50% of cancers. Moreover, *TP53* mutations occur in virtually every type of cancer, including carcinomas of the lung, colon, and breast—the three leading causes of cancer death. In most cases, mutations are present in both *TP53* alleles and are acquired in somatic cells (not inherited in

the germline). Less commonly, individuals inherit one mutated *TP53* allele. As in the case of the *RB* tumor suppressor and retinoblastoma, inheritance of a mutated copy of *TP53* predisposes individuals to malignant tumors because only one additional "hit" in the remaining normal allele is needed to abrogate *TP53* function. Individuals with these inherited mutations, said to have the *Li-Fraumeni syndrome,* have a 25-fold greater chance of developing a malignant tumor by age 50 than the general population. In contrast to individuals who inherit a mutant *RB* allele, the spectrum of tumors that develop in persons with Li-Fraumeni syndrome is broad; the most common types are sarcomas, breast cancers, leukemias, brain tumors, and carcinomas of the adrenal cortex. Persons with Li-Fraumeni syndrome often develop cancer at younger ages and are more likely to suffer from multiple primary tumors of varying types than are normal individuals.

These mutational data, while impressive, only begin to tell the tale of altered *TP53* function in cancer. *TP53* encodes the protein p53, which is tightly regulated at several levels. Analogous to RB, many tumors lacking *TP53* mutations have instead other mutations affecting proteins that regulate p53 function. For example, MDM2 and related proteins of the MDM2 family stimulate the degradation of p53; these proteins are frequently overexpressed in malignancies with normal *TP53* alleles. Indeed, the *MDM2* gene is amplified in 33% of human sarcomas, leading to a functional deficiency of p53 in these tumors. Like RB, the transforming proteins of several DNA viruses bind p53 and promote its degradation. Best known of these viral oncoproteins is the E6 protein of high-risk HPVs, which have causative roles in cervical carcinoma and a subset of squamous cell carcinomas of the head and neck.

The frequent loss of p53 function in human tumors reflects its critical role in preventing cancer development. p53 is the focal point of a large network of signals that sense cellular stress, primarily DNA damage, but also shortened telomeres, hypoxia, and stress caused by excessive pro-growth signaling, as may occur in cells bearing mutations in genes such as *RAS* and *MYC*. In nonstressed, healthy cells, p53 is held at bay through its aforementioned association with MDM2, an enzyme that ubiquitinates p53, leading to its degradation by the proteasome. As a result, p53 is virtually undetectable in normal cells. In stressed cells, however, p53 is released from the inhibitory effects of MDM2 via two major mechanisms, which vary depending on the nature of the stress.

- *DNA damage and hypoxia*. The key initiators of p53 activation following DNA damage or hypoxic stress are two related protein kinases, ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and Rad3 related (ATR). As the name implies, *ATM* was originally identified in individuals with ataxia-telangiectasia, which is caused by germline mutations in *ATM*. Affected patients suffer from an inability to repair certain kinds of DNA damage and have an increased incidence of cancer. The types of damage sensed by ATM and ATR are different, but the downstream effects are similar. Once triggered, both ATM and ATR stimulate the phosphorylation of p53 and MDM2. These posttranslational modifications disrupt the binding and degradation of p53 by MDM2, allowing p53 to accumulate.
- *"Oncogenic" stress*. Activation of oncoproteins such as RAS leads to sustained, supraphysiologic signaling through pro-growth pathways such as the MAPK and PI3K/AKT cascades. Through incompletely understood mechanisms, these aberrant signals create cellular stress and lead to increased expression of p14/ARF, which is encoded by the *CDKN2A* tumor suppressor gene. p14/ARF binds MDM2 and displaces p53, again allowing p53 levels to rise in the cell.

Once activated, p53 thwarts neoplastic transformation by inducing transient cell cycle arrest, senescence (permanent cell cycle arrest), or programmed cell death (apoptosis) (Fig. 7.27). p53 is a transcription factor that binds DNA in a sequence-specific fashion and activates the transcription of hundreds of target genes with p53-binding regulatory elements. The target genes that execute the functions of p53 are not completely defined but appear to fall into three major categories: (1) genes that cause cell cycle arrest, (2) genes that cause apoptosis, and (3) genes that enhance catabolic metabolism or inhibit anabolic metabolism. The last group of genes makes intuitive sense; there is no need for a cell that has stopped its cell cycle progression to continue to synthesize macromolecules (e.g., lipids and proteins) that are needed for cell growth and division.

Once p53 accumulates in a cell to levels that are sufficient to activate the transcription of target genes, several different outcomes are possible, each more serious than the last with respect to the ultimate fate of the affected cell.

- *Transient p53-induced cell cycle arrest.* Rapid onset, p53-mediated cell cycle arrest may be considered a primordial response to DNA damage. It occurs late in G_1 phase and is caused in part by p53-dependent transcription of the *CDKN1A* gene, which encodes the CDK inhibitor p21. p21 (like p16) inhibits CDK4/D cyclin complexes, thereby maintaining RB in an active, hypophosphorylated state and blocking the progression of cells from G_1 phase to S phase. This pause in cell cycling is welcome, as it gives the cells "breathing time" to repair DNA damage. p53 also helps the process by inducing proteins such as GADD45 (growth arrest and DNA damage) that enhance DNA repair. If DNA damage is repaired successfully, the signals responsible for p53 stabilization cease and p53 levels fall, releasing the cell cycle block. The cells may then revert to a normal state.
- *p53-induced senescence*. Senescence is a state of permanent cell cycle arrest characterized by specific changes in morphology and gene expression that differentiate it from reversible cell cycle arrest. How cells become fixed in the senescence state is unclear. One plausible idea is that senescence is the product of epigenetic changes that result in the formation of heterochromatin at key loci, including genes that are required for progression of cells from G_1 phase to S phase. Like other p53 responses, senescence may be stimulated in response to a variety of stresses, such as unopposed oncogene signaling, hypoxia, and shortened telomeres. Senescent cells, while not normal, cannot divide and therefore cannot develop into tumors.
- *p53-induced apoptosis.* Apoptosis of cells with irreversible DNA damage is the ultimate protective mechanism against neoplastic transformation. p53 directs the transcription

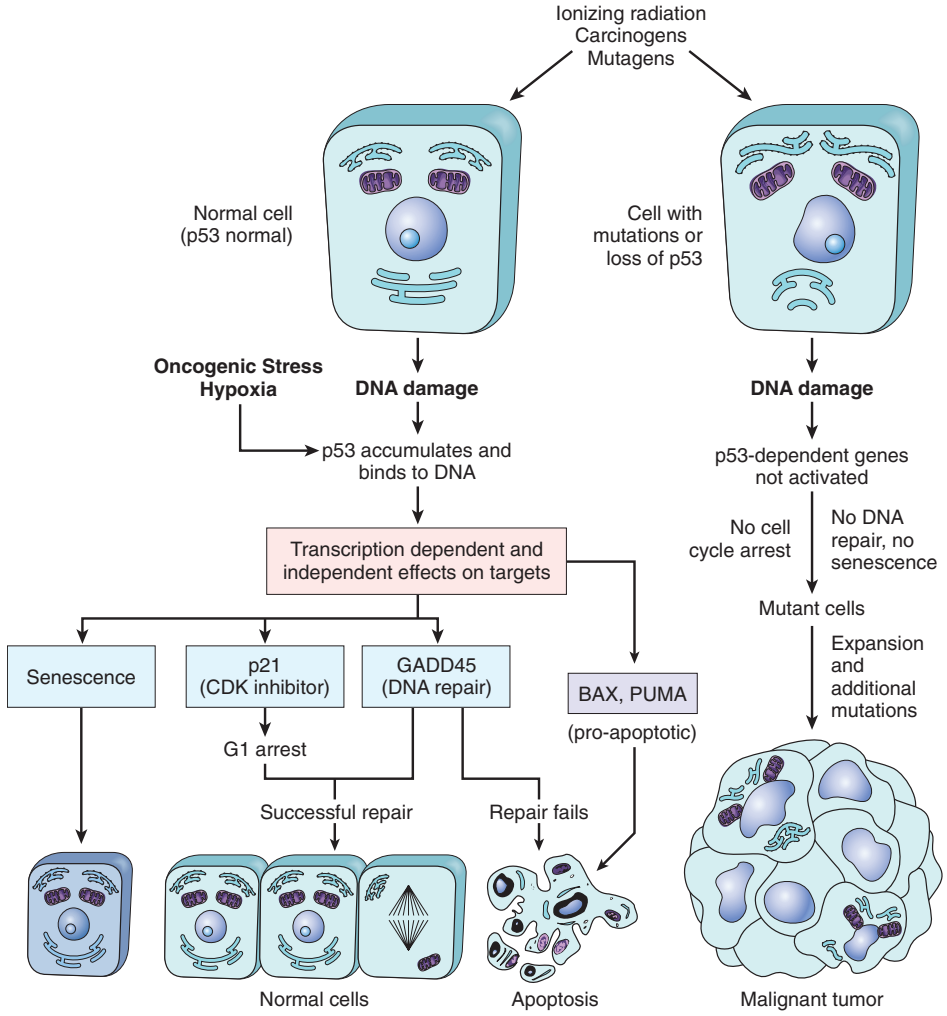

Figure 7.27 The role of p53 in maintaining the integrity of the genome. Activation of normal p53 by DNA-damaging agents or by hypoxia leads to cell cycle arrest in G_1 and induction of DNA repair by transcriptional upregulation of the cyclin-dependent kinase inhibitor *CDKN1A* (encoding the cyclin-dependent kinase inhibitor p21) and *GADD45* genes. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, p53 triggers either apoptosis or senescence. In cells with loss or mutations of the p53 gene, DNA damage does not induce cell cycle arrest or DNA repair, and genetically damaged cells proliferate, giving rise eventually to malignant neoplasms.

of several pro-apoptotic genes such as *BAX* and *PUMA* (described later), which are believed to tip the balance in favor of cell death via the intrinsic (mitochondrial) pathway.

What determines whether a cell repairs its DNA, becomes senescent, or undergoes apoptosis is uncertain, but both the duration and the level of p53 activation may be deciding factors. It appears that the affinity of p53 for its binding sites in the promoters and enhancers of DNA repair genes is higher than its affinity for binding sites in pro-apoptotic genes. Thus the DNA repair pathway is stimulated first as p53 begins to accumulate. If p53 is sustained at this level due to ineffective DNA repair or other chronic stresses (e.g., that induced by a potentially oncogenic RAS mutation), epigenetic silencing of genes that are needed for cell cycle progression occurs, leading to senescence. Alternatively, if enough p53 accumulates to stimulate the transcription of the pro-apoptotic genes, the cell dies. While this scheme seems to be generally correct, cell type–specific variations in response to p53 activation have been observed that are not easily explained, with some cell types succumbing rapidly to apoptosis and others opting mainly for senescence. Thus there is still much to be learned about the nuances of p53 function.

With loss of p53 function, DNA damage goes unrepaired, driver mutations accumulate in oncogenes and other cancer genes, and the cell marches along a dangerous path leading to malignant transformation. Moreover, once a cancer is established, its p53 status has important therapeutic implications. Irradiation and conventional chemotherapy, the two common modalities of cancer treatment, mediate their effects

by inducing DNA damage and subsequent apoptosis. Tumors with wild-type *TP53* alleles are more likely to be killed by such therapy than tumors with mutated *TP53* alleles. Such is the case with testicular germ cell tumors and childhood acute lymphoblastic leukemias, cancers with excellent clinical outcomes that usually have wild-type *TP53* alleles. In contrast, tumors such as lung cancers and colorectal cancers, which frequently carry *TP53* mutations, are relatively resistant to chemotherapy and irradiation. A second, less obvious but even more nefarious effect is that cells with defective p53 acquire a mutator phenotype, a tendency to acquire mutations at a high rate. Particularly in advanced stage tumors with mutator phenotypes, it is very likely (and perhaps inevitable) that genetically distinct subclones will arise by chance that are resistant to any single therapy, whether radiation, conventional chemotherapy, or molecularly targeted cancer drugs. This theme is discussed later when the enabling properties of genomic instability are discussed more broadly.

KEY CONCEPTS

P53, GUARDIAN OF THE GENOME

- The p53 protein is the central monitor of stress in the cell and can be activated by anoxia, inappropriate signaling by mutated oncoproteins, or DNA damage. p53 controls the expression and activity of proteins involved in cell cycle arrest, DNA repair, cellular senescence, and apoptosis.
- DNA damage is sensed by complexes containing kinases of the ATM/ATR family; these kinases phosphorylate p53, liberating it from inhibitors such as MDM2. Active p53 then upregulates the expression of proteins such as the cyclin-dependent kinase inhibitor p21, thereby causing cell-cycle arrest at the G_1/S checkpoint. This pause allows cells to repair DNA damage.
- If DNA damage cannot be repaired, p53 induces additional events that lead to cellular senescence or apoptosis.
- The majority of human cancers demonstrate biallelic loss-of-function mutations in *TP53*. Rare patients with Li-Fraumeni syndrome inherit one defective copy of *TP53* and have a very high incidence of a wide variety of cancers.
- Like RB, p53 is inactivated by viral oncoproteins, such as the E6 protein of HPV.

Other Tumor Suppressor Genes. The full panoply of tumor suppressor genes is still being defined. Often, they are disabled because they are the targets of recurrent chromosomal deletions, which are now being systematically identified and characterized by sequencing of cancer genomes. Tumor suppressor genes all appear to impact one or more of the hallmarks of cancer. Some that are associated with well-defined clinical syndromes (Table 7.7) or that serve to highlight mechanisms by which tumor suppressors function are described next; others that are organ- or tumor-specific are mentioned in the relevant chapters that follow.

APC: Gatekeeper of Colonic Neoplasia. **Adenomatous polyposis coli (APC) is a member of the class of tumor suppressors that function by downregulating growth-promoting signaling pathways.** Germline loss-of-function mutations involving the *APC* locus on chromosome 5q21 are associated with *familial adenomatous polyposis*, an autosomal dominant disorder in which individuals inheriting one mutant allele develop thousands of adenomatous polyps in the colon during their teens or 20s (Chapter 17). Almost invariably, one or more of these polyps undergoes malignant transformation, giving rise to colon cancer. As with other tumor suppressor genes, both copies of *APC* must be lost for an adenoma to arise. As discussed later, several additional mutations must then occur for adenomas to progress to cancers. In addition to these hereditary forms of colon cancer, 70% to 80% of nonfamilial colorectal carcinomas and sporadic adenomas also show acquired defects involving both APC genes, firmly implicating APC loss of function in the pathogenesis of colonic tumors.

APC is a component of the WNT signaling pathway, which has a major role in controlling cellular growth and differentiation during embryonic development (Fig. 7.28). WNT molecules signal by binding to cell surface receptors of the frizzled (FRZ) family. This stimulates several pathways, the central one involving APC and β-catenin. A major function of the APC protein is to hold β-catenin activity in check. In the absence of WNT signaling, APC participates in the formation a "destruction complex" that leads to the proteasomal degradation of β-catenin. WNT signaling blocks the formation of the destruction complex, stabilizing β-catenin and allowing it to translocate from the cytoplasm to the nucleus. Here, it forms a transcription activation complex with a DNA-binding factor called TCF that promotes the growth of colonic epithelial cells by increasing the expression of *MYC, cyclin D1,* and other genes. Because loss of APC function disrupts the destruction complex, cells that lose APC behave as if they are being continuously stimulated by WNT and show elevated expression of genes that are regulated by β-catenin. The importance of the β-catenin complex in tumorigenesis is attested to by the fact that many colon tumors with normal *APC* genes harbor β-catenin mutations that prevent its APC-dependent destruction, again leading to its accumulation and increased expression of β-catenin–dependent target genes. Thus β-catenin, the target of APC, is itself a proto-oncoprotein. Dysregulation of the APC/β-catenin pathway is not restricted to colon cancers; for example, gain-of-function mutations in β-catenin are present in approximately 20% of hepatocellular carcinomas.

E-Cadherin. β-catenin also binds to the cytoplasmic tail of E-cadherin, a cell surface protein that maintains intercellular adhesiveness. Loss of cell-cell contact, such as following epithelial wounding or injury, disrupts the interaction between E-cadherin and β-catenin. Like WNT signaling, this in turn allows β-catenin to translocate to the nucleus and stimulate genes that promote proliferation, an appropriate response to injury that can help repair a wound. Reestablishment of these E-cadherin contacts as the wound heals leads to sequestration of β-catenin at the membrane and reduces proliferative signaling; these cells are said to be "contact-inhibited." Loss of contact inhibition, by mutation of the E-cadherin/β-catenin axis or by other changes, is a characteristic of many carcinomas. Furthermore, loss of E-cadherin can contribute to the malignant phenotype by allowing easy disaggregation of cells, which can then invade locally or metastasize. Reduced cell surface expression of E-cadherin has been noted in many carcinomas including

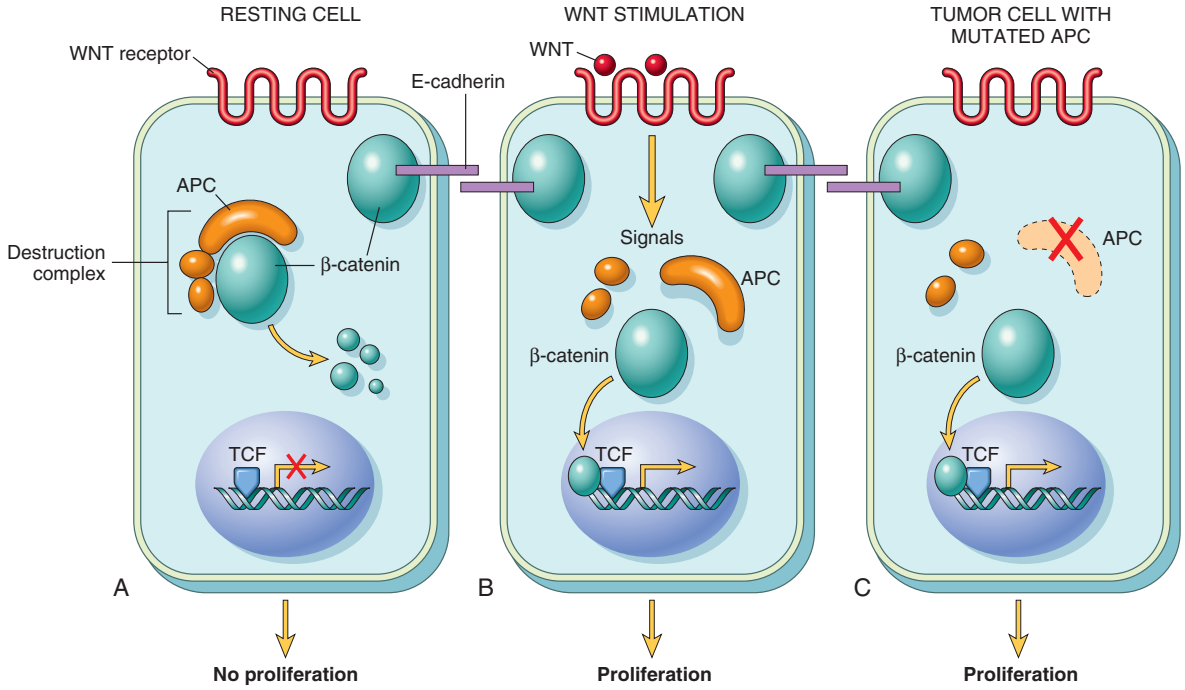

Figure 7.28 The role of adenomatous polyposis coli *(APC)* in regulating the stability and function of β-catenin. APC and β-catenin are components of the WNT signaling pathway. (A) In resting colonic epithelial cells (not exposed to WNT), β-catenin forms a macromolecular complex containing the APC protein. This complex leads to the destruction of β-catenin, and intracellular levels of β-catenin are low. (B) When normal colonic epithelial cells are stimulated by WNT molecules, the *destruction complex* is deactivated, β-catenin degradation does not occur, and cytoplasmic levels increase. β-Catenin translocates to the nucleus, where it binds to TCF, a transcription factor that activates genes involved in cell cycle progression. (C) When *APC* is mutated or absent, as frequently occurs in colonic polyps and cancers, the destruction of β-catenin cannot occur. β-Catenin translocates to the nucleus and coactivates genes that promote entry into the cell cycle, and cells behave as if they are under constant stimulation by the WNT pathway.

those that arise in the esophagus, colon, breast, ovary, and prostate. Germline loss-of-function mutations of the E-cadherin gene, known as *CDH1,* are associated with familial gastric carcinoma, and a proportion of sporadic gastric carcinomas are also associated with loss of E-cadherin expression, which can occur due to mutation of the *CDH1* gene or other indirect mechanisms.

CDKN2A. As mentioned earlier, the *CDKN2A* gene encodes two protein products: the p16/INK4a cyclin-dependent kinase inhibitor, which blocks CDK4/cyclin D–mediated phosphorylation of RB, thereby reinforcing the G_1/S checkpoint; and p14/ARF, which activates the p53 pathway by inhibiting MDM2 and preventing destruction of p53. p16 also appears to be important in induction of cellular senescence (described later). Germline mutations in *CDKN2A* are associated with familial forms of melanoma, and sporadic mutations of this locus have been detected in bladder cancer, head and neck tumors, acute lymphoblastic leukemia, and cholangiocarcinoma. In some tumors, such as cervical cancer, p16 is often silenced by hypermethylation of the gene rather than mutation (see "Epigenetic Changes"). Other cyclin-dependent kinase inhibitors also function as tumor suppressors and are frequently mutated or otherwise silenced in many human malignancies.

TGF-β Pathway. In most normal epithelial, endothelial, and hematopoietic cells, TGF-β is a potent inhibitor of proliferation. It regulates cellular processes by binding to TGF-β receptors, thereby stimulating intracellular signals that involve transcriptional regulators of the SMAD protein family. Under normal circumstances, these signals turn on antiproliferative genes (e.g., genes for cyclin-dependent kinase inhibitors) and turn off genes that drive cell growth (e.g., *MYC,* cyclins, and cyclin-dependent kinases). As can be inferred from our earlier discussion, these changes result in decreased phosphorylation of RB and cell cycle arrest.

In many forms of cancer these growth-inhibiting effects are impaired by loss-of-function mutations in the TGF-β signaling pathway. Mutations affecting TGF-β receptors are common in cancers of the colon, stomach, and endometrium, while mutational inactivation of SMAD4 is common in pancreatic cancers. In many other cancers, loss of TGF-β–mediated growth inhibition occurs at the level of key target genes; examples include mutations that lead to loss of p21 function and/or persistent expression of *MYC.* In such cases, other preserved elements of the TGF-β–induced program of gene expression may actually facilitate acquisition of cancer hallmarks, such as immune evasion or angiogenesis. Thus TGF-β signaling is a double-edged sword that can prevent or promote tumor growth, depending on the state of other genes in the cell.

PTEN. Phosphatase and tensin homologue (PTEN) is a membrane-associated phosphatase encoded by a gene on chromosome 10q23 that is mutated in Cowden syndrome,

an autosomal dominant disorder marked by frequent benign growths such as skin appendage tumors and an increased incidence of epithelial cancers, particularly of the breast (Chapter 21), endometrium, and thyroid. As already mentioned, PTEN acts as a tumor suppressor by serving as a brake on the PI3K/AKT signaling cascade. *PTEN* gene function is lost in many cancers through deletion, deleterious point mutations, or epigenetic silencing.

VHL. Germline loss-of-function mutations of the von Hippel–Lindau *(VHL)* gene on chromosome 3p are associated with hereditary renal cell carcinoma and several other tumors and proliferations. Somatic mutations of *VHL* also occur in a subset of sporadic renal cell carcinomas (Chapter 20). VHL is a component of a protein complex that covalently links ubiquitin chains to specific protein substrates, thereby promoting their degradation by the proteasome. A critical substrate for the VHL ubiquitin ligase is the transcription factor hypoxia-inducible transcription factor 1α (HIF1α). In the presence of oxygen, HIF1α is hydroxylated and binds to VHL, leading to its ubiquitination and degradation. In hypoxic environments the hydroxylation reaction does not occur, and HIF1α escapes recognition by VHL. As a result, HIF1α accumulates in the nuclei of hypoxic cells and turns on target genes, including genes encoding vascular endothelial growth factor (VEGF), a critical angiogenesis factor; PDGF, a potent mitogen; and the glucose transporter GLUT1 and several glycolytic enzymes, factors that contribute to Warburg metabolism (described later). Thus, VHL is part of the system that regulates cellular responses to oxygen levels. Loss-of-function mutations in *VHL* prevent the ubiquitination and degradation of HIF1α, even under normoxic conditions, and are accordingly associated with increased levels of angiogenic growth factors and alterations in cellular metabolism that favor growth.

STK11. The *STK11* gene, also known as *LKB1,* encodes a serine/threonine kinase that is an important regulator of cellular metabolism. Germline loss-of-function *STK11* mutations cause Peutz-Jeghers syndrome, an autosomal dominant disorder associated with benign polyps of the gastrointestinal tract and an increased risk of multiple epithelial cancers, particularly gastrointestinal and pancreatic carcinomas. STK11 has pleiotropic effects on multiple facets of cellular metabolism including glucose uptake, gluconeogenesis, protein synthesis, mitochondrial biogenesis, and lipid metabolism. Sporadic *STK11* loss-of-function mutations are found in diverse carcinomas, a finding pointing to the important role of altered cellular metabolism in the establishment and maintenance of the transformed state (discussed later).

KEY CONCEPTS

MECHANISM OF ACTION OF MAJOR TUMOR SUPPRESSOR GENES

APC: encodes a factor that negatively regulates the WNT pathway in colonic epithelium by promoting the formation of a complex that degrades β-catenin.
- Germline loss-of-function mutations cause familial adenomatous polyposis, an autosomal dominant disorder associated with development of thousands of colonic polyps and early-onset colon carcinoma.
- Acquired somatic *APC* mutations are found in about 70% of sporadic colon carcinomas.

E-cadherin: cell adhesion molecule that plays an important role in contact-mediated growth inhibition of epithelial cells; also binds and sequesters β-catenin, a signaling protein that functions in the WNT pathway.
- Germline loss-of-function mutations in the E-cadherin gene *(CDH1)* cause autosomal dominant familial gastric carcinoma.
- Loss of *CDH1* expression is seen in many sporadic carcinomas; these are associated with loss of contact inhibition, loss of cohesiveness, increased invasiveness, and increased WNT signaling.

CDKN2A: a complex locus that encodes two tumor suppressive proteins, p16/INK4a, a cyclin-dependent kinase inhibitor that augments RB function, and ARF, which stabilizes p53.
- Germline loss-of-function mutations cause autosomal dominant familial melanoma.
- Biallelic loss of function is seen in diverse cancers including leukemias, melanomas, and carcinomas.

TGF-β pathway: potent inhibitor of cellular proliferation in normal tissues.
- Frequent loss-of-function mutations involving TGF-β receptors (colon, stomach, endometrium) or downstream signal transducers (SMADs, pancreas) in diverse carcinomas.
- Complex role in carcinogenesis; may also have a pro-oncogenic role by enhancing the immune evasiveness of tumors.

PTEN: encodes a lipid phosphatase that is an important negative regulator of PI3K/AKT signaling.
- Germline loss-of-function mutations cause Cowden syndrome, an autosomal dominant disorder associated with a high risk of breast and endometrial carcinoma.
- Biallelic loss of function common in diverse cancers.

VHL: encodes a component of a ubiquitin ligase complex that is responsible for degradation of hypoxia-induced factors (HIFs), transcription factors that alter gene expression in response to hypoxia.
- Germline loss-of-function mutations cause von Hippel–Lindau syndrome, an autosomal dominant disorder associated with a high risk of renal cell carcinoma and pheochromocytoma.
- Acquired biallelic loss-of-function mutations are common in sporadic renal cell carcinoma.

Growth-Promoting Metabolic Alterations: The Warburg Effect

Even in the presence of ample oxygen, cancer cells demonstrate a distinctive form of cellular metabolism characterized by high levels of glucose uptake and increased conversion of glucose to lactose (fermentation) via the glycolytic pathway. This phenomenon, called the *Warburg effect* and also known as *aerobic glycolysis,* has been recognized for many years (Otto Warburg received the Nobel Prize in 1931 for discovery of the effect that bears his name). Clinically the "glucose hunger" of tumors is used to visualize them via positron emission tomography (PET) scanning, in which patients are injected with ^{18}F-fluorodeoxyglucose, a nonmetabolizable derivative of glucose that is preferentially taken up into tumor cells (as well as normal, actively dividing

tissues such as the bone marrow). Most tumors are PET-positive, and rapidly growing ones are markedly so.

At the heart of the Warburg effect lies a simple question: why is it advantageous for a cancer cell to rely on seemingly inefficient glycolysis (which generates two molecules of ATP per molecule of glucose) instead of oxidative phosphorylation (which generates 36 molecules of ATP per molecule of glucose)? While pondering this question, it is important to recognize that rapidly growing normal cells, such as in embryonic tissues, also rely on aerobic fermentation. Thus, "Warburg metabolism" is not cancer-specific, but instead is a general property of growing cells that is exploited by cancer cells.

The answer to this riddle is surprisingly simple: **aerobic glycolysis provides rapidly dividing tumor cells with metabolic intermediates that are needed for the synthesis of cellular components, whereas mitochondrial oxidative phosphorylation does not.** The reason growing cells rely on aerobic glycolysis becomes readily apparent when one considers that a growing cell has a strict biosynthetic requirement; it must duplicate all of its cellular components—DNA, RNA, proteins, lipid, and organelles—before it can divide and produce two daughter cells. Recall that the net effect of oxidative phosphorylation is to take a molecule of glucose, $C_6H_{12}O_6$, and combine it with six molecules of O_2 to produce six molecules of H_2O and six molecules of CO_2, which are lost through respiration. Thus, while "pure" oxidative phosphorylation yields abundant ATP, it fails to produce any carbon moieties that can be used to build cellular components that are needed for growth (proteins, lipids, and nucleic acids). Even cells that are not actively growing must shunt some metabolic intermediates away from oxidative phosphorylation to synthesize macromolecules that are needed for cellular maintenance.

By contrast, in actively growing cells, only a small fraction of the cellular glucose is shunted through the oxidative phosphorylation pathway, such that on average each molecule of glucose that is metabolized produces approximately four molecules of ATP (instead of the two molecules that would be produced by "pure" glycolysis). Presumably, this balance in glucose utilization (heavily biased toward aerobic fermentation, with a bit of oxidative phosphorylation) hits a metabolic "sweet spot" that is optimal for growth. It follows that growing cells do rely on mitochondrial metabolism. However, the main metabolic function of mitochondria in growing cells is not to generate ATP, but rather to carry out reactions that generate intermediates that can be diverted for use as precursors in the synthesis of cellular building blocks. For example, lipid biosynthesis requires acetyl coenzyme A (acetyl-CoA), and acetyl-CoA is largely synthesized in growing cells from intermediates such as citrate that are generated in mitochondria.

So how is this reprogramming of metabolism triggered in growing normal and malignant cells? As might be guessed, **metabolic reprogramming is produced by signaling cascades downstream of growth factor receptors, the very same pathways that are deregulated by mutations in oncogenes and tumor suppressor genes in cancers.** Thus, whereas in rapidly growing normal cells aerobic glycolysis ceases when the tissue is no longer growing, in cancer cells this reprogramming persists due to the action of oncogenes and the loss of tumor suppressor gene function. Some of the important points of crosstalk between pro-growth signaling factors and cellular metabolism are shown in Fig. 7.29 and include the following:

- *Receptor tyrosine kinase/PI3K/AKT signaling.* PI3K/AKT signaling upregulates the activity of glucose transporters and multiple glycolytic enzymes, thus increasing glycolysis; promotes shunting of mitochondrial intermediates to pathways leading to lipid biosynthesis; and stimulates factors that are required for protein synthesis. In addition, receptor tyrosine kinases phosphorylate and inhibit pyruvate kinase, which catalyzes the last step in the glycolytic pathway, the conversion of phosphoenolpyruvate to pyruvate. This creates a damming effect that leads to the buildup of upstream glycolytic intermediates, which are siphoned off for synthesis of DNA, RNA, and protein.
- *MYC.* As mentioned, pro-growth pathways upregulate expression of the transcription factor MYC, which drives changes in gene expression that support anabolic metabolism and cell growth. Among the most important metabolic factors that are upregulated by MYC are multiple glycolytic enzymes and glutaminase, which is required for mitochondrial utilization of glutamine, another important source of intermediates needed for biosynthesis of cellular components.

The flip side of the coin is that tumor suppressors often inhibit metabolic pathways that support growth. We have already discussed how the STK11 tumor suppressor antagonizes metabolic changes that produce Warburg metabolism. Indeed, it may be that many (and perhaps all) tumor suppressors that induce growth arrest suppress the Warburg effect. For example, p53, arguably the most important tumor suppressor, upregulates target genes that collectively inhibit glucose uptake, glycolysis, lipogenesis, and the generation of NADPH (a key cofactor needed for the biosynthesis of macromolecules). Thus, it is clear that the functions of many oncoproteins and tumor suppressors are inextricably intertwined with cellular metabolism.

Autophagy. Autophagy is a state of severe nutrient deficiency in which cells not only arrest their growth but also cannibalize their own organelles, proteins, and membranes as carbon sources for energy production (see Fig. 7.29 and Chapter 2). If this adaptation fails, the cells die of starvation. Tumor cells often seem to be able to grow under marginal environmental conditions without triggering autophagy, suggesting that the pathways that induce autophagy are disabled. In keeping with this, several genes that promote autophagy are tumor suppressors, meaning that the loss of autophagy enhances tumor growth. Whether autophagy is always bad from the vantage point of the tumor, however, is a matter of active investigation and debate. For example, under conditions of severe nutrient deprivation, tumor cells may use autophagy to become "dormant," a state of metabolic hibernation that allows cells to survive hard times for long periods. Such cells are believed to be resistant to therapies that kill actively dividing cells and could therefore be responsible for therapeutic failures. Thus, autophagy may be a tumor's friend or foe depending on how the signaling pathways that regulate it are "wired" in a given tumor.

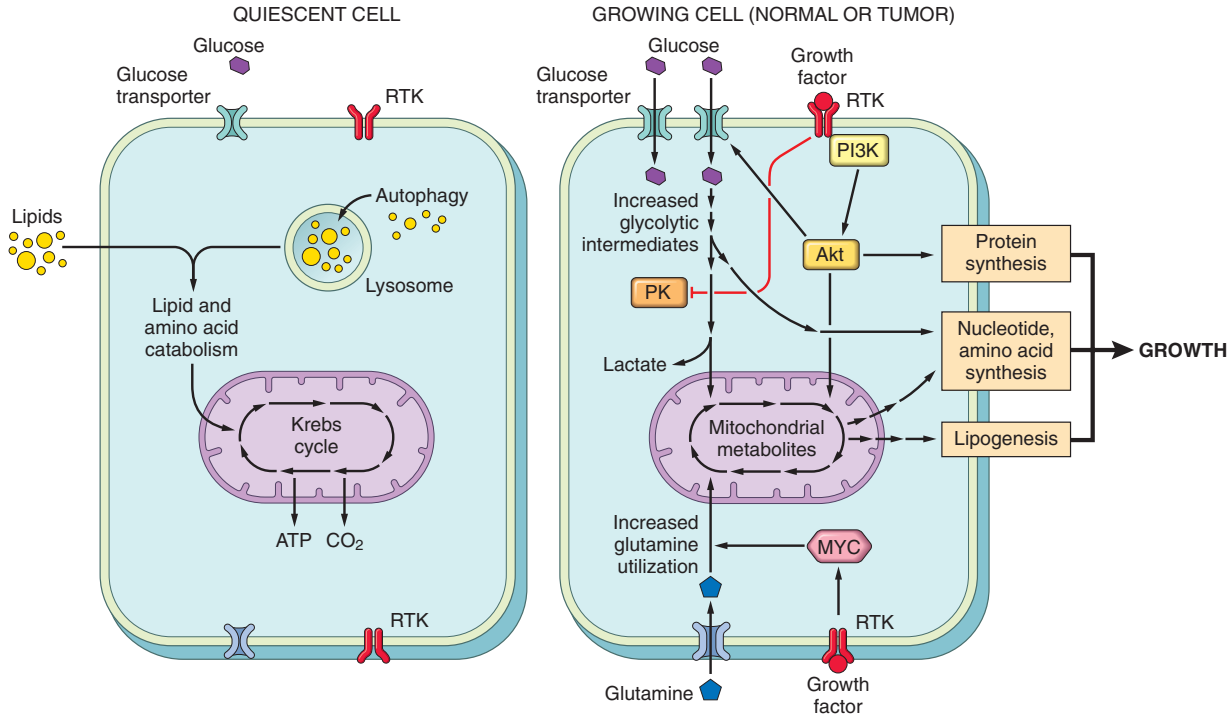

Figure 7.29 Metabolism and cell growth. Quiescent cells rely mainly on the Krebs cycle for adenosine triphosphate *(ATP)* production; if starved, autophagy (self-eating) is induced to provide a source of fuel. When stimulated by growth factors, normal cells markedly upregulate glucose and glutamine uptake, yielding glycolytic and Krebs cycle metabolic intermediates that provide carbon sources for synthesis of nucleotides, proteins, and lipids. In cancers, oncogenic mutations involving growth factor signaling pathways and other key factors such as MYC deregulate these metabolic pathways.

Oncometabolism. A surprising group of genetic alterations discovered through tumor genome sequencing studies consists of mutations in enzymes that participate in the Krebs cycle. Of these, mutations in isocitrate dehydrogenase (IDH) have garnered the most interest, as they have revealed a new mechanism of oncogenesis termed *oncometabolism* (Fig. 7.30).

The proposed steps in the oncogenic pathway involving IDH are as follows:

- IDH acquires a mutation that leads to a specific amino acid substitution involving residues in its active site. As a result, the mutated protein loses it IDH function and instead acquires a new enzymatic activity that catalyzes the production of 2-hydroxyglutarate (2-HG).
- 2-HG in turn acts as an inhibitor of several other enzymes that require a metabolite called α-ketoglutarate as a cofactor. Among the proteins that are inhibited by 2-HG are several members of the TET family, including TET2.
- TET2 is one of several factors that enhance DNA methylation, which you will recall is an epigenetic modification that controls normal gene expression and often goes awry in cancer. According to the model, loss of TET2 activity leads to abnormal patterns of DNA methylation.
- Abnormal DNA methylation in turn leads to misexpression of cancer genes, which drive cellular transformation and oncogenesis. Some data suggest that the net effect of TET2 loss in lineages in which TET2 is a tumor suppressor is the upregulation of RAS and receptor tyrosine kinase signaling.

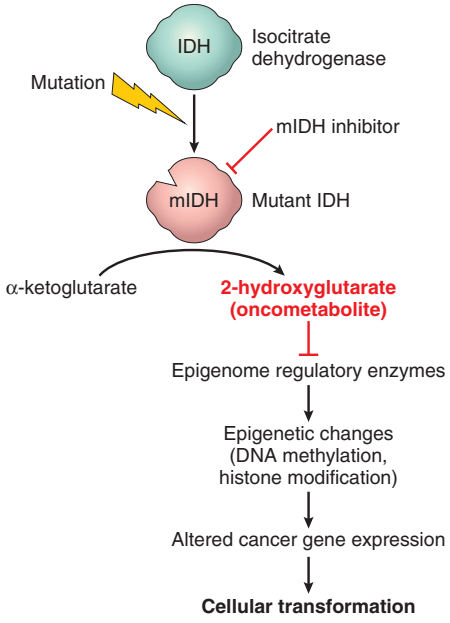

Figure 7.30 Proposed action of the oncometabolite 2-hydroxyglutarate in cancer cells with mutated isocitrate dehydrogenase *(mIDH)*. *IDH,* Isocitrate dehydrogenase.

Thus, according to this scenario, mutated IDH acts by producing 2-HG, which is considered a prototypical *oncometabolite*. Oncogenic IDH mutations occur in a diverse collection of cancers including a sizable fraction of cholangiocarcinomas, gliomas, acute myeloid leukemias, and sarcomas. Of clinical significance, because the mutated IDH proteins have an altered structure, it has been possible to develop drugs that inhibit mutated IDH and not the normal IDH enzyme. These drugs are now being used to treat leukemias with IDH mutations. This story exemplifies how detailed understanding of oncogenic mechanisms can yield entirely new kinds of anticancer drugs.

KEY CONCEPTS

ALTERED CELLULAR METABOLISM

- Warburg metabolism is a form of pro-growth metabolism favoring glycolysis over oxidative phosphorylation. It is induced in normal cells by exposure to growth factors and becomes fixed in cancer cells due to the action of certain driver mutations.
- Many oncoproteins (RAS, MYC, mutated growth factor receptors) induce or contribute to Warburg metabolism, and many tumor suppressors (*PTEN*, *NF1*, *p53*) oppose it.
- Stress may induce cells to consume their components in a process called autophagy. Cancer cells may accumulate mutations to avoid autophagy or may corrupt the process to provide nutrients for continued growth and survival.
- Some oncoproteins such as mutated IDH act by causing the formation of high levels of "oncometabolites" that alter the epigenome, thereby leading to changes in gene expression that are oncogenic.

Evasion of Cell Death

Tumor cells frequently contain mutations in genes that result in resistance to apoptotic cell death. As discussed in Chapter 2, apoptosis, or regulated cell death, refers to an orderly dismantling of cells into component pieces, which are then efficiently consumed by macrophages without stimulating inflammation. You will recall there are two pathways that lead to apoptosis: (1) the extrinsic (death receptor) pathway, triggered by death receptors of the tumor necrosis factor (TNF) receptor family, such as FAS, and their ligands; and (2) the intrinsic (mitochondrial) pathway, initiated by various stresses, such as the absence of growth factors and DNA damage. The intrinsic pathway appears to be the primary arbitrator of life and death in cancer cells, as cancer cells are subject to a number of intrinsic stresses that can initiate apoptosis, particularly DNA damage, but also metabolic disturbances stemming from dysregulated growth, hypoxia caused by insufficient blood supply, and in some cancers increased amounts of misfolded proteins. These stresses are enhanced manyfold when tumors are treated with chemotherapy or radiation (which kill tumor cells mainly by inducing apoptosis). Thus there is strong selective pressure, both before and after therapy, for cancer cells to develop mechanisms to evade apoptosis. This occurs mainly by way of acquired mutations and changes in gene expression that disable key components of the intrinsic pathway or that reset the balance of regulatory factors so as to favor cell survival in the face of intrinsic stresses (Fig. 7.31).

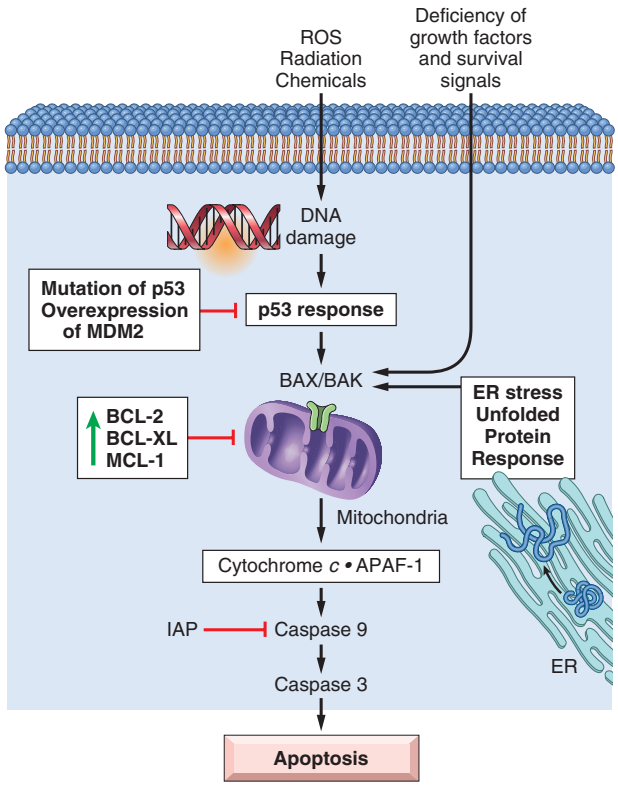

Figure 7.31 Intrinsic pathway of apoptosis and major mechanisms used by tumor cells to evade cell death. The most important mechanisms involve loss of p53 function, either through mutation or through antagonism by MDM2, and reduced egress of cytochrome *c* from mitochondria as a result of upregulation of anti-apoptotic factors that stabilize the mitochondrial membrane such as BCL2, BCL-XL, and MCL-1. Less commonly, tumors suppress apoptosis by unregulating members of the inhibitor of apoptosis *(IAP)* family.

Before delving into modes of resistance to apoptosis, a brief review of the intrinsic pathway is in order. Activation of this pathway leads to permeabilization of the mitochondrial outer membrane and release of molecules, such as cytochrome *c,* that initiate apoptosis. The integrity of the mitochondrial outer membrane is determined by a delicate balancing act between pro-apoptotic and anti-apoptotic members of the BCL2 protein family. The pro-apoptotic proteins BAX and BAK are required for apoptosis and directly promote mitochondrial permeabilization. Their action is inhibited by the anti-apoptotic members of this family, which are exemplified by BCL2 and BCL-XL. A third set of proteins, so-called *BH3-only proteins,* which include BIM, BAD, BID, and PUMA, shift the balance between the pro-apoptotic and anti-apoptotic family members by neutralizing the actions of anti-apoptotic proteins like BCL2 and BCL-XL, thereby promoting apoptosis. When the sum total of all BH3 proteins expressed "overwhelms" the anti-apoptotic BCL2/ BCL-XL protein barrier, BAX and BAK are activated and form pores in the mitochondrial membrane. This allows cytochrome *c* to leak into the cytosol, where it

binds to APAF-1 and activates caspase-9, which in turn cleaves and activates the executioner caspases. Another check on this system consists of a group of proteins called *inhibitor of apoptosis proteins (IAPs)*, which block the action of caspase-9.

Within this framework, it is possible to illustrate the two major mechanisms by which apoptosis is avoided by cancer cells (see Fig. 7.31):

- *Loss of TP53 function*. While *TP53* is commonly mutated in cancers at diagnosis, the frequency of *TP53* mutations is even higher in tumors that relapse after therapy, probably because of their ability to convey resistance to genotoxic therapies. Other lesions in cancer impair p53 function indirectly, most notably amplification of *MDM2*, which encodes an inhibitor of p53. Loss of p53 function prevents the upregulation of PUMA, a pro-apoptotic BH3-only protein, in response to DNA damage and other stresses, allowing cells to survive that otherwise would be killed.
- *Overexpression of anti-apoptotic members of the BCL2 family*. Overexpression of BCL2 is a common event leading to the protection of tumor cells from apoptosis. One of the best-understood examples is found in follicular lymphoma (Chapter 12), a B-cell tumor carrying a characteristic (14;18)(q32;q21) translocation that fuses the *BCL2* gene to the transcriptionally active immunoglobulin heavy chain gene. The resulting overabundance of BCL2 protects the transformed lymphocytes from apoptosis. Because BCL2-overexpressing follicular lymphomas arise in large part through reduced cell death rather than explosive cell proliferation, they tend to be indolent (slow-growing). In other tumors such as chronic lymphocytic leukemia (Chapter 12), it appears that BCL2 is upregulated due to loss of specific micro-RNAs that normally restrain BCL2 levels. Many other mechanisms leading to overexpression of anti-apoptotic members of the BCL2 family have been proposed in a wide variety of cancers.

Recognition of the mechanisms by which cancers evade cell death has stimulated several lines of targeted drug development. Restoration of p53 function in *TP53*-mutated tumors is a daunting problem (because of the inherent difficulty of "fixing" defective genes) but is possible in tumors in which p53 is inactive because of overexpression of its inhibitor, MDM2. Indeed, inhibitors of MDM2 that reactivate p53 and induce apoptosis in tumors with *MDM2* gene amplification are being tested in clinical trials. Even more impressive results have been generated with drugs that inhibit the function of anti-apoptotic members of the BCL2 family, particularly BCL2 itself. These drugs have potent activity against certain tumors characterized by BCL2 overexpression (such as chronic lymphocytic leukemia) and are beoming a standard part of the treatment of particular cancers.

KEY CONCEPTS

EVASION OF CELL DEATH

- Apoptosis can be initiated through intrinsic or extrinsic pathways, both of which result in the activation of a proteolytic cascade of caspases that destroys the cell.
- Lesions that incapacitate the intrinsic (mitochondrial) pathway appear to be most common in cancers.
- In greater than 85% of follicular B-cell lymphomas, the anti-apoptotic gene *BCL2* is overexpressed due to a (14;18) translocation.
- Overexpression of other BCL2 family members is also linked to cancer cell survival and drug resistance.

Limitless Replicative Potential: The Stem Cell–Like Properties of Cancer Cells

All cancers contain cells that are immortal and have limitless replicative potential. Some cell lines established from cancers have now been proliferating ceaselessly in laboratories for more than 60 years, and it is reasonable to expect that they will continue to grow for as long as there are scientists to tend to them. How can it be that cancer cells have seemingly discovered the proverbial fountain of eternal youth? The answers are not completely known, but three interrelated factors appear to be critical to the immortality of cancer cells: (1) evasion of senescence, (2) evasion of mitotic crisis, and (3) the capacity for self-renewal.

- *Evasion of senescence.* As discussed in Chapter 2, most normal human cells have the capacity to divide 60 to 70 times. After this, the cells become senescent, permanently leaving the cell cycle and never dividing again. The mechanisms that produce senescence are not well understood, but the senescent state is associated with upregulation of p53 and INK4a/p16 (perhaps in response to the accumulation of DNA damage over time). These are believed to contribute to senescence in part by maintaining RB in a hypophosphorylated state, which favors cell cycle arrest. As already discussed, the RB-dependent G_1/S cell cycle checkpoint is disrupted in virtually all cancers by a wide variety of acquired genetic and epigenetic aberrations that may allow cells to bypass senescence.
- *Evasion of mitotic crisis.* While cells that are resistant to senescence have increased replicative capacity, they are not immortal; instead, they eventually enter a phase referred to as *mitotic crisis* and die. Mitotic crisis has been ascribed to progressive shortening of *telomeres*, special DNA sequences at the ends of chromosomes that bind several types of protective protein complexes (Chapter 2). Most somatic cells do not express *telomerase*, the enzyme that is responsible for the maintenance of telomeres, and with each cell division their telomeres shorten. When the telomeric DNA is completely eroded, the exposed chromosome ends are "sensed" as double-stranded DNA breaks. If the affected cells have functional p53, the cell arrests its growth and may undergo apoptosis, but if p53 is dysfunctional, the nonhomologous end-joining pathway is activated and may join the "naked" ends of two chromosomes. The resulting dicentric chromosomes are broken during attempted mitotic segregation during anaphase, producing new double-stranded DNA breaks. The snowballing genomic damage caused by repeated "bridge-fusion-breakage" cycles eventually produces mitotic catastrophe and cell death (Fig. 7.32). Telomerase is expressed at very low levels in most somatic cells, and thus proliferating cells that escape from senescence are

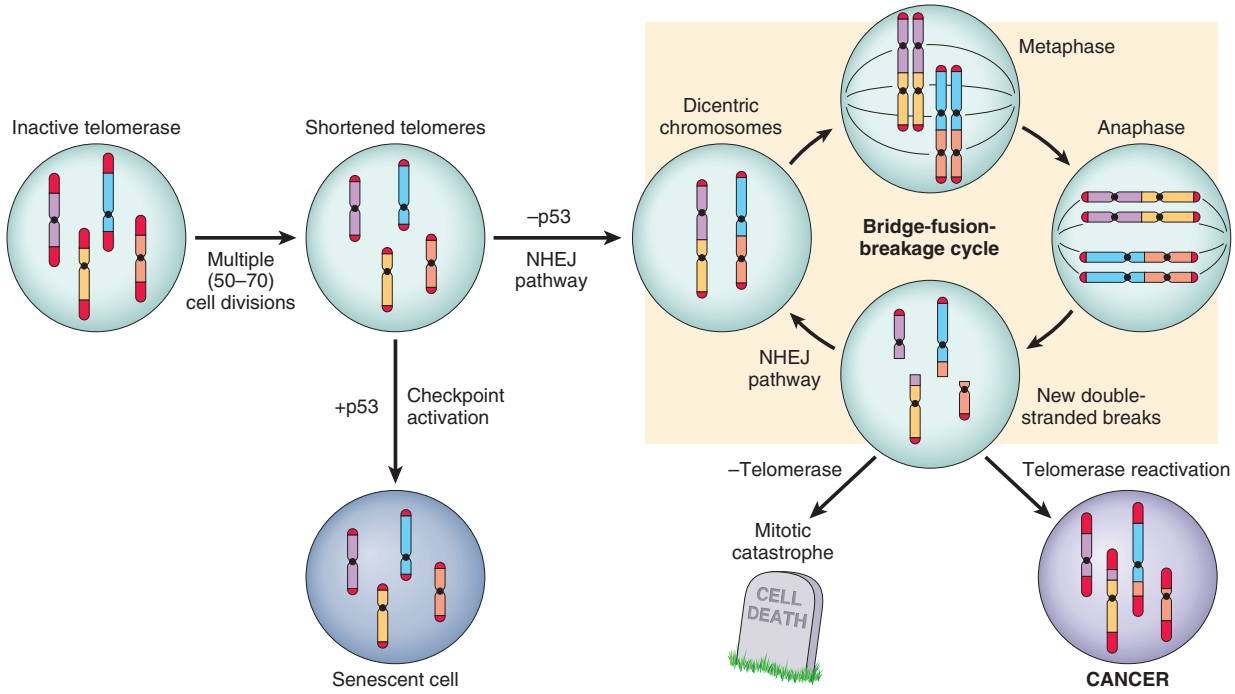

Figure 7.32 Escape of cells from senescence and mitotic catastrophe caused by telomere shortening. Replication of somatic cells, which do not express telomerase, leads to shortened telomeres. In the presence of competent checkpoints, cells undergo arrest and enter nonreplicative senescence. In the absence of checkpoints, DNA repair pathways such as the nonhomologous end joining *(NHEJ)* pathway are inappropriately activated, leading to the formation of dicentric chromosomes. At mitosis the dicentric chromosomes are pulled apart, generating random double-stranded breaks, which then activate DNA repair pathways, leading to the random association of double-stranded ends and the formation, again, of dicentric chromosomes. Cells undergo numerous rounds of this bridge-fusion-breakage cycle, which generates massive chromosomal instability and numerous mutations. If cells fail to reexpress telomerase, they eventually undergo mitotic catastrophe and death. Reexpression of telomerase allows the cells to escape the bridge-fusion-breakage cycle, thus promoting their survival and tumorigenesis.

likely to expire in this fashion. However, cells in crisis that reactivate telomerase can restore their telomeres and survive. Such cells may have suffered damage to oncogenes and tumor suppressor genes during crisis and are at high risk for malignant transformation. Alternatively, cancers may arise from stem cells (described later), which are long-lived in part because they express telomerase. Whatever the mechanism, telomere maintenance is seen in virtually all types of cancers. In 85% to 95% of tumors it is due to expression of telomerase. The remaining tumors use another mechanism to maintain their telomeres termed alternative lengthening of telomeres that depends on DNA recombination.

- *Self-renewal.* Unlike most cells, tissue stem cells and germ cells express telomerase, making them resistant to mitotic crisis, and also somehow avoid the genetic and epigenetic alterations that trigger senescence. In addition, long-lived stem cells possess another critical property, the capacity for self-renewal. In simple terms, self-renewal means that each time a stem cell divides at least one of the two daughter cells remains a stem cell. In a symmetric division, both daughter cells remain stem cells; such divisions may occur during embryogenesis, when stem cell pools are expanding, or during times of stress. In an asymmetric division, only one daughter cell remains a stem cell; in such circumstances, the non–stem cell daughter proceeds along some differentiation pathway, losing "stemness" but gaining one or more functions in the process. Cells in "transit" to a differentiated state are often highly proliferative, but they eventually differentiate, stop dividing, and may eventually become senescent or undergo apoptosis. The continued growth and maintenance of many tissues that contain short-lived cells, such as the formed elements of the bone marrow and blood and the epithelial cells of the gastrointestinal tract and skin, depend on a resident population of tissue stem cells that are capable of self-renewal. Following on this logic, because cancers are immortal and have limitless proliferative capacity, they too must contain cells that self-renew—so-called *cancer stem cells*. While there remains debate about the identity and number of stemlike cells in particular cancers, it is accepted that cells resembling stem cells must exist in all cancers.

Another open question is whether cancer stem cells arise from the transformation of tissue stem cells or from the conversion of conventional somatic cells to transformed cells with the acquired property of "stemness." The answer seems to be that both scenarios occur in different types of tumors, as exemplified by chronic myeloid leukemia and acute promyelocytic leukemia (Fig. 7.33). Recall also that expression of a small number of transcription factors can

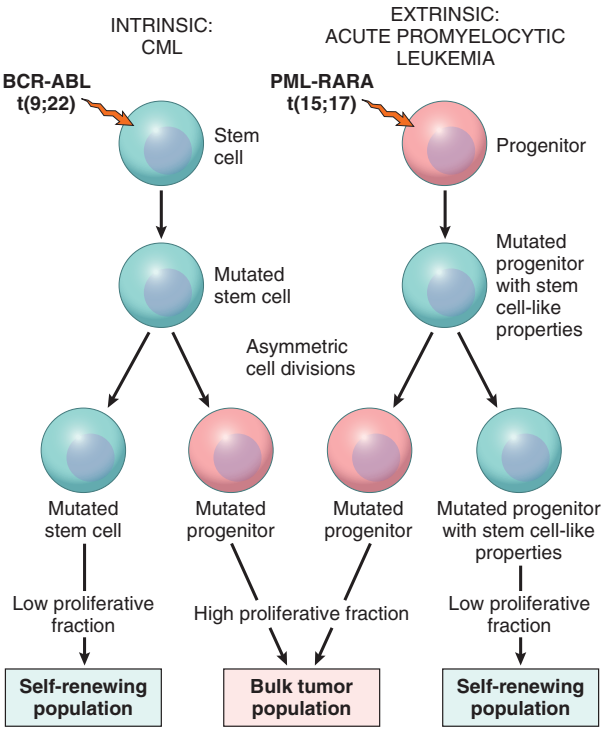

Figure 7.33 Origins of cells with self-renewing capacity in cancer. Cancer stem cells can arise from transformed tissue stem cells (e.g., hematopoietic stem cells in chronic myeloid leukemia *[CML]*) with intrinsic "stemness" or from proliferating cells that acquire a mutation that confers stemness (e.g., granulocyte progenitors in acute promyelocytic leukemia). In both instances, the cancer stem cells undergo asymmetric cell divisions that give rise to committed progenitors that proliferate more rapidly than the cancer stem cells; as a result, most of the malignant cells in both tumors lack self-renewing capacity.

result in the epigenetic reprogramming of a differentiated somatic cell such as a fibroblast into a pluripotent stem cell. Thus it is easy to imagine how mutations leading to misexpression of certain key transcription factors, such as MYC, might convert a somatic cell into a transformed cell with a capacity for self-renewal. A corollary of this idea is that, unlike normal stem cells and their more differentiated progeny, which have a fixed parent–offspring relationship, cancer cells within a tumor may be able to dedifferentiate to a stem cell–like state. Indeed, there is evidence that cancers can repopulate their stem cell pools from non–stem cell populations, further complicating efforts to precisely define and selectively target cancer stem cells.

Despite these uncertainties, the concept of cancer stem cells has important implications for cancer therapy. Most notably, if cancer stem cells are essential for tumor persistence, it follows that these cells must be eliminated to eradicate the tumor. It is hypothesized that like normal stem cells, cancer stem cells have a high intrinsic resistance to conventional therapies due to a low rate of cell division and the expression of factors such as multiple drug resistance-1 (MDR1) that counteract the effects of chemotherapeutic drugs. Thus the limited success of current therapies may in part be explained by their failure to kill the malignant stem cells that lie at the root of cancer.

KEY CONCEPTS

LIMITLESS REPLICATIVE POTENTIAL

- At least some cells in all cancers must be stem cell–like; these cells are sometimes referred to as cancer stem cells. These may arise through transformation of a normal stem cell or through acquired genetic lesions that impart a stem-like state on a more mature cell.
- Cancer cells acquire lesions that inactivate senescence signals and reactivate telomerase, which act together to convey limitless replicative potential.

Angiogenesis

Even if a solid tumor possesses all the genetic aberrations that are required for malignant transformation, it cannot enlarge beyond 1 to 2 mm in diameter unless it has the capacity to induce angiogenesis. Like normal tissues, the "health" of tumors requires delivery of oxygen and nutrients and removal of waste products; presumably the 1- to 2-mm size limit is the maximal distance across which oxygen, nutrients, and waste can diffuse from existing blood vessels. Growing cancers stimulate neoangiogenesis, during which new vessels sprout from capillaries (Chapter 3). Neovascularization supplies needed nutrients and oxygen, and proliferating endothelial cells also stimulate the growth of adjacent tumor cells by secreting growth factors such as insulin-like growth factors (IGFs) and PDGF. While the resulting tumor vasculature is effective at delivering nutrients and removing wastes, it is not entirely normal; the vessels are leaky and dilated and have a haphazard pattern of connection, features that can be appreciated on angiograms. By permitting tumor cells ready access to these abnormal vessels, angiogenesis also contributes to metastasis. Angiogenesis is thus an essential facet of malignancy.

How do growing tumors develop a blood supply? The current paradigm is that **angiogenesis is controlled by a balance between angiogenesis promoters and inhibitors; in angiogenic tumors this balance is skewed in favor of promoters.** Early in their development, most human tumors do not induce angiogenesis. Starved of nutrients, these tumors remain small or in situ, possibly for years, until an *angiogenic switch* terminates this stage of quiescence. The molecular basis of the angiogenic switch involves increased local production of angiogenic factors and/or loss of angiogenic inhibitors. The sources of these factors include tumor cells, infiltrating inflammatory cells (e.g., macrophages) or other tumor-associated stromal cells, and the ECM. In the case of tumor cells, several alterations enhance the production of pro-angiogenic factors:

- *Relative lack of oxygen due to hypoxia stabilizes HIF1α,* an oxygen-sensitive transcription factor mentioned earlier that activates the transcription of the pro-angiogenic cytokines VEGF and basic fibroblast growth factor (bFGF). These factors create an angiogenic gradient that stimulates the proliferation of endothelial cells and guides the growth of new vessels toward the tumor (Chapter 3).
- *Driver mutations in certain tumor suppressors and oncogenes favor angiogenesis.* For example, p53 stimulates the expression of anti-angiogenic molecules such as thrombospondin-1

and represses the expression of pro-angiogenic molecules such as VEGF. Thus, loss of p53 not only removes cell cycle checkpoints and alters tumor cell metabolism but also provides a more permissive environment for angiogenesis. Conversely, gain-of-function mutations in *RAS* or *MYC* upregulate the production of VEGF.

- *Proteases,* which may be elaborated by either tumor cells or by stromal cells, also influence the local balance of angiogenic and antiangiogenic factors. Many proteases release bFGF from the ECM, which constitutes a storage site for this factor, while other proteases release antiangiogenic factors such as angiostatin and endostatin through proteolytic cleavage of plasminogen and collagen, respectively.

The idea that angiogenesis is essential for solid tumors to grow to clinically significant sizes provided a powerful impetus for the development of therapeutic agents that block angiogenesis. These agents are now a part of the armamentarium that oncologists use against cancers. A cardinal example is bevacizumab, a monoclonal antibody that neutralizes VEGF activity and is approved for treatment of multiple cancers. However, angiogenesis inhibitors have not been nearly as effective as was hoped; they can prolong life, but usually for only a few months and at very high financial cost. The mechanisms that underlie the persistence and ultimate progression of cancers in the face of angiogenesis inhibitors are not yet clear. Improvements will be possible only with greater understanding of the "escape routes" through which tumor cells sidestep the effects of the angiogenesis inhibitors that are now in use.

KEY CONCEPTS

ANGIOGENESIS

- Vascularization of tumors is essential for their growth and is controlled by the balance between angiogenic and anti-angiogenic factors that are produced by tumor and stromal cells.
- Hypoxia triggers angiogenesis through the actions of HIF1α on the transcription of the pro-angiogenic factor VEGF.
- Many other factors regulate angiogenesis; for example, p53 induces synthesis of the angiogenesis inhibitor thombospondin-1, while RAS, MYC, and MAPK signaling all upregulate VEGF expression and stimulate angiogenesis.
- VEGF inhibitors are used to treat a number of advanced cancers and prolong the clinical course, but are not curative.

Invasion and Metastasis

Invasion and metastasis are the major causes of cancer-related morbidity and mortality and hence are the subjects of intense investigation. Local invasion of tumor cells may damage or destroy vital structures and is a prerequisite for distant spread. Studies in mice and humans reveal that although many of these locally invasive cells enter the bloodstream each day, very few produce metastases. Why is the metastatic process so inefficient? The answer is uncertain but undoubtedly relates to the complexity of the process. For cancer cells to emerge from a primary mass, enter blood vessels or lymphatics, and produce a secondary growth at a distant site, they must go through a series of steps that involve an intricate interplay between tumor cells and many different types of host cells and factors (summarized in Fig. 7.34). At each point in this sequence, the breakaway cells must overcome the challenges of avoiding immune defenses (discussed later) and adapting to a microenvironment (e.g., lymph node, bone marrow, or brain) that is quite different from that of the site of origin of the tumor. The complexity of this series of events may explain why individual "metastasis genes" have not been found; it may be that the "metastatic phenotype" requires the

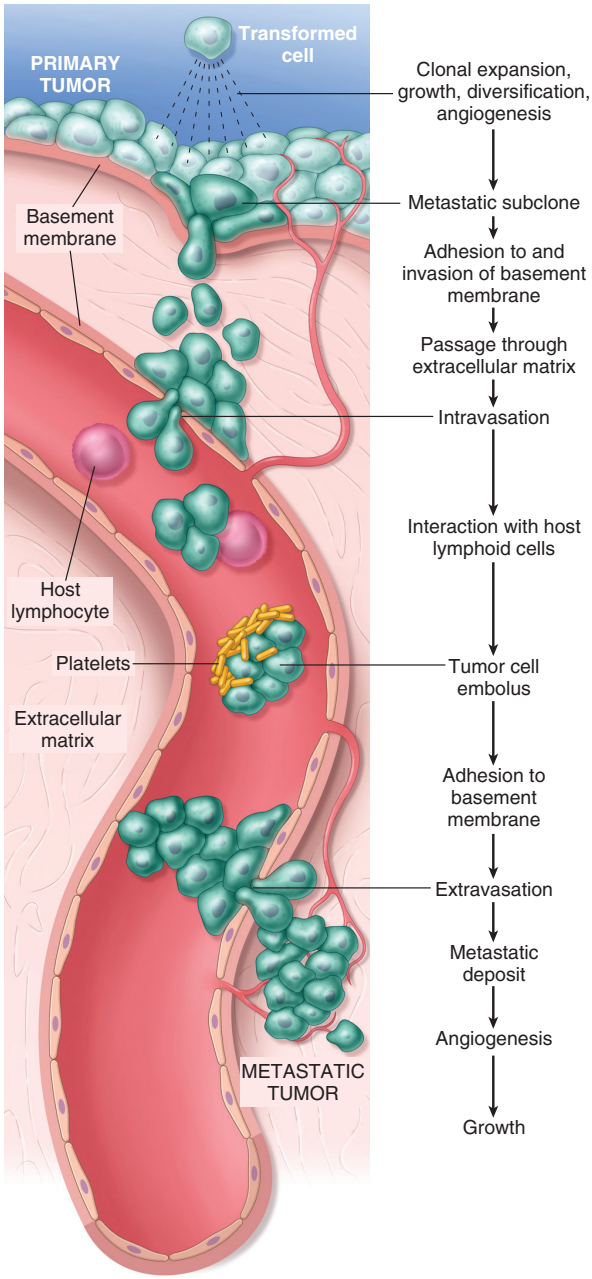

Figure 7.34 The metastatic cascade. Sequential steps involved in the hematogenous spread of a tumor.

accumulate complementary genetic and epigenetic alterations that collectively promote the metastatic cascade. This set of "skills" may be present only in rare cells, or might even require a collaboration between subclones, with each providing some needed function. This latter idea implies that successful metastases may arise from cells that migrate as cohesive groups, for which there is some evidence.

In the discussion that follows, the metastatic cascade is divided into two phases: (1) invasion of the ECM and (2) vascular dissemination, tissue homing, and colonization. Throughout, we touch on some of the proposed molecular mechanisms that underlie the process.

Invasion of Extracellular Matrix

The structural organization and function of normal tissues is determined by interactions between cells and the ECM. As discussed in Chapter 1, tissue compartments are separated from each other by two types of ECM, basement membrane and interstitial connective tissue, each made up of different combinations of collagens, glycoproteins, and proteoglycans. As shown in Fig. 7.34, tumor cells interact with the ECM at several stages in the metastatic cascade. To metastasize, carcinoma cells must breach the underlying basement membrane, traverse the interstitial connective tissue, and ultimately gain access to the circulation by penetrating the vascular basement membrane. This process is repeated in reverse when tumor cells extravasate at a distant site. Invasion of the ECM initiates the metastatic cascade and is an active process that can be resolved into several steps (Fig. 7.35):

- "Loosening up" of tumor cell–tumor cell interactions
- Degradation of ECM
- Attachment to "remodeled" ECM components
- Migration and invasion of tumor cells

Dissociation of cancer cells from one another is often the result of alterations in intercellular adhesion molecules and is the first step in the process of invasion. Normal epithelial cells are tightly glued to each other and to the ECM by a variety of adhesion molecules. As discussed earlier, *E-cadherins* are transmembrane glycoproteins that mediate the homotypic adhesion of epithelial cells, serving both to hold the cells together and to relay signals between cells. In several epithelial tumors including certain adenocarcinomas of the stomach and breast, E-cadherin function is lost due to pathogenic mutations, and in many other epithelial cancers it is hypothesized that E-cadherin expression is silenced, at least transiently, through a process called *epithelial-mesenchymal transition (EMT)*. It is postulated that EMT is integral to the metastasis of carcinomas, particularly breast and prostate cancers. EMT is controlled by the transcription factors SNAIL and TWIST and is defined not only by the downregulation of epithelial markers (e.g., E-cadherin) but also by the concomitant upregulation of mesenchymal markers (e.g., vimentin and smooth muscle actin), changes that are believed to favor the development of a promigratory phenotype that is essential for metastasis.

Degradation of the basement membrane and interstitial connective tissue is the second step in invasion. Tumor cells may accomplish this by secreting proteolytic enzymes or by inducing stromal cells (e.g., fibroblasts and inflammatory cells) to do so. Many different proteases such as matrix metalloproteinases (MMPs), cathepsin D, and urokinase plasminogen activator are overexpressed in tumors and have been implicated in tumor cell invasion. MMPs regulate tumor invasion not only by remodeling the basement membrane and interstitial connective tissue, but also by releasing factors that contribute to the malignant behavior

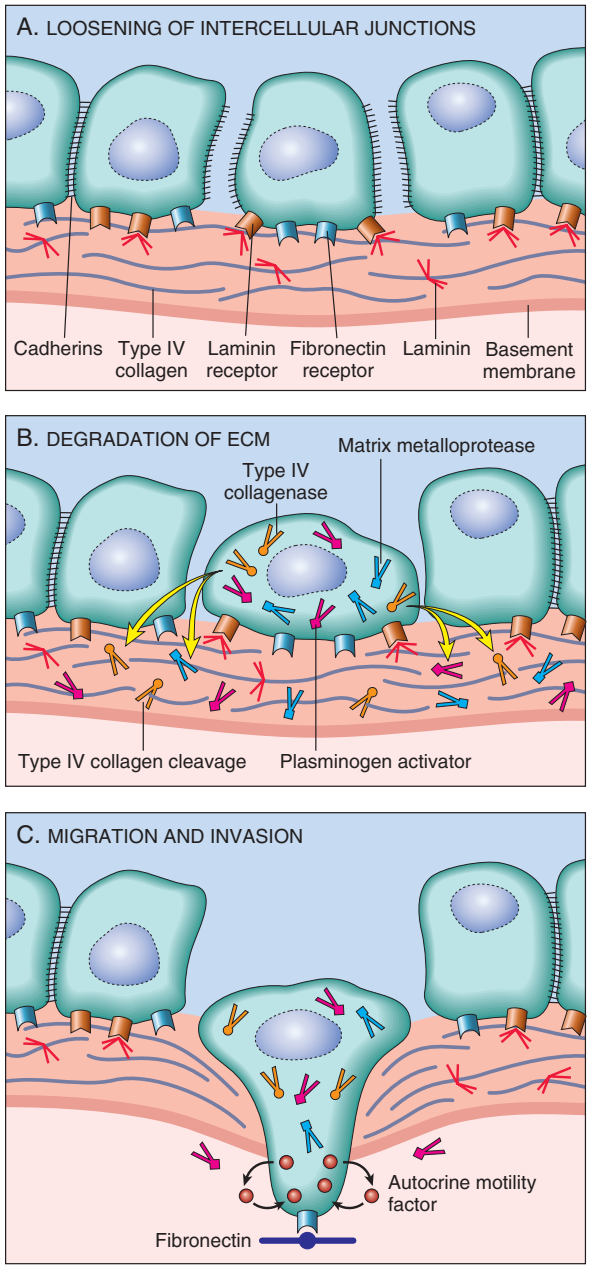

Figure 7.35 Sequence of events in the invasion of epithelial basement membranes by tumor cells. Tumor cells detach from each other because of reduced adhesiveness and attract inflammatory cells. Proteases secreted from tumor cells and inflammatory cells degrade the basement membrane. Binding of tumor cells to proteolytically generated binding sites and tumor cell migration follow. *ECM*, Extracellular matrix.

of cancers. For example, MMP-9, a gelatinase that cleaves type IV collagen found within the epithelial and vascular basement membrane, also stimulates the release of VEGF from ECM-sequestered pools and generates collagen and proteoglycan cleavage products with chemotactic, angiogenic, and growth-promoting effects. Benign tumors of the breast, colon, and stomach have little MMP-9 activity, whereas their malignant counterparts overexpress this enzyme. Concurrently the concentrations of metalloproteinase inhibitors are also reduced in many cancers, further tilting the balance toward tissue degradation.

The third step in invasion involves changes in how tumor cells attach to ECM proteins. Tumor cells demonstrate complex changes in the expression of integrins, which you will recall are transmembrane proteins that participate in adhesion of cells to other cells and to ECM (Chapter 3). In normal epithelial cells, integrins that bind basement membrane laminin and collagens are strictly restricted to the basal aspect of the cell; these receptors help to maintain the cells in a resting, polarized state. Loss of adhesion in normal cells leads to induction of apoptosis, but free tumor cells are resistant to this form of cell death (termed *anoikis,* meaning without a home), in part because of expression of other integrins that mitigate the loss of adhesion to ECM, apparently by transmitting signals that promote cell survival. Additionally, the matrix itself is modified in ways that promote invasion and metastasis. For example, cleavage of the basement membrane proteins collagen IV and laminin by MMP-2 or MMP-9 generates novel sites that bind to receptors on tumor cells and stimulate migration.

Locomotion is the final step of invasion, propelling tumor cells through the degraded basement membranes and zones of matrix proteolysis. Migration is a multistep process that involves many families of receptors and several signaling pathways that eventually impinge on the actin cytoskeleton. Cells must attach to the matrix at their leading edge, detach from the matrix at their trailing edge, and contract the actin cytoskeleton to ratchet forward. Such movement seems to be stimulated and directed by several types of factors, which likely vary among different types of tumors. These include:

- Tumor cell–derived cytokines including chemokines and growth factors (e.g., insulin-like growth factors), which act as autocrine motility factors
- Cleavage products of matrix components (e.g., collagen, laminin)
- Stromal cell–derived paracrine factors such as hepatocyte growth factor/scatter factor, which binds to the receptor tyrosine kinase MET on tumor cells and stimulates motility

This initial phase of metastasis culminates in penetration through the endothelial basement membrane and transmigration into the vascular space. Throughout this phase of the process, tumor cells interact not only with ECM but also with several types of stromal cells, including innate and adaptive immune cells, fibroblasts, and endothelial cells. Details remain to be worked out, but it is clear that "successful" invaders induce signals that modify stromal cells in ways that support their malignant behavior. For example, under the influence of invading cancer cells, so-called cancer-associated fibroblasts alter their expression of genes that encode ECM molecules, proteases, protease inhibitors, and various growth factors. It is easy to imagine that as tumors evolve over time, they come to be dominated by cancer cells that are most effective at co-opting the complex, ever-changing tumor microenvironment to serve their malignant purposes.

Vascular Dissemination, Homing, and Colonization

Once in the circulation, tumor cells are vulnerable to destruction by a variety of mechanisms including mechanical shear stress, apoptosis due to anoikis, and innate and adaptive immune defenses. Nevertheless, viable circulating tumor cells are not rare in patients with solid tumors such as carcinomas. What then separates those few cells that give rise to metastases from the large number of cells that fail? Also, what determines why metastases ultimately appear where they do in the body?

While definitive answers to these questions are not known, clues have emerged. One idea that has gained support proposes that the circulating cells that establish metastases are much more likely to migrate as multicellular aggregates than as single cells. Clumping of tumor cells in the blood is promoted by homotypic interactions as well as heterotypic interactions between tumor cells and blood elements, particularly platelets (see Fig. 7.34), which are believed to enhance tumor cell survival in the circulation. Tumor cells may also express anionic substances such as polyphosphate that activate factor XII (contact factor), resulting in fibrin deposition and further stabilization of tumor emboli, which may enhance the ability of the cells to arrest en masse within capillary beds. Another potential advantage possessed by tumor cells circulating as a group is that they may be far more likely than any single cell to possess all the properties that are needed to establish a metastasis. Among these essential attributes is the presence of cells with stem cell–like properties, which may contribute not only to the relentless growth of metastatic lesions but also to the "plasticity" that is needed for metastatic cells to adapt to growth in a new microenvironment.

Where circulating tumor cells arrest and eventually form clinically significant metastatic deposits appears to relate to three factors: (1) location and vascular drainage of the primary tumor; (2) tropism of particular kinds of tumor cells for specific tissues; and (3) escape from tumor dormancy. The first is a matter of simple anatomy; thus colon carcinomas are far more likely to give rise to metastases in the liver, the first organ downstream of the tumor, than to metastases elsewhere. However, many exceptions to this "rule" exist. For example, carcinomas of the prostate and breast preferentially spread to bone, bronchogenic carcinomas tend to involve the adrenals and the brain, and neuroblastomas spread to the liver and bones. Such organ tropism may be related to the following mechanisms:

- Tumor cells may express adhesion molecules whose ligands are found preferentially on the endothelial cells of the target organ. Of interest in this regard is the CD44 adhesion molecule, which is expressed on normal T lymphocytes and is used by these cells to migrate to selective sites in lymphoid tissues. Such migration is accomplished by the binding of CD44 to hyaluronate on high endothelial venules. Solid tumors also often express

CD44, which appears to enhance their spread to lymph nodes and other metastatic sites.
- Some cancer cells express chemokine receptors, which may guide tumor cells to tissues expressing chemokines, much in the way chemokines normally act as attractants for cells of the immune system.
- Some tissues may provide a favorable "soil" for the growth of tumor seedlings. According to this "seed-soil" hypothesis, originally proposed by Paget, the ability of tumor cells originating from a particular site to adapt to a foreign environment may be limited to certain tissue types. A corollary of this idea is that target tissues in which metastasis is rare despite a rich vascular supply (e.g., skeletal muscle, spleen) constitute nonpermissive environments—"unfavorable soil," so to speak.

Once arrested at distant sites, extravasation of tumor cells involves transmigration between endothelial cells followed by egress through the basement membrane. Little is known about how this process occurs, and the mechanisms may differ depending on whether the endothelium is fenestrated (as in tissues such as the liver and bone marrow) or is held together by tight junctions (as in the brain). Extravasation requires the action of adhesion molecules (integrins, laminin receptors), proteolytic enzymes, and chemokines, which may be derived from tumor cells or from innate immune cells such as monocytes and neutrophils.

Even when metastatic cells take root and survive within distant tissues, they may fail to grow. This phenomenon, called *tumor dormancy,* is well described in melanoma and in breast and prostate cancer. Although the molecular mechanisms of productive colonization are still being unraveled, a consistent theme seems to be that tumor cells secrete cytokines, growth factors, and ECM molecules that act on the resident stromal cells, which in turn make the metastatic site habitable for the cancer cell. For example, breast cancer cells that are metastatic to bone often secrete parathyroid hormone–related protein (PTHRP), which stimulates osteoblasts to make RANK ligand (RANKL). RANKL then activates osteoclasts, which degrade the bone matrix and release growth factors embedded within it, like IGF and TGF-β. These in turn bind to receptors on the cancer cells, activating signaling pathways that support the growth and survival of the cancer cells. It is likely that many similar feedback loops between metastatic tumor cells and stromal cells await discovery.

KEY CONCEPTS

INVASION AND METASTASIS

- Ability to invade tissues, a hallmark of malignancy, occurs in four steps: loosening of cell-cell contacts, degradation of ECM, attachment to novel ECM components, and migration of tumor cells.
- Cell-cell contacts are lost by the inactivation of E-cadherin through a variety of pathways.
- Basement membrane and interstitial matrix degradation is mediated by proteolytic enzymes secreted by tumor cells and stromal cells, such as MMPs and cathepsins.
- Proteolytic enzymes also release growth factors sequestered in the ECM and generate chemotactic and angiogenic fragments from cleavage of ECM glycoproteins.
- The metastatic site of many tumors can be predicted by the location of the primary tumor. Many tumors arrest in the first capillary bed they encounter (lung and liver, most commonly).
- Some tumors show organ tropism, probably due to expression of adhesion or chemokine receptors whose ligands are expressed by endothelial cells at the metastatic site.
- Genes that promote epithelial-mesenchymal transition (EMT), like *TWIST* and *SNAIL*, may be important metastasis genes in epithelial tumors.

Evasion of Immune Surveillance

Long one of the "holy grails" of oncology, the promise of therapies that enable the host immune system to recognize and destroy cancer cells is finally coming to fruition, largely due to a clearer understanding of the ways by which cancer cells evade the host response. Paul Ehrlich first conceived the idea that tumor cells can be recognized as "foreign" and eliminated by the immune system. Subsequently, Lewis Thomas and Macfarlane Burnet formalized this concept by coining the term *immune surveillance,* which implies that a normal function of the immune system is to constantly "scan" the body for emerging malignant cells and destroy them. This idea has been supported by many observations—the direct demonstration of tumor-specific T cells and antibodies in patients; data showing that the density and quality of immune infiltrates in cancers often correlate with outcome; the increased incidence of certain cancers in immunodeficient people and mice; and most recently and most directly, the response of advanced cancers to therapeutic agents that act by stimulating latent host T-cell responses (described later).

Assuming the immune system is capable of recognizing and eliminating nascent cancers, it follows that the tumors that grow out in immunocompetent individuals must be composed of cells that are either invisible to the host immune system or that activate mechanisms that suppress host immunity. In support of this concept, it is now evident that therapeutic agents that neutralize these mechanisms can lead to tumor regression, even in patients with advanced cancers. These encouraging clinical responses constitute strong evidence that evasion of host immunity is indeed a hallmark of many, if not all, human cancers.

The following section explores some of the important questions about tumor immunity: What is the nature of tumor antigens? What host effector systems recognize tumor cells? How do tumors evade these host mechanisms? And how can immune reactions against tumors be exploited therapeutically?

Tumor Antigens

Malignant tumors express various types of molecules that may be recognized by the immune system as foreign antigens (Fig. 7.36). Tumor antigens that elicit an immune response have been demonstrated in many experimentally induced tumors and in some human cancers. It appears that protein antigens that elicit CD8+ cytotoxic T-cell

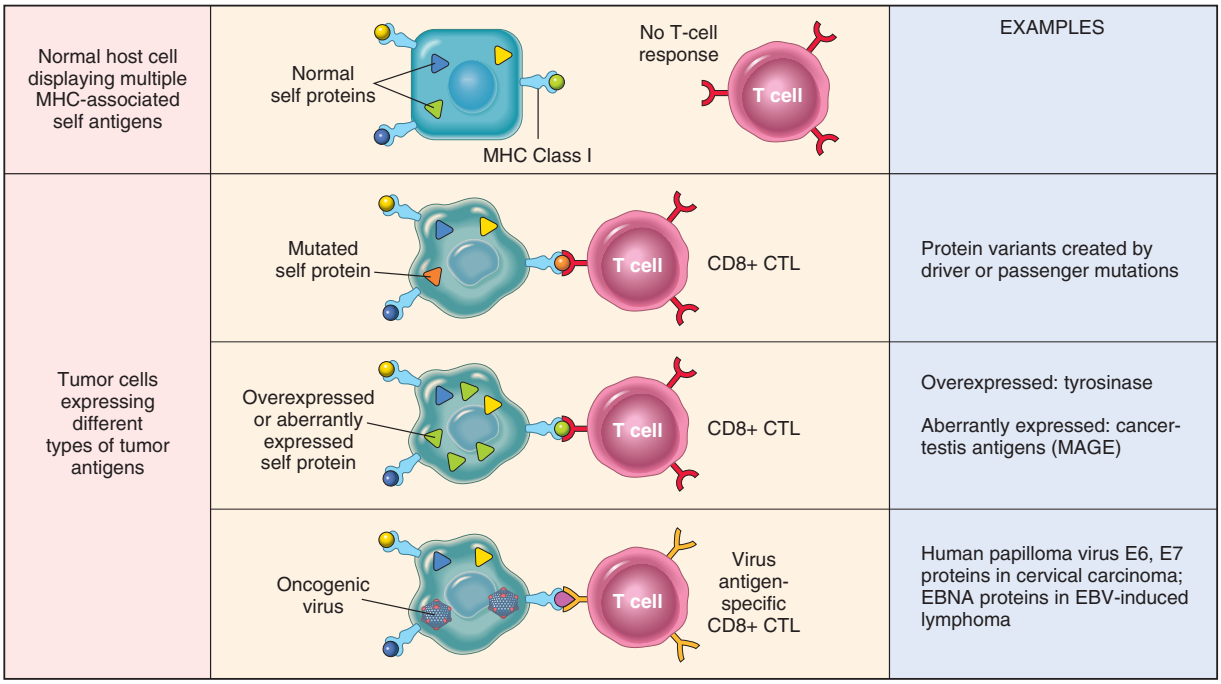

Figure 7.36 Tumor antigens recognized by CD8+ T cells. *EBV,* Epstein-Barr virus; *MHC,* major histocompatibility complex. (Modified from Abbas AK, Lichtman AH: *Cellular and Molecular Immunology,* ed 5, Philadelphia, 2003, WB Saunders.)

responses are most relevant for protective antitumor immunity. These protein antigens can be classified according to their source and molecular structure as follows:

- *Neoantigens produced from genes bearing passenger and driver mutations.* As discussed earlier, neoplastic transformation results from driver mutations in cancer genes; these mutated genes encode variant proteins that have never been seen by the immune system and may thus be recognized as nonself. Additionally, because of the enabling hallmark feature of genetic instability, cancers often have a high burden of passenger mutations throughout their genomes. Although these mutations are neutral in terms of cancer cell fitness and thus unrelated to the transformed phenotype, some by chance fall in the coding sequences of genes and give rise to protein variants that serve as tumor antigens (provided the mutation is in an epitope that binds to the MHC molecules of the individual). Notably, the size of the mutational load in a particular tumor, as deduced from DNA sequencing of tumor genomes, correlates well with the strength of the host CD8+ cytotoxic T-cell response and the effectiveness of immunomodulatory therapies (described later).
- *Overexpressed or aberrantly expressed normal cellular proteins.* Tumor antigens may also be normal cellular proteins that are overexpressed or aberrantly expressed in tumor cells. An example of an overexpressed protein that may be antigenic is tyrosinase, an enzyme involved in melanin biosynthesis that is expressed only in normal melanocytes and melanomas. It may be surprising that the immune system is able to respond to this normal self antigen. The probable explanation is that tyrosinase is normally produced in such small amounts and in so few healthy cells that it is not recognized by the immune system and fails to induce tolerance. Another group of tumor antigens are proteins that are aberrantly expressed in cancer cells at levels much greater than those seen in normal tissues. This may occur because of gene amplification or because of acquired epigenetic alterations that reactivate genes that are normally silenced in adult tissues. Some genes in this class encode *cancer-testis antigens,* proteins that are normally expressed only in testicular germ cells. Although the protein is present in the testis, it is not expressed on the cell surface in an antigenic form because sperm do not express major histocompatibility complex (MHC) class I antigens. Thus, for all practical purposes, these antigens are tumor-specific. Prototypic of this group is the melanoma antigen gene (MAGE) family. Although originally described in melanomas, MAGE antigens are expressed by a variety of tumor types.
- *Tumor antigens produced by oncogenic viruses.* In several cancers associated with ongoing active or latent viral infections (described later), the responsible viruses encode viral proteins that are recognized as foreign by the immune system. Cytotoxic T lymphocytes (CTLs) play a vital role in surveillance against virus-induced tumors by recognizing viral antigens and killing virus-infected cells. The importance of this immune mechanism is made evident by the high incidence of virally induced cancers that is observed in patients with inherited or acquired T-cell immunodeficiency. Many of these tumors are caused by Epstein-Barr virus (EBV) or HPV, DNA viruses that carry potent viral oncogenes.

Antitumor Effector Mechanisms

The principal immune mechanism of tumor eradication is killing of tumor cells by CTLs specific for tumor antigens. This mechanism predominates because the majority of tumor neoantigens consist of mutated gene products or viral proteins that are endogenously synthesized and presented in the context of MHC class I molecules, enabling their recognition by CTLs. Although sera from cancer patients may contain antibodies that recognize tumors, there is little evidence that they have any protective role under physiologic conditions. The cellular effectors that mediate immunity are described in Chapter 6. Here we focus on the CTL response to tumor antigens.

CTL responses against tumors are initiated by recognition of tumor antigens on host antigen presenting cells (APCs). Many cancers, particularly those that are rapidly growing, have a high fraction of cells that are undergoing cell death, either by apoptosis or necrosis. Dendritic cells and macrophages in the tumor microenvironment ingest tumor cells or released tumor antigens and migrate to draining lymph nodes. Here, they present the antigens in the context of MHC class II molecules and, through a mechanism called cross-presentation, in the context of MHC class I molecules (Fig. 7.37), allowing the antigens to be recognized by naïve CD8+ CTLs. Activation of antigen-specific CTLs also requires costimulatory molecules, which are upregulated on APCs presumably by "danger signals" released from damaged or necrotic tumor cells (DAMPs). Once activated by interaction with APCs, tumor-specific CTLs can migrate from lymph nodes to the tumor and kill tumor cells, directly and serially, without any assistance from other cell types.

The ability of CTLs to kill tumor cells independent of other cell types and factors underlies the ferocious antitumor activity of CTLs engineered to express chimeric antigen receptors (so-called CAR-T cells) against lineage-specific surface antigens found on certain tumors. For example, CAR-T cells specific for B-cell antigens are highly active against B-cell tumors but also annihilate normal B cells and release sufficient inflammatory cytokines to cause substantial morbidity and sometimes the death of the patient.

Although CTLs appear to have a preeminent role, other mechanisms also may play a role in tumor immunity. Antitumor CD4+ T-cell responses have been detected in patients, and increased numbers of CD4+ effector T cells, especially Th1 cells, in tumor infiltrates have been associated with a better prognosis in certain cancers, such as colorectal carcinoma. In experimental systems, natural killer (NK) cells and activated macrophages are capable of killing tumor cells. After activation with interleukin (IL)-2 and IL-15, NK cells can lyse a wide range of human tumors, including those that are nonimmunogenic for T cells due to loss of expression of MHC class I molecules. The ability of NK cells to kill tumor cells requires no prior sensitization, suggesting that they might constitute a first line of defense. While the importance of NK cells in host responses against spontaneous tumors is still not well established, cytokines that activate NK cells are being used for immunotherapy. Activated macrophages also exhibit cytotoxicity against tumor cells in vitro. Interferon-γ, a cytokine secreted by T cells and NK cells, is a potent activator of macrophages and may allow macrophages to kill tumors by mechanisms similar to those used to kill microbes (e.g., production of reactive oxygen species; see Chapter 3).

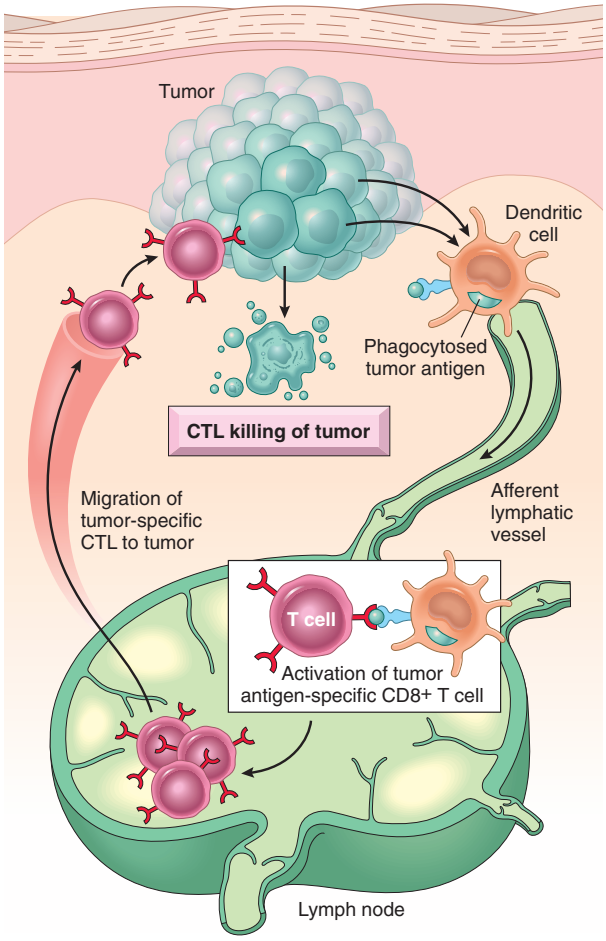

Figure 7.37 Cross-presentation of tumor antigens and induction of CD8+ cytotoxic T cell antitumor response. (Modified from Abbas AK, Lichtman AH: *Cellular and Molecular Immunology*, ed 8, Philadelphia, 2017, Elsevier.)

Mechanisms of Immune Evasion by Cancers

Immune responses often fail to check tumor growth because cancers evade immune recognition or resist immune effector mechanisms. Since cancer is all too common in persons who do not suffer from any overt immunodeficiency, it is evident that tumor cells must have ways to escape or evade the immune system in immunocompetent hosts. Several mechanisms appear to be operative (Fig. 7.38).

- *Selective outgrowth of antigen-negative variants.* During tumor progression, strongly immunogenic antigen-expressing subclones may be eliminated, and tumor cells that survive are those that have lost their antigens. If tumor cells express a large number of neoantigens, it is unlikely that all can be lost, but the same goal may be accomplished by other strategies.

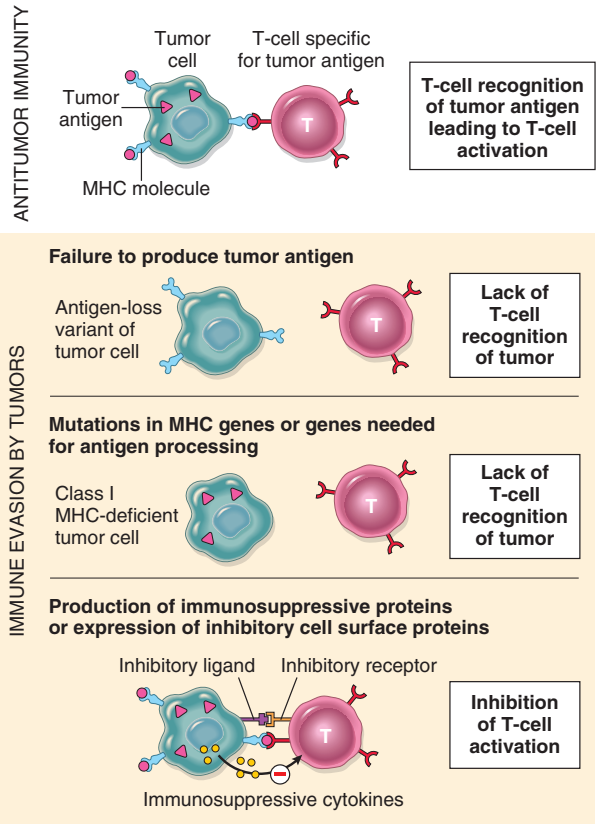

Figure 7.38 Mechanisms by which tumors evade the immune system. Tumors may evade immune responses by losing expression of antigens or major histocompatibility complex *(MHC)* molecules or by producing immunosuppressive cytokines or ligands such as PD-L1 for inhibitory receptors on T cells. (From Abbas AK, Lichtman AH, Pillai S: *Cellular and Molecular Immunology*, ed 7, Philadelphia, 2012, WB Saunders.)

- *Loss or reduced expression of MHC molecules.* Tumor cells may fail to express normal levels of HLA class I molecules, thereby losing the ability to display cytosolic antigens and escaping attack by cytotoxic T cells. Such cells, however, may trigger NK cells if the tumor cells express ligands for NK cell activating receptors.
- *Engagement of pathways that inhibit T-cell activation.* Tumor cells actively inhibit tumor immunity by upregulating negative regulatory "checkpoints" that suppress immune responses. Through a variety of mechanisms, tumor cells may promote the expression of the inhibitory receptor CTLA-4 on tumor-specific T cells. CTLA-4 binds to and removes its ligands, the B7 molecules, from APCs, thus reducing the engagement of the activating costimulatory receptor CD28. This not only prevents sensitization but also may induce long-lived unresponsiveness in tumor-specific T cells. Tumor cells also may upregulate the expression of PD-L1 and PD-L2, cell surface proteins that activate the programmed death-1 (PD-1) receptor on effector T cells. PD-1, like CTLA-4, inhibits T-cell activation. In some instances, overexpression of PD-L1 and PD-L2 is caused by amplification or translocation of the PD-L1 and PD-L2 genes, placing them in the pantheon of true oncogenes. Based on promising results in clinical trials, antibodies that restore T-cell function by blocking CTLA-4, PD-L1, or inhibitory PD-1 receptors are now approved for treatment of patients with advanced stage solid tumors and certain forms of lymphoma. The success of these agents has led to a new paradigm in cancer immunotherapy, sometimes called "checkpoint blockade," which is centered on the idea that agents that remove the "brakes" (checkpoints) imposed by tumors on host antitumor immune responses can be highly effective in treating cancer (Fig. 7.39).
- *Secretion of immunosuppressive factors.* Tumors may secrete several products that inhibit the host immune response. TGF-β is secreted in large quantities by many tumors and is a potent immunosuppressant. Many other soluble factors produced by tumors are also suspected of inhibiting the host immune response, including IL-10, prostaglandin E_2, certain metabolites derived from tryptophan, and VEGF, which can inhibit the movement of T cells from the vasculature into the tumor bed.
- *Induction of regulatory T cells (Tregs).* Some studies suggest that tumors produce factors that favor the development of immunosuppressive Tregs, which could also contribute to "immunoevasion."

Thus, it seems that there is no dearth of mechanisms by which tumor cells can outwit the host immune system. Nevertheless, the aforementioned response of tumors to immunomodulatory agents, such as antibodies that block CTLA-4 and PD-1, has generated tremendous excitement around the potential of rationally designed cancer immunotherapy. One of the remarkable features of this therapy is that it can achieve long-term remission and possibly even cures (which is not likely with any other treatment), perhaps because long-lived tumor-specific memory T cells are activated in the treated patients. The major challenges now are to determine which immune evasion mechanisms are most important in the cancers of individual patients and to develop a broader set of therapies that stymie various evasion mechanisms and thereby induce effective host immunity. In this regard, the treatment of human cancers with highly specific checkpoint inhibitor antibodies affords the opportunity to develop a mechanistic understanding of certain aspects of tumor response and resistance. Lessons learned to date from clinical trials conducted with these inhibitors include the following:

- *Tumor neoantigen burden is a good predictor of response.* Tumors that have deficiencies in mismatch repair enzymes, which normally correct errors in DNA replication that lead to point mutations, have the highest mutation burdens of all cancers, and these cancers are most likely to respond to checkpoint blockade therapy. Based on this observation, anti–PD-1 therapy is now approved for all metastatic tumors that have mismatch repair deficiency, which leads to a high mutational burden, regardless of histologic type.
- *Only a subset of tumors (25% to 40%) responds to checkpoint inhibitors,* probably because nonresponding tumors rely on evasion strategies other than engaging checkpoint pathways. For example, tumors that do not express PD-L1, or do not have exhausted PD-1–positive CD8+ T-cell infiltrates, are not likely to respond to anti–PD-1 or

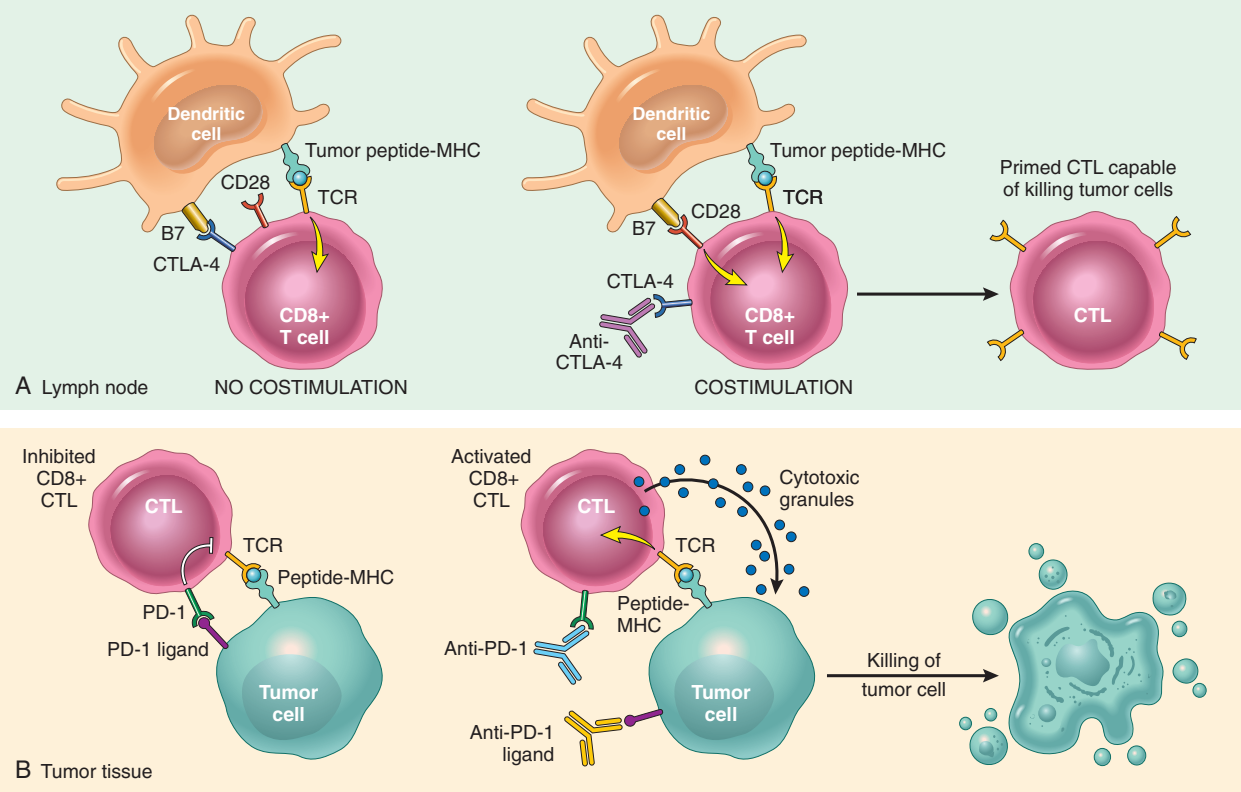

Figure 7.39 Activation of host antitumor immunity by checkpoint inhibitors. (A) Blockade of the CTLA4 surface molecule with an inhibitor antibody allows cytotoxic CD8+ T cells *(CTLs)* to engage B7 family coreceptors, leading to T-cell activation. (B) Blockade of PD-1 receptor or PD-1 ligand by inhibitory antibodies abrogates inhibitory signals transmitted by PD-1, again leading to activation of CTLs. *MHC*, Major histocompatibility complex. (From Abbas AK, Lichtman AH, Pillai S: *Cellular and Molecular Immunology*, ed 9, Philadelphia, 2017, Elsevier.)

anti–PD-L1 therapy. Investigators are currently studying which biomarkers will predict responsiveness to different checkpoint blockade approaches.

- *Combination of checkpoint inhibitors with other therapeutic agents.* The combined use of different checkpoint inhibitors, or of checkpoint inhibitors with other types of therapeutic agents (e.g., drugs that target oncoproteins like tyrosine kinases), will likely be necessary to achieve higher rates of therapeutic success. The first successful example of this is the combined use of anti–CTLA-4 and anti–PD-1 to treat melanoma, an approach that is more effective than anti–CTLA-4 alone. This reflects the fact that the mechanisms by which CTLA-4 and PD-1 inhibit T-cell activation are different (Fig. 7.39). There are numerous ongoing or planned clinical trials using combinations of checkpoint blockade together with other kinds of therapeutic agents.
- *The most common toxicities associated with checkpoint blockade are autoimmunity and/or inflammatory damage to organs.* This is predictable because the physiologic function of inhibitory receptors and ligands is to maintain tolerance to self antigens. A wide range of organs may be affected including colon, lungs, endocrine organs, heart, and skin, each requiring different clinical interventions, sometimes including cessation of the potentially life-saving checkpoint blockade therapy.

KEY CONCEPTS

EVASION OF IMMUNE SURVEILLANCE

- Tumor cells can be recognized by the immune system as nonself and destroyed.
- Antitumor activity is mediated by predominantly cell-mediated mechanisms. Tumor antigens are presented on the cell surface by MHC class I molecules and are recognized by CD8+ CTLs.
- The different classes of tumor antigens include products of mutated proto-oncogenes, tumor suppressor genes, overexpressed or aberrantly expressed proteins, and tumor antigens produced by oncogenic viruses.
- Immunosuppressed patients have an increased risk for development of cancer, particularly types caused by oncogenic DNA viruses.
- In immunocompetent patients, tumors may avoid the immune system by several mechanisms including selective outgrowth of antigen-negative variants, loss or reduced expression of histocompatibility antigens, and immunosuppression mediated by expression of certain factors (e.g., TGF-β, PD-1 ligand) by the tumor cells.
- Antibodies that overcome mechanisms of immune evasion involving "immune checkpoints" are approved for treatment of patients with advanced cancer and are most likely to be effective against cancers with a high mutational burden.

Genomic Instability

Genetic aberrations that increase mutation rates are very common in cancers and expedite the acquisition of driver mutations that are required for transformation and subsequent tumor progression. Although exposure to environmental agents that are mutagenic (e.g., chemicals, radiation, sunlight) is unavoidable, cancers are relatively rare outcomes of these encounters. This state of affairs results from the ability of normal cells to repair DNA damage, the death of cells with irreparable damage (see "Evasion of Cell Death" earlier), and other mechanisms such as oncogene-induced senescence and immune surveillance.

Several mechanisms contribute to genomic instability in cancer cells. We previously discussed how p53 protects the genome from potentially oncogenic damage by arresting cell division to provide time for repair of DNA damage and by initiating apoptosis in irreparably damaged cells. Cancers with loss of p53 function not only accumulate point mutations but also are strongly associated with aneuploidy, which may take the form of deletions, amplifications, and complex chromosomal rearrangements. These genomic aberrations may occur in cells with defective telomeres during break-fusion-breakage cycles (see Fig. 7.32) or may be created by other types of chromosomal "catastrophes" (such as chromothrypsis, described later) that lead to DNA breaks in multiple chromosomes. In the absence of p53 function, cells with severely damaged genomes that normally would be eliminated persist and stitch their chromosome back together in an error-prone way using the nonhomologous end-joining pathway.

TP53 is the most commonly mutated gene in cancer, and loss of p53 function is thus the preeminent source of genomic instability in cancers. In the following sections, we discuss two other classes of proteins that normally function to protect against genomic instability: DNA repair factors and DNA polymerase itself. As with p53, dysfunction of these factors leads to more rapid accumulation of genomic damage (a "mutator" phenotype), speeding cancer development and progression. Finally, we will describe a special type of regulated genomic instability specific to lymphoid cells that also is a source of oncogenic mutations.

DNA Mismatch Repair Factors. DNA mismatch repair proteins work together to act as "spell checkers" during the process of DNA replication. For example, if there is an erroneous pairing of G with T rather than the normal A with T, the mismatch-repair factors correct the defect. With "proofreading" function lost, errors accumulate throughout the genome. Some of these errors may by chance create driver mutations, and with time a cancer may result. One of the hallmarks of mismatch-repair defects is *microsatellite instability*. Microsatellites are tandem repeats of one to six nucleotides found throughout the genome. In normal people the length of these microsatellites remains constant. However, if mismatch repair is defective, these satellites are unstable and increase or decrease in length, creating mutated alleles.

Hereditary nonpolyposis colon cancer (HNPCC) syndrome (also known as Lynch syndrome) is an autosomal dominant disorder associated with carcinomas that arise predominantly in the cecum and proximal colon (Chapter 17). Individuals with HNPCC syndrome inherit one abnormal copy of a mismatch repair gene. Trouble arises when cells acquire somatic loss-of-function mutations, presumably at random, in their single normal alleles. Germline loss-of-function mutations in at least four different genes can produce HNPCC syndrome. The most commonly affected genes are *MSH2* and *MLH1*, each accounting for approximately 30% of HNPCC syndrome. Microsatellite instability also is observed in about 15% of sporadic colon cancers and less frequently in many other cancer types. In sporadic cancers, defects in mismatch repair usually stem from epigenetic silencing of the *MLH1* gene, rather than somatic mutations.

Nucleotide Excision Repair Factors. UV radiation causes cross-linking of pyrimidine residues, preventing normal DNA replication. Such DNA damage is repaired by the nucleotide excision repair system. Several genes are involved in nucleotide excision repair. Inherited loss-of-function mutations in any of these genes gives rise to a syndrome called *xeroderma pigmentosum* that is marked by an extraordinarily high risk of skin cancers, specifically squamous cell carcinoma and basal cell carcinoma.

Homologous Recombination Repair Factors. Other types of DNA damage, particularly covalent DNA cross-links and double-stranded DNA breaks, are repaired through a complex process called homologous recombination. Several disorders caused by defects in homologous recombination factors are associated with an increased risk of cancer, as follows:

- *Bloom syndrome* is an autosomal recessive disorder caused by loss-of-function mutations in a helicase that is required for homologous recombination repair. Affected individuals have developmental anomalies and an increased risk of developing many different types of cancer.
- *Ataxia telangiectasia* is an autosomal recessive disorder caused by defects in *ATM*, a gene encoding a kinase that acts upstream of p53. This syndrome is characterized by neurodegeneration (particularly of the cerebellum, hence the ataxia), immunodeficiency, hypersensitivity to radiation (due to an inability to repair double-stranded DNA breaks), and predisposition to cancer, particularly certain forms of leukemia and lymphoma. Somatic driver mutations in *ATM* also are common in certain types of lymphoid neoplasms.
- *Fanconi anemia* is an autosomal recessive disorder that may be caused by mutations in more than a dozen different genes, each encoding a protein that participates in a pathway that repairs DNA cross-links through homologous recombination. It is characterized by developmental abnormalities (short stature, skeletal abnormalities), hypersensitivity to chemotherapeutic agents that cross-link DNA, and increased risk of bone marrow failure (aplasia) and leukemia.
- *Familial breast cancer* often is associated with inherited defects in genes that are required for homologous recombination repair. Mutations in two genes, *BRCA1* and *BRCA2*, account for 25% of cases. Certain germline *BRCA2* mutations cause Fanconi anemia, and it appears that BRCA proteins and Fanconi proteins function cooperatively in a DNA damage response network linked to homologous recombination repair. Defects in this pathway lead to the activation of the salvage

nonhomologous end-joining pathway, formation of dicentric chromosomes, bridge-fusion-breakage cycles, and aneuploidy, just as occurs in p53-deficient cells that undergo telomere shortening (see Fig. 7.32). In addition to breast cancer, women with *BRCA1* mutations have a substantially higher risk of epithelial ovarian cancers, and men have a slightly higher risk of prostate cancer. Mutations in the *BRCA2* gene are associated with a broader spectrum of cancers including breast cancer in men and women as well as cancers of the ovary, prostate, pancreas, bile ducts, stomach, melanocytes, and B lymphocytes.

DNA Polymerase. Under normal circumstances, cellular DNA polymerases involved in DNA replication have a very low rate of error, defined as addition of nucleotide that does not match its partner on the template strand of DNA. This fidelity stems in part from an inherent exonuclease activity that allows DNA polymerase to pause, excise mismatched bases, and insert the proper nucleotide before proceeding down the template strand. Subsets of certain cancers, most often endometrial carcinomas and colon cancers, harbor mutations in DNA polymerase that result in a loss of this "proofreading" function and the accumulation of numerous point substitutions. Cancers with DNA polymerase mutations are the most heavily mutated of all human cancers and, presumably because of a high burden of neoantigens, appear to have excellent responses to immune checkpoint inhibitors.

Regulated Genomic Instability in Lymphoid Cells. A special type of DNA damage plays a central role in the pathogenesis of tumors of B and T lymphocytes. As described in Chapter 6, adaptive immunity relies on the ability of B and T cells to diversify their antigen receptor genes. Developing B and T cells both express a pair of gene products, RAG1 and RAG2, that carry out V(D)J segment recombination, permitting the assembly of functional antigen receptor genes. In addition, after encountering antigen, mature B cells express a specialized enzyme called activation-induced cytosine deaminase (AID), which is required for both immunoglobulin gene class switch recombination and somatic hypermutation. These processes are associated with AID-induced DNA breaks or nucleotide substitutions, both of which are prone to errors such as translocations and mutations that cause lymphoid neoplasms (Chapter 13).

KEY CONCEPTS

GENOMIC INSTABILITY AS ENABLER OF MALIGNANCY

- Persons with inherited mutations of genes involved in DNA repair systems are at greatly increased risk for the development of cancer.
- Patients with HNPCC syndrome have defects in the mismatch repair system, leading to development of carcinomas of the colon. These patients' genomes show microsatellite instability, characterized by changes in length of short repeats throughout the genome.
- Patients with xeroderma pigmentosum have a defect in the nucleotide excision repair pathway and are at increased risk for the development of cancers of the skin exposed to UV light because of an inability to repair pyrimidine dimers.
- Syndromes involving defects in the homologous recombination DNA repair system constitute a group of disorders—Bloom syndrome, ataxia-telangiectasia, and Fanconi anemia—that are characterized by developmental disorders and hypersensitivity to DNA-damaging agents, such as ionizing radiation. *BRCA1* and *BRCA2*, which are mutated in familial breast cancers, are involved in DNA repair.
- Mutations in DNA polymerase that abolish proofreading function leads to genomic instability in subsets of colonic and endometrial carcinomas.
- T and B cells undergo regulated genomic instability during somatic gene rearrangements. Errors in this process are an important cause of lymphoid neoplasms.

Cancer-Enabling Inflammation

Infiltrating cancers provoke a chronic inflammatory reaction, leading some to liken them to "wounds that do not heal." In patients with advanced cancers, this inflammatory reaction can be so extensive as to cause systemic signs and symptoms such as anemia (Chapter 14), fatigue, and cachexia (described later). However, studies carried out on cancers in animal models suggest that inflammatory cells also modify the tumor cells and the local microenvironment to enable many of the hallmarks of cancer. These effects may stem from direct interactions between inflammatory cells and tumor cells or through indirect effects of inflammatory cells on other resident stromal cells, particularly cancer-associated fibroblasts and endothelial cells. Proposed cancer-enabling effects of inflammatory cells and resident stromal cells include the following:

- *Release of factors that promote proliferation.* Infiltrating leukocytes and activated stromal cells secrete a wide variety of growth factors such as EGF, as well as proteases that can liberate growth factors from the ECM.
- *Removal of growth suppressors.* The growth of epithelial cells is normally suppressed by cell–cell and cell–ECM interactions. Proteases released by inflammatory cells can degrade the adhesion molecules that mediate these interactions, removing a barrier to growth.
- *Enhanced resistance to cell death.* Recall that detachment of epithelial cells from basement membranes and from cell–cell interactions leads to a form of cell death called *anoikis*. It is suspected that tumor-associated macrophages prevent anoikis by expressing adhesion molecules such as integrins that promote direct physical interactions with the tumor cells. There is also substantial evidence that stromal cell–cancer cell interactions increase the resistance of cancer cells to chemotherapy, presumably by activating signaling pathways that promote cell survival in the face of stresses such as DNA damage.
- *Inducing angiogenesis.* Inflammatory cells release numerous factors, including VEGF, which stimulate angiogenesis.
- *Activating invasion and metastasis.* Proteases released from macrophages foster tissue invasion by remodeling the ECM, while factors such as TNF and EGF may directly stimulate tumor cell motility. As mentioned, other factors released from stromal cells, such as TGF-β, may promote

EMT, which is considered to be a key event in the process of invasion and metastasis.

- *Evading immune destruction.* A variety of soluble factors released by macrophages and other stromal cells may contribute to the immunosuppressive microenvironment of tumors, including TGF-β and a number of other factors that either favor the recruitment of immunosuppressive Tregs or suppress the function of CD8+ cytotoxic T cells. Furthermore, there is abundant evidence in murine cancer models and emerging evidence in human disease that advanced cancers contain alternatively activated (M2) macrophages (Chapter 3), cells induced by cytokines such as IL-4 and IL-13. These macrophages produce cytokines that promote angiogenesis, fibroblast proliferation, and collagen deposition, all of which are commonly observed in invasive cancers. In addition, macrophages may suppress effective host immune responses to cancer cells by expressing the immune checkpoint factor PD-L1 and through other mechanisms that remain to be fully elucidated.

Although a thorough understanding of how cancers "manipulate" inflammatory cells to support their growth and survival remains elusive, there is substantial interest in the development of therapies directed at tumor-induced inflammation and its downstream consequences. Of note in this regard, antiinflammatory cyclooxygenase-2 (COX-2) inhibitors have been shown to decrease the incidence of colonic adenomas and are approved for treatment of patients with familial adenomatous polyposis.

Dysregulation of Cancer-Associated Genes

The genetic damage that activates oncogenes or inactivates tumor suppressor genes may be subtle (e.g., point mutations) or may involve segments of chromosomes large enough to be detected in a routine karyotype. Activation of oncogenes and loss of function of tumor suppressor genes by mutations were discussed earlier in this chapter. Here we discuss chromosomal abnormalities and epigenetic changes that contribute to carcinogenesis and then briefly touch on the role of noncoding RNAs.

Chromosomal Changes

Certain chromosomal abnormalities are highly associated with particular neoplasms and inevitably lead to the dysregulation of genes with an integral role in the pathogenesis of that tumor type. Specific recurrent chromosomal abnormalities have been identified in most leukemias and lymphomas, many sarcomas, and an increasing number of carcinomas. In addition, whole chromosomes may be gained or lost. Although changes in chromosome number (aneuploidy) and structure are generally considered to be late phenomena in cancer progression, in some cases (e.g., in cells that have lost their telomeres; see Fig. 7.32), it can be an early event that initiates the transformation process.

Historically, chromosomal changes in cancer were identified through karyotyping, the morphologic identification of metaphase chromosomes prepared from clinical specimens. Today, however, cancer cell karyotypes are being reconstructed in research laboratories from deep sequencing of cancer cell genomes, and it is possible that conventional karyotyping will be supplanted by other methods even in clinical laboratories in the years to come. Whatever technology is used, the study of chromosomal changes in tumor cells is important. First, genes in the vicinity of recurrent chromosomal breakpoints or deletions are very likely to be either oncogenes (e.g., *MYC, BCL2, ABL*) or tumor suppressor genes (e.g., *APC, RB*). Second, certain karyotypic abnormalities have diagnostic value or important prognostic or therapeutic implications. For example, tests that detect and quantify *BCR-ABL* fusion genes or their mRNA products are essential for the diagnosis of CML and are used to monitor the response to BCR-ABL kinase inhibitors. Many other chromosomal aberrations that are characteristic of specific tumor types are presented in later chapters.

Table 7.8 Selected Examples of Oncogenes Created by Translocations

Malignancy	Translocation	Affected Genes
Chronic myeloid leukemia (CML)	(9;22)(q34;q11)	*ABL* 9q34 *BCR* 22q11
Acute myeloid leukemia (AML)	(8;21)(q22;q22) (15;17)(q22;q21)	*AML* 8q22 *ETO* 21q22 *PML* 15q22 *RARA* 17q21
Burkitt lymphoma	(8;14)(q24;q32)	*MYC* 8q24 *IGH* 14q32
Mantle cell lymphoma	(11;14)(q13;q32)	*CCND1* 11q13 *IGH* 14q32
Follicular lymphoma	(14;18)(q32;q21)	*IGH* 14q32 *BCL2* 18q21
Ewing sarcoma	(11;22)(q24;q12)	*FLI1* 11q24 *EWSR1* 22q12
Prostatic adenocarcinoma	(7;21)(p22;q22) (17;21)(p21;q22)	*TMPRSS2* (21q22.3) *ETV1* (7p21.2) *ETV4* (17q21)

Chromosomal Translocations. Any type of chromosomal rearrangement—translocations, inversions, amplifications, and even small deletions—can activate proto-oncogenes, but chromosomal translocation is the most common mechanism. Notable examples of oncogenes activated by chromosomal translocations are listed in Table 7.8. Translocations can activate proto-oncogenes in two ways:

- By promoter or enhancer substitution, in which the translocation results in overexpression of a proto-oncogene by swapping its regulatory elements with those of another gene, typically one that is highly expressed.
- By formation of a fusion gene in which the coding sequences of two genes are fused in part or in whole, leading to the expression of a novel chimeric protein with oncogenic properties.

Overexpression of a proto-oncogene caused by translocation is exemplified by Burkitt lymphoma. Virtually all Burkitt lymphomas have a translocation involving chromosome 8q24, where the *MYC* gene resides, and one of the three chromosomes that carry an immunoglobulin gene. At its normal locus, *MYC* is tightly controlled and is most highly expressed in actively dividing cells. In Burkitt lymphoma the most common translocation moves the *MYC*-containing

segment of chromosome 8 to chromosome 14q32 (see Fig. 7.23), placing it close to the immunoglobulin heavy chain *(IGH)* gene. The genetic notation for the translocation is t(8;14)(q24;q32). The molecular mechanisms of the translocation-mediated overexpression of *MYC* are variable, as are the precise breakpoints within the *MYC* gene. In most cases the translocation removes regulatory sequences of the *MYC* gene and replaces them with the control regions of the *IGH* gene, which is highly expressed in B cells. The *MYC* coding sequences remain intact, and the MYC protein is constitutively expressed at high levels. The almost invariable presence of *MYC* translocations in Burkitt lymphomas attests to the importance of *MYC* overactivity in the pathogenesis of this tumor.

There are many other examples of translocations involving oncogenes and antigen receptor loci in lymphoid tumors. For these (or any other) translocations to occur, double-stranded DNA breaks must occur simultaneously in at least two places in the genome, and the free DNA ends must then be joined to create two new derivative chromosomes. In lymphoid cells, most of these molecular misadventures are believed to occur during attempts at normal antigen receptor gene recombination (which occurs in both B- and T-cell progenitors) or class-switch recombination (which is confined to antigen-stimulated mature B cells). Not unexpectedly, tumors with translocations involving immunoglobulin genes are always of B-cell origin, and tumors with translocations involving T-cell receptor genes are always of T-cell origin. The affected genes are diverse, but as with translocations involving *MYC,* the net effect is overexpression of some protein with oncogenic activity.

The *Philadelphia chromosome,* characteristic of CML and a subset of B-cell acute lymphoblastic leukemias (Chapter 13), provides the prototypic example of a chromosomal rearrangement that creates a fusion gene encoding a chimeric oncoprotein. In this instance the two chromosome breaks lie within the *ABL* gene on chromosome 9 and within the *BCR (b*reakpoint *c*luster *r*egion) gene on chromosome 22 (see Fig. 7.23). Nonhomologous end-joining then leads to a reciprocal translocation that creates an oncogenic *BCR-ABL* fusion gene on the derivative chromosome 22 (the so-called Philadelphia chromosome). *BCR-ABL* fusion genes encode chimeric BCR-ABL proteins with constitutive tyrosine kinase activity. Since the discovery of *BCR-ABL* in CML, many other fusion oncogenes encoding constitutively active tyrosine kinases have been described in a broad array of human cancers. Like BCR-ABL, these fusion proteins drive oncogenic signaling pathways and have sometimes proven to be targets of effective therapies.

Other oncogenic fusion genes encode nuclear factors that regulate transcription or chromatin structure. In contrast to overactive tyrosine kinases, less is known about how nuclear fusion oncoproteins contribute to cancer. An exception with important clinical consequences is found in *acute promyelocytic leukemia* (APML). APML is virtually always associated with a reciprocal translocation between chromosomes 15 and 17 that produces a *PML-RARA* fusion gene (Fig. 7.40). How this fusion gene functions is now reasonably well understood.

- The fusion gene encodes a chimeric protein consisting of part of a protein called PML and part of the retinoic acid receptor-α (RARα). Normal RARα binds to DNA and activates transcription in the presence of retinoids. Among the RARα responsive genes are a number that are needed for the differentiation of myeloid progenitors into neutrophils.
- The PML-RARα oncoprotein has diminished affinity for retinoids, such that at physiologic levels retinoids do not bind to PML-RARα to any significant degree. In this "unliganded" state, it retains the capacity to bind DNA, but instead of activating transcription, it inhibits transcription through recruitment of transcriptional repressors. This interferes with the expression of genes that are needed for differentiation, leading to a "pile-up" of proliferating myeloid progenitors that replace normal bone marrow elements.
- When given in pharmacologic doses, all-*trans* retinoic acid (ATRA) binds to PML-RARα and causes a conformational change that results in the displacement of repressor complexes and the recruitment of different complexes that activate transcription. There also is evidence that ATRA-bound PML-RARA complexes are degraded more rapidly. These changes overcome the block in gene expression, causing the neoplastic myeloid progenitors to differentiate into neutrophils and die (as normal mature neutrophils do), clearing the marrow over several days and allowing for recovery of normal hematopoiesis.

This highly effective therapy is the first example of *differentiation therapy,* in which immortal tumor cells are induced to differentiate into their mature progeny, which have limited lifespans. It has also spurred efforts to develop drugs that target other nuclear oncoproteins, despite the inherent difficulty of the problem.

Deletions. Chromosomal deletions are another very common structural abnormality in tumor cells. Deletion of specific regions of chromosomes is associated with the loss of particular tumor suppressor genes.

As we discussed earlier, deletions involving chromosome 13q14, the site of the *RB* gene, are associated with retinoblastoma, and deletion of the *VHL* tumor suppressor gene on chromosome 3p is a common event in renal cell carcinomas. Sequencing of cancer cell genomes has revealed many more examples of deletions involving tumor suppressor genes, as well as small insertions of DNA from one site into another. Not all deletions lead to loss of gene function, however; a subset activates oncogenes through the same mechanisms as chromosomal translocations. For example, about 25% of T-cell acute lymphoblastic leukemias have small deletions of chromosome 1 that juxtapose the *TAL1* proto-oncogene with a nearby active promoter, leading to overexpression of the TAL1 transcription factor. Many other examples of "addition by genomic subtraction" have now been discovered through sequencing of cancer genomes.

Gene Amplification. Overexpression of oncogenes may also result from reduplication and amplification of their DNA sequences. Such amplification may produce up to several hundred copies of the oncogene in the tumor cell. In some cases the amplified genes produce chromosomal changes that can be identified microscopically. Two mutually exclusive patterns are seen: (1) multiple small extrachromosomal structures called *double minutes* and (2) *homogeneous*

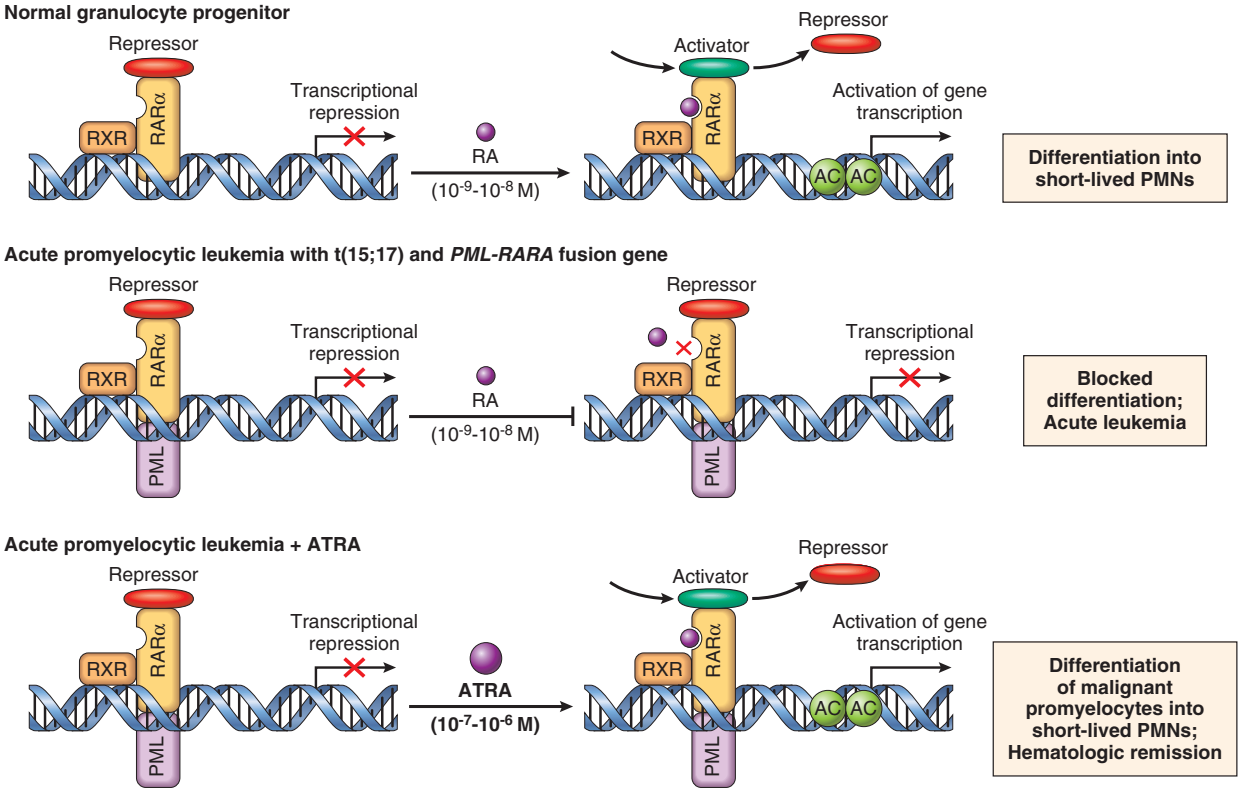

Figure 7.40 Molecular pathogenesis of acute promyelocytic leukemia *(PML)* and basis for response to all-*trans* retinoic acid *(ATRA)*. *PMN*, Polymorphonuclear neutrophil; *RA*, retinoic acid; *RXR*, binding partner for normal RARa and PML-RARa fusion protein encoded by a chimeric gene created by the (15;17) translocation in acute promyelocytic leukemia.

staining regions. The latter derive from the insertion of the amplified genes into new chromosomal locations, which may be distant from the normal location of the involved oncogene. The affected chromosomal regions lack a normal pattern of light- and dark-staining bands, appearing homogeneous in karyotypes (see Fig. 7.24). From a clinical perspective the most important amplifications are *NMYC* in neuroblastoma and *ERBB2* in breast cancers. *NMYC* is amplified in 25% to 30% of neuroblastomas, and its amplification is associated with poor prognosis. *ERBB2* amplification occurs in about 20% of breast cancers. As already mentioned, antibody therapy directed against the HER2 receptor encoded by *ERBB2* is an effective therapy for this molecular subset of breast cancers.

Complex Chromosomal Rearrangements. The true extent of chromosome rearrangements in cancer is only now coming into view thanks to sequencing of entire cancer cell genomes, which allows for comprehensive "reconstruction" of chromosomes from DNA sequences. This exercise has revealed not only a large number of simple rearrangements (e.g., small deletions, duplications, or inversions), but also much more dramatic chromosomal "catastrophes" that stem from chromothrypsis (literally, chromosome shattering). *Chromothrypsis* is observed in 1% to 2% of cancers as a whole and is particularly common in osteosarcomas and gliomas. It appears to result from a single event in which dozens to hundreds of chromosome breaks occur in a single chromosome or several chromosomes. The genesis of these breaks is uncertain, but DNA repair mechanisms are activated that stitch the pieces together in a haphazard way, creating many rearrangements, deletions, and even amplifications. It is hypothesized that such catastrophic events may by chance mutate multiple cancer genes simultaneously thereby expediting the process of carcinogenesis.

KEY CONCEPTS

GENETIC LESIONS IN CANCER

- Tumor cells may acquire several types of oncogenic mutations including point mutations and other nonrandom chromosomal abnormalities, such as translocations, deletions, and gene amplifications.
- Translocations contribute to carcinogenesis by overexpression of oncogenes or generation of novel fusion proteins with altered signaling capacity. Deletions frequently cause loss of tumor suppressor gene function and occasionally activate proto-oncogenes. Gene amplification generally increases the expression and function of oncogenes.
- Genomic sequencing has revealed numerous "cryptic" (subcytogenetic) rearrangements, mainly small deletions and insertions ("indels"), as well as chromothrypsis, in which a chromosome is "shattered" and then reassembled in a haphazard way.

Epigenetic Changes

Epigenetic changes have been implicated in many aspects of the malignant phenotype including the expression of cancer genes, the control of differentiation and self-renewal, and drug sensitivity and drug resistance. As discussed in Chapter 1, "epigenetics" refers to factors other than the sequence of DNA that regulate gene expression (and thereby cellular phenotype). Recall that epigenetic mechanisms include histone modifications catalyzed by enzymes associated with chromatin regulatory complexes; DNA methylation, a modification created by DNA methyltransferases; and other alterations that regulate the higher order organization of DNA (e.g., looping of enhancer elements onto gene promoters).

It has been recognized for more than a hundred years that the nuclei of cancer cells display abnormal morphologies, which (as discussed earlier) may take the form of hyperchromasia, chromatin clumping, or chromatin clearing (so-called vesicular nuclear chromatin). These altered appearances stem from disturbances of chromatin organization. One of the most notable findings emerging from the sequencing of cancer genomes has been the identification of numerous mutations involving genes that encode epigenetic regulatory proteins (Table 7.9). As a result, it is now suspected that the altered morphologic appearance of cancer cells reflects acquired genetic defects in factors that maintain the epigenome. Indeed, methods that allow genome-wide assessments have begun to reveal widespread alterations in cancer cell epigenomes, which can be broadly divided into the following categories:

- *Silencing of tumor suppressor genes by local hypermethylation of DNA.* Some cancer cells exhibit selective hypermethylation of the promoters of tumor suppressor genes that results in their transcriptional silencing. Typically, hypermethylation occurs on only one allele, and the function of the other copy of the affected tumor suppressor gene is lost through another mechanism, such as a disabling point mutation or a deletion. One of several examples of a tumor suppressor gene that is hypermethylated in several cancers is *CDKN2A,* which you will recall is a complex locus that encodes two tumor suppressors, p14/ARF and p16/INK4a, that enhance p53 and RB activity, respectively.
- *Global changes in DNA methylation.* In addition to local hypermethylation of tumor suppressor genes, many tumors exhibit abnormal patterns of DNA methylation throughout their genomes. Tumors commonly exhibiting abnormal DNA methylation, such as acute myeloid leukemia, sometimes have mutations in genes encoding DNA methyltransferases or other proteins that influence DNA methylation (see Table 7.9), suggesting that the observed alterations have a genetic basis. The most obvious potential consequence of global changes in methylation is altered expression of multiple genes, which may be overexpressed or underexpressed compared to normal depending on the nature of local changes.
- *Changes in histones.* You will recall that histones are responsible for "packaging" of DNA and that changes in histone positioning or posttranslational modifications (so-called histone marks) regulate gene transcription. Cancer cells often demonstrate changes in histones near genes that influence cellular behavior. As with DNA methylation, in many instances these alterations appear to have a genetic basis, being attributable to mutations in proteins that "write," "read," and "erase" histone marks or that position nucleosomes on DNA (see Table 7.9). Remarkably, in some cancers, driver mutations occur in the histone genes themselves. Details have yet to emerge, but it is certain that these lesions alter the expression of genes that contribute to the malignant phenotype.

Table 7.9 Examples of Epigenomic Regulatory Genes That Are Mutated in Cancer

Gene(s)	Function	Tumor (Approximate Frequency of Mutation)
DNMT3A	DNA methylation	Acute myeloid leukemia (20%)
MLL1	Histone methylation	Acute leukemia in infants (90%)
MLL2	Histone methylation	Follicular lymphoma (90%)
CREBBP/ EP300	Histone acetylation	Diffuse large B-cell lymphoma (40%)
ARID1A	Nucleosome positioning/ chromatin remodeling	Ovarian clear cell carcinoma (60%), endometrial carcinoma (30%–40%)
SNF5	Nucleosome positioning/ chromatin remodeling	Malignant rhabdoid tumor (100%)
PBRM1	Nucleosome positioning/ chromatin remodeling	Renal carcinoma (30%)
H3F3A, HIST1H3B	Histone H3 variants (nucleosome components)	Pediatric gliomas (30%–80%, depending on anatomic location)

Much remains to be deciphered about the state of the "epigenome" in various cancers and its contribution to the malignant state, but several aspects of the relationship merit emphasis.

- *The lineage-specificity of certain oncogenes and tumor suppressor genes has an epigenetic basis.* You may have noticed that tumor suppressors and oncoproteins can be broadly divided into two classes, those that are mutated or otherwise dysregulated in many cancers (e.g., RAS, MYC, p53) and those that are mutated in a restricted subset of tumors (e.g., RB in retinoblastoma, VHL in renal cell carcinoma, APC in colon carcinoma) and are thus lineage-restricted. A cancer cell's lineage or differentiation state, like that of normal cells, is dependent on epigenetic modifications that produce a pattern of gene expression that characterizes that particular cell type. It follows that lineage-restricted cancer genes act only within epigenetic contexts in which key oncogenic targets are controlled by these genes. At its extremes, this allows some genes, such as those encoding Notch receptors, to act as a tumor suppressor in one lineage and behave as an oncogene in another. Thus, the *NOTCH1* gene is one of the most commonly mutated tumor suppressor genes in squamous

cell carcinoma of the skin (in which the mutations result in loss of function and lead to impaired differentiation) and is also the most commonly mutated oncogene in T-cell acute lymphoblastic leukemia (in which mutations in different parts of the gene result in gain of function and drive the expression of pro-growth genes such as *MYC*).

- *The epigenome is a therapeutic target.* Because the epigenetic state of a cell depends on reversible modifications that are carried out by enzymes (which are generally good drug targets), there is intense interest in developing drugs that target epigenomic modifiers in cancer and other diseases. Inhibitors of histone deacetylases, chromatin erasers that remove acetyl groups from histones, are approved for use in certain lymphoid tumors, and DNA methylation inhibitors are used to treat myeloid tumors, based in part on the idea that these drugs may reactivate tumor suppressor genes. Other drugs that target specific chromatin writers and chromatin readers are now being tested in clinical trials.
- *Cancers likely exhibit considerable epigenetic heterogeneity.* Just as genomic instability gives rise to genetic heterogeneity in cancers, it is feared that cancers will also prove to have extensive epigenetic heterogeneity from cell to cell within individual tumors. One consequence of such heterogeneity may be drug resistance. For example, epigenetic alterations can lead to the resistance of lung cancer cells to inhibitors of EGF receptor signaling. When the inhibitors are removed, the lung cancer cells revert to their prior inhibitor-sensitive state. If widespread, such epigenetic plasticity may join genetic heterogeneity as yet another barrier to the development of curative cancer therapies.

Noncoding RNAs and Cancer

Noncoding RNAs participate in carcinogenesis by regulating the expression of protein-coding cancer-associated genes. The best characterized of these noncoding RNAs are microRNAs. As discussed in Chapter 1, microRNAs (miRs) are small noncoding, single-stranded RNAs, approximately 22 nucleotides in length, that mediate sequence-specific inhibition of mRNAs. Given that miRs control normal cell survival, growth, and differentiation, it is not surprising that they play a role in carcinogenesis. Altered miR expression, sometimes stemming from amplifications and deletions of miR loci, has been identified in many cancers. Decreased expression of certain miRs increases the translation of oncogenic mRNAs; such miRs have tumor suppressive activity. For example, deletions affecting miR-15 and miR-16 are among the most frequent genetic lesions in chronic lymphocytic leukemia, a common tumor of older adults (Chapter 13). In this tumor, loss of these mIRs leads to upregulation of the anti-apoptotic protein BCL-2, enhancing tumor cell survival. Conversely, overexpression of other miRs represses the expression of tumor suppressor genes; such miRs promote tumor development and are referred to as *onco-miRs*. One example of an onco-miR is miR-155, which is overexpressed in many human B-cell lymphomas and indirectly upregulates a large number of genes that promote proliferation, including *MYC*.

The involvement of miRs may be the proverbial tip of the iceberg with respect to the role of noncoding RNAs in cancer. Systematic genomic analyses have revealed that more than 60% of the genome is transcribed into RNAs, most of which are noncoding and believed to have regulatory functions (Chapter 1).

Molecular Basis of Multistep Carcinogenesis

Given that malignant tumors must acquire multiple "hallmarks" of cancer, it follows that cancers result from the stepwise accumulation of multiple mutations that act in complementary ways to produce a fully malignant tumor. The notion that malignant tumors arise from a sequential accumulation of cancer-promoting alterations is supported by epidemiologic, experimental, and molecular studies, and the study of oncogenes and tumor suppressor genes has provided a firm molecular footing for the concept of multistep carcinogenesis. Genome-wide sequencing of cancers has revealed as few as 10 or so mutations in certain leukemias to many thousands of mutations (most of which are passengers rather than drivers) in tumors that arise following chronic exposure to carcinogens, such as lung cancers associated with cigarette smoking. A more direct answer to the question "how many mutations does it take to establish a fully malignant tumor?" comes from experimental attempts to transform normal human cells with combinations of oncogenes, some derived from transforming viruses (described later). For example, normal human epithelial cells can be transformed by the following combination of events: (1) activation of RAS; (2) inactivation of RB; (3) inactivation of p53; (4) inactivation of PP2A, a tumor suppressive phosphatase that is a negative regulator of many signaling pathways; and (5) constitutive expression of telomerase. Cells bearing all of these alterations are immortal and produce invasive, fully malignant growths when injected into immunodeficient mice.

Unlike in the laboratory, these events presumably never occur simultaneously during the natural development of a human cancer, but instead occur in a stepwise fashion. What is the evidence that this is so? A classic example of incremental acquisition of the malignant phenotype is found in colon carcinoma. Many of these cancers evolve through a series of morphologically identifiable stages, most notably the formation of adenomas that progressively enlarge and ultimately undergo malignant transformation (Chapter 17). Molecular analyses of proliferations at each stage has indeed shown that precancerous lesions have fewer mutations than adenocarcinomas and that certain mutations tend to occur early (e.g., mutations in the tumor suppressor gene *APC*) or late (e.g., mutations in *TP53*) in the process (discussed in detail in Chapter 17). Similar evidence for stepwise progression exists for other recognizable precursor lesions to epithelial cancers, such as dysplasias of the cervix, epidermis, and oral mucosa, and hyperplasias of the endometrium. These are also described in subsequent chapters.

CARCINOGENIC AGENTS AND THEIR CELLULAR INTERACTIONS

More than 200 years ago the London surgeon Sir Percival Pott correctly attributed scrotal skin cancer in chimney sweeps to chronic exposure to soot. Based on this observation,

Table 7.10 Major Chemical Carcinogens

Direct-Acting Carcinogens
Alkylating Agents
β-Propiolactone Dimethyl sulfate Diepoxybutane Anticancer drugs (cyclophosphamide, chlorambucil, nitrosoureas, and others)
Acylating Agents
1-Acetyl-imidazole Dimethylcarbamyl chloride
Procarcinogens That Require Metabolic Activation
Polycyclic and Heterocyclic Aromatic Hydrocarbons
Benz[*a*]anthracene Benzo[*a*]pyrene Dibenz[*a,h*]anthracene 3-Methylcholanthrene 7,12-Dimethylbenz[*a*]anthracene
Aromatic Amines, Amides, Azo Dyes
2-Naphthylamine (β-naphthylamine) Benzidine 2-Acetylaminofluorene Dimethylaminoazobenzene (butter yellow)
Natural Plant and Microbial Products
Aflatoxin B_1 Griseofulvin Cycasin Safrole Betel nuts
Others
Nitrosamine and amides Vinyl chloride, nickel, chromium Insecticides, fungicides Polychlorinated biphenyls

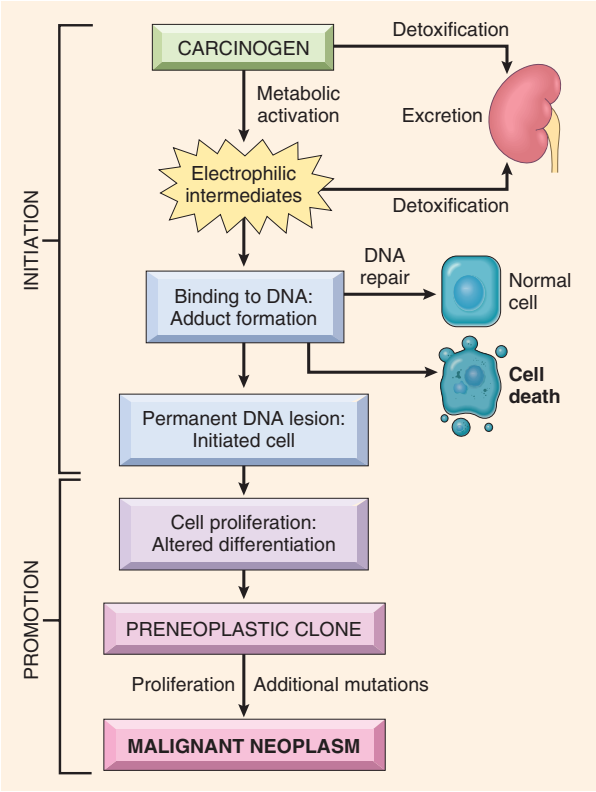

Figure 7.41 General schema of events in chemical carcinogenesis. Note that promoters cause clonal expansion of the initiated cell, thus producing a preneoplastic clone. Further proliferation induced by the promoter or other factors causes accumulation of additional mutations and emergence of a malignant tumor.

the Danish Chimney Sweeps Guild ruled that its members must bathe daily. No public health measure since that time has achieved as much in controlling a form of cancer! Subsequently, hundreds of chemicals have been shown to be carcinogenic in animals. Some of the major agents are listed in Table 7.10.

Chemical Carcinogenesis

As discussed earlier, carcinogenesis is a multistep process. This is readily demonstrated in experimental models of chemical carcinogenesis, in which the stages of initiation and promotion during cancer development were first described. The classic experiments that allowed the distinction between initiation and promotion were performed on mouse skin and revealed the following concepts relating to the initiation-promotion sequence: *Initiation* results from exposure of cells to a sufficient dose of a carcinogenic agent. It causes permanent DNA damage (mutations). *Promoters* can induce tumors to arise from initiated cells, but they are not tumorigenic by themselves. Application of promoters leads to proliferation and clonal expansion of initiated (mutated) cells. Driven to proliferate, subclones of the initiated cells suffer various additional mutations, and eventually a cancerous clone with all the hallmark characteristics emerges. It is likely that many factors contributing to oncogenesis in humans also act by stimulating proliferation and thus can be thought of conceptually as tumor promoters; examples include unopposed estrogenic stimulation of the endometrium and breast and chronic inflammatory processes associated with tissue repair (e.g., inflammatory bowel disease, chronic hepatitis, and Barrett esophagus).

Although the concepts of initiation and promotion have been derived largely from experiments in mice, they are useful concepts when considering the roles of certain factors that contribute to human cancers. With this brief overview, initiation and promotion can be examined in more detail (Fig. 7.41). All initiating chemical carcinogens are highly reactive electrophiles (have electron-deficient atoms) that can react with nucleophilic (electron-rich) atoms in the cell. Their targets are DNA, RNA, and proteins, and in some cases these interactions cause cell death. Initiation, obviously, inflicts nonlethal damage to the DNA that is repaired in some error-prone fashion. The mutated cell then passes on the DNA lesions to its daughter cells. Chemicals that can cause initiation of carcinogenesis fall into two categories: direct acting and indirect acting.

Direct-Acting Carcinogens

***Direct-acting carcinogens* do not require metabolic conversion to become carcinogenic.** Most are weak carcinogens, but some are important because they are cancer chemotherapeutic drugs (e.g., alkylating agents). Tragically, in some instances these agents have cured, controlled, or delayed recurrence of certain types of cancer (e.g., leukemia, lymphoma, or breast carcinoma), only to evoke a second form of cancer, usually acute myeloid leukemia. The risk of induced cancer is low, but its existence dictates judicious use of such agents.

Indirect-Acting Carcinogens

***Indirect-acting carcinogens* require metabolic conversion to become active carcinogens; the carcinogenic products are called *ultimate carcinogens*.** Most chemical carcinogens act indirectly and require metabolic activation for conversion into ultimate carcinogens (Fig. 7.41). Some of the most potent indirect chemical carcinogens—the polycyclic hydrocarbons—are present in fossil fuels. Others, for example, benzo[*a*]pyrene (the active component of soot, which Potts showed to be carcinogenic), are formed during the high-temperature combustion of tobacco in cigarettes and are implicated in the causation of lung cancer. Polycyclic hydrocarbons also are produced from animal fats during the process of broiling or grilling meats and are present in smoked meats and fish. The aromatic amines and azo dyes are another class of indirect-acting carcinogens that were widely used in the past in the aniline dye and rubber industries. Many other occupational carcinogens are listed in Table 7.10.

Most indirect carcinogens are metabolized by *cytochrome P-450–dependent monooxygenases*. The genes that encode these enzymes are polymorphic, and the activity and inducibility of these enzymes vary significantly among individuals (described further in Chapter 9). Because these enzymes are essential for the activation of procarcinogens, the susceptibility to carcinogenesis is related in part to the particular polymorphic variants that an individual inherits. Thus it may be possible to assess cancer risk in a given individual by genetic analysis of such enzyme polymorphisms.

The metabolism of polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene by the product of the P-450 gene, *CYP1A1,* provides an instructive example. Approximately 10% of the white population carry a highly inducible form of this gene. Light smokers with the susceptible *CYP1A1* genotype have a sevenfold higher risk of developing lung cancer compared with smokers without the permissive genotype. Metabolic pathways also are involved in the inactivation (detoxification) of certain procarcinogens or their derivatives, and variation in these pathways also may influence cancer risk.

Molecular Targets of Chemical Carcinogens. Because malignant transformation results from mutations, it comes as no surprise that most chemical initiating agents target DNA and are mutagenic. There is no single or unique alteration associated with cancer initiation. Nor is there any apparent predisposition for initiators to cause mutations in particular genes; presumably, mutations occur throughout the genome, and cells that by chance suffer damage to the "usual suspects"—oncogenes and tumor suppressors such as *RAS* and *TP53*—gain a potential selective advantage and are at risk for subsequent transformation.

This is not to say that mutations induced by carcinogens occur in an entirely random fashion. Because of their chemical structures, some carcinogens interact preferentially with particular DNA sequences or bases and thus produce mutations that are clustered at "hotspots" or that are enriched for particular base substitutions. This phenomenon is illustrated by a mutational "hotspot" associated with exposure to *aflatoxin* B_1, a naturally occurring agent produced by some strains of the mold *Aspergillus*. *Aspergillus* grows on improperly stored grains and nuts, and there is a strong correlation between the dietary level of this food contaminant and the incidence of hepatocellular carcinoma in parts of Africa and the Far East. Interestingly, aflatoxin B_1–associated hepatocellular carcinomas tend to have a particular mutation in *TP53*, a G:C→T:A transversion in codon 249 that produces an arginine-to-serine substitution in the p53 protein that interferes with its function. In contrast, *TP53* mutations are infrequent in liver tumors from areas where aflatoxin contamination of food does not occur, and few of these mutations involve codon 249. Similarly, lung cancers associated with smoking have a 10-fold higher mutational burden on average than lung cancers in nonsmokers, and these excess mutations are strongly skewed toward particular base substitutions known to be caused by carcinogens in cigarette smoke (the proverbial "smoking gun"). Sequencing of cancer genomes has revealed several dozen other mutational "signatures." One of these signatures reflects exposure to chemotherapeutic agents, but the rest are largely unexplained, suggesting that other carcinogenic agents lurk in the environment, awaiting discovery.

Additional potential carcinogens in the workplace and at home include vinyl chloride, arsenic, nickel, chromium, insecticides, fungicides, and polychlorinated biphenyls. Nitrites used as food preservatives also have caused concern, as they react with amines contained in the food to form nitrosoamines, which are suspected carcinogens.

KEY CONCEPTS

CHEMICAL CARCINOGENESIS

- Chemical carcinogens have highly reactive electrophile groups that directly damage DNA, leading to mutations and eventually cancer.
- Direct-acting agents do not require metabolic conversion to become carcinogenic, while indirect-acting agents are not active until converted to an ultimate carcinogen by endogenous metabolic pathways. Hence, polymorphisms of endogenous enzymes such as cytochrome P-450 may influence carcinogenesis.
- After exposure of a cell to a mutagen or an initiator, tumorigenesis can be enhanced by exposure to promoters, which stimulate proliferation of the mutated cells.
- Examples of human carcinogens are direct-acting agents (e.g., alkylating agents used for chemotherapy), indirect-acting agents (e.g., benzo[*a*]pyrene, azo dyes, aflatoxin), and promoters or agents that cause pathologic hyperplasias of the endometrium or regenerative activity in the liver.

Radiation Carcinogenesis

Radiant energy, in the form of the UV rays of sunlight or as ionizing electromagnetic and particulate radiation, is mutagenic and carcinogenic. UV light exposure causes skin cancers, and ionizing radiation exposure from medical or occupational exposure, nuclear plant accidents, and atomic bomb detonations is associated with a variety of cancers. Although the contribution of ionizing radiation to the total human burden of cancer is probably small, those cancers that do occur may arise decades later, and long periods of observation are necessary to ascertain its full effect. An increased incidence of breast cancer has become apparent decades after women were exposed during childhood to atomic bomb tests. The incidence peaked during 1988–1992 and then declined. Moreover, radiation may have additive or synergistic effects with other potentially carcinogenic factors.

Ultraviolet Rays

Exposure to UV rays derived from the sun, particularly in fair-skinned individuals, is associated with an increased incidence of squamous cell carcinoma, basal cell carcinoma, and melanoma of the skin. The degree of risk depends on the type of UV rays, the intensity of exposure, and skin pigmentation, the latter reflecting the quantity of light-absorbing melanin in the skin. Thus, persons of European origin with fair skin that sunburns easily and stalwartly refuses to tan and who live in locales receiving a great deal of sunlight (e.g., Queensland, Australia, close to the equator) have the highest incidence of skin cancers in the world. The UV portion of the solar spectrum can be divided into three wavelength ranges: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). Of these, UVB is believed to be responsible for the induction of cutaneous cancers. UVC, although a potent mutagen, is not considered significant because it is filtered out by the ozone layer surrounding the earth (hence concerns about ozone depletion).

UVB light is carcinogenic because of its ability to cause pyrimidine dimers to form in DNA. Absorption of the energy in a photon of UV light by DNA produces a chemical reaction that leads to covalent cross-linking of pyrimidine bases, particularly adjacent thymidine residues in the same strand of DNA. This distorts the DNA helix and prevents proper pairing of the dimer with bases in the opposite DNA strand. Pyrimidine dimers are repaired by the nucleotide excision repair pathway, a process that may involve 30 or more proteins. It is postulated that with excessive sun exposure, the capacity of the nucleotide excision repair pathway is overwhelmed, and error-prone nontemplated DNA repair mechanisms become operative. These allow the cell to survive but also introduce mutations that, in some instances, lead to cancer. The importance of the nucleotide excision repair pathway of DNA repair is most graphically illustrated by the high frequency of cancers in individuals with the hereditary disorder *xeroderma pigmentosum* (discussed previously). Controversy about the role of UV exposure in the etiology of melanoma was put to rest by sequencing of melanoma genomes. This revealed that melanomas arising in sun-exposed skin harbor enormous numbers of mutations bearing the signature of error-prone repair of pyrimidine dimers, confirming that UV light has an important causative role in this potentially lethal cancer.

Ionizing Radiation

Electromagnetic (x-rays, γ rays) and particulate (α particles, β particles, protons, neutrons) radiations are all carcinogenic. The evidence is voluminous, and a few examples suffice. Many individuals pioneering the use of x-rays developed skin cancers. Miners of radioactive elements in central Europe and the Rocky Mountain region of the United States have a 10-fold higher incidence of lung cancers than the rest of the population. Most telling is the follow-up of survivors of the atomic bombs dropped on Hiroshima and Nagasaki. Initially there was a marked increase in the incidence of certain forms of leukemia after an average latent period of about 7 years. Subsequently the incidence of many solid tumors with longer latent periods (e.g., carcinomas of the breast, colon, thyroid, and lung) increased. Of great concern in the current era of widespread use of computed tomography (CT) scans are studies that have shown that children who get two or three CT scans have a threefold higher risk of leukemia, and those who receive five to 10 such scans have a threefold higher risk of brain tumors. The overall risk in children is very low (roughly one excess leukemia and one excess brain tumor over 10 years per 10,000 CT scans), but nevertheless emphasizes the need to minimize radiation exposure whenever possible.

In humans, for reasons that are not clear, there is a hierarchy of tissue vulnerability to radiation-induced cancers. Most frequent are myeloid leukemias (tumors of granulocytes and their precursors; see Chapter 13). Cancer of the thyroid follows closely but only in young patients. In the intermediate category are cancers of the breast, lungs, and salivary glands. In contrast, skin, bone, and the gastrointestinal tract are relatively resistant to radiation-induced neoplasia, even though gastrointestinal epithelial cells are vulnerable to the acute cell-killing effects of radiation, and the skin is "first in line" for all external radiation. Nonetheless, the physician must not forget: practically *any* cell can be transformed into a cancer cell by sufficient exposure to radiant energy.

KEY CONCEPTS

RADIATION CARCINOGENESIS

- Ionizing radiation causes chromosome breakage, translocations, and, less frequently, point mutations, leading to genetic damage and carcinogenesis.
- UV rays induce the formation of pyrimidine dimers within DNA, leading to mutations. Therefore UV rays can give rise to skin cancers. Individuals with defects in the repair of pyrimidine dimers suffer from xeroderma pigmentosa and are at particularly high risk.
- Exposure to radiation during imaging procedures such as CT scans is linked to a very small, but measurable, increase in cancer risk in children.

Microbial Carcinogenesis

Many RNA and DNA viruses have proved to be oncogenic in animals as disparate as frogs and primates. Despite intense scrutiny, however, only a few viruses have been linked with human cancer. Our discussion focuses on human oncogenic viruses as well as the role of the bacterium *H. pylori* in gastric cancer. A common theme in the pathogenesis

of microbial carcinogenesis is that the infecton triggers cell proliferation, which is initially polyclonal but with time becomes monoclonal by acquisition of driver mutations in rapidly dividing cells.

Oncogenic RNA Viruses

Human T-Cell Leukemia Virus Type 1. Although the study of animal retroviruses has provided spectacular insights into the molecular basis of cancer, only one human retrovirus, human T-cell leukemia virus type 1 (HTLV-1), is firmly implicated in the pathogenesis of cancer in humans.

HTLV-1 causes adult T-cell leukemia/lymphoma (ATLL), a tumor that is endemic in certain parts of Japan, the Caribbean basin, South America, and Africa and found sporadically elsewhere, including the United States. Worldwide, it is estimated that 15 to 20 million people are infected with HTLV-1. Similar to human immunodeficiency virus (HIV), which causes AIDS, HTLV-1 has tropism for CD4+ T cells, and hence this subset of T cells is the major target for neoplastic transformation. Human infection requires transmission of infected T cells via sexual intercourse, blood products, or breastfeeding. Leukemia develops in only 3% to 5% of the infected individuals, typically after a long latent period of 40 to 60 years.

There is little doubt that HTLV-1 infection of T lymphocytes is necessary for leukemogenesis, but the molecular mechanisms of transformation are not defined. In contrast to several murine retroviruses, HTLV-1 does not contain an oncogene, and no consistent integration next to a proto-oncogene has been discovered. In leukemic cells, however, viral integration shows a clonal pattern. In other words, although the site of viral integration in host chromosomes is random (the viral DNA is found at different locations in different cancers), the site of integration is identical within all cells of a given cancer. This would not occur if HTLV-1 were merely a passenger that infects cells after transformation; rather, it means that HTLV-1 must have been present at the moment of transformation, placing it at the "scene of the crime."

The HTLV-1 genome contains the *gag, pol, env,* and long-terminal-repeat regions typical of all retroviruses, but, in contrast to other leukemia viruses, it contains two other genes referred to as *tax* and *HBZ*. Several aspects of HTLV-1's transforming activity may be attributable to the protein products of these genes. Tax is essential for viral replication because it stimulates transcription of viral RNA from the 5′ long terminal repeat. HBZ is a transcription factor, and Tax and HBZ alter the transcription of host cell genes and interact with certain host cell signaling proteins. In doing so, they appear to contribute to the acquisition of cancer hallmarks, though the mechanisms remain unclear; effects on intracellular signals that regulate growth and cell survival, induction of genomic instability, and inhibition of senescence all have been suggested. Whatever the actual mechanism, it is quite inefficient, given the typical latency period of many decades between infection and the development of leukemia, which appears in only a small subset of infected individuals.

Oncogenic DNA Viruses

As with RNA viruses, several oncogenic DNA viruses that cause tumors in animals have been identified. Of the various human DNA viruses, five—HPV, EBV, HBV, Merkel cell polyomavirus, and human herpesvirus 8 (HHV8, also called Kaposi sarcoma herpesvirus)—have been implicated in the causation of human cancer. Merkel cell polyomavirus has been identified in Merkel cell carcinomas and is described in Chapter 25. HHV8 is discussed in Chapters 6 and 11. Although not a DNA virus, HCV is also associated with cancer and is discussed here briefly.

Human Papillomavirus. At least 70 genetically distinct types of HPV have been identified. Some types (e.g., 1, 2, 4, and 7) cause benign squamous papillomas (warts) in humans. In contrast, high-risk HPVs (e.g., types 16 and 18) have been implicated in the genesis of squamous cell carcinomas of the cervix, anogenital region, and head and neck (particularly tumors arising in the tonsillar mucosa). These cancers are sexually transmitted diseases caused by chronic HPV infection. In contrast to cervical cancers, genital warts have low malignant potential and are associated with low-risk HPVs, predominantly HPV-6 and HPV-11.

What explains the variation in cancer risk among HPV strains? In benign warts, the HPV genome is maintained in a nonintegrated episomal form, while in cancers the HPV genome is integrated into the host genome, suggesting that integration of viral DNA is one factor. As with HTLV-1, the site of viral integration in host chromosomes is random, but the pattern of integration is clonal. Integration always occurs in a fashion that interrupts the viral DNA within the E1/E2 open reading frame, leading to loss of the E2 viral repressor and increased expression of the HPV E6 and E7 genes, which are responsible for the oncogenic potential of HPV (Fig. 7.42).

- *Oncogenic activities of E6.* The E6 protein binds to and mediates the degradation of p53 and stimulates the expression of telomerase reverse transcriptase (TERT), the catalytic subunit of telomerase, which you will recall contributes to the immortalization of cells. E6 from high-risk HPV types has a higher affinity for p53 than E6 from

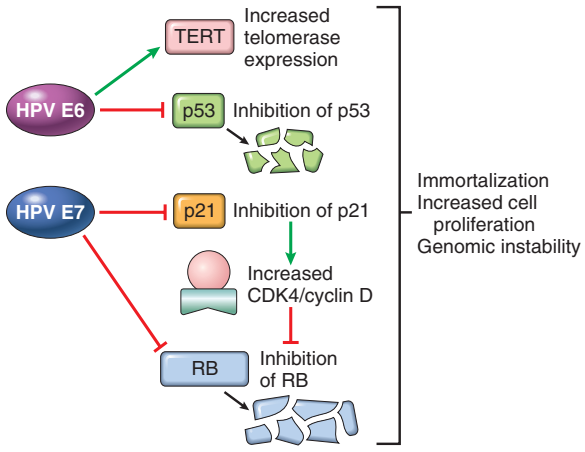

Figure 7.42 Transforming effects of human papillomavirus *(HPV)* E6 and E7 proteins. The net effect of HPV E6 and E7 proteins is to immortalize cells and remove the restraints on cell proliferation (see Fig. 7.26). *TERT,* Telomerase reverse transcriptase. (Modified from Münger K, Howley PM: Human papillomavirus immortalization and transformation functions, *Virus Res* 89:213–228, 2002.)

low-risk HPV types, a distinction that likely accounts for the difference in cancer risk.

- *Oncogenic activities of E7.* The E7 protein has effects that complement those of E6, all of which are centered on speeding cells through the G_1/S cell cycle checkpoint. It binds to the RB protein and displaces the E2F transcription factors that are normally sequestered by RB, promoting progression through the cell cycle. As with E6 proteins and p53, E7 proteins from high-risk HPV types have a higher affinity for RB than do E7 proteins from low-risk HPV types. E7 also inactivates the CDK inhibitors p21 and p27. Finally, E7 proteins from high-risk HPVs (types 16, 18, and 31) also bind and presumably activate cyclins A and E.

To summarize, **high-risk HPV types express oncogenic proteins that inactivate tumor suppressors, activate cyclins, inhibit apoptosis, and combat cellular senescence.** Thus, it is evident that HPV proteins promote many of the hallmarks of cancer. The primacy of HPV infection in the causation of cervical cancer is confirmed by the effectiveness of HPV vaccines in preventing cervical cancer. However, infection with HPV itself is not sufficient for carcinogenesis. For example, when human keratinocytes are transfected with DNA from HPV types 16, 18, or 31 in vitro, they are immortalized but do not form tumors. Cotransfection with a mutated *RAS* gene results in full malignant transformation. In addition to such genetic cofactors, HPV in all likelihood also acts in concert with environmental factors. These include cigarette smoking, coexisting microbial infections, dietary deficiencies, and hormonal changes, all of which have been implicated in the pathogenesis of cervical cancers. A high proportion of women infected with HPV clear the infection by immunologic mechanisms, but others do not because of acquired immune abnormalities, such as those that result from HIV infection, or for unknown reasons. As might be expected, women who are coinfected with high-risk HPV types and HIV have an elevated risk of cervical cancer.

Epstein-Barr Virus. EBV, a member of the herpesvirus family, was the first virus linked to a human tumor, Burkitt lymphoma. Since its initial discovery 50 years ago, EBV has been implicated in the pathogenesis of a diverse collection of human tumors including various lymphomas, several carcinomas, and even rare sarcomas. The most common EBV-associated tumors are lymphomas derived from B cells and nasopharyngeal carcinoma; other EBV-associated neoplasms are discussed elsewhere in this book.

The manner in which EBV causes B-cell tumors such as Burkitt lymphoma is complex and incompletely understood, but best appreciated by considering its effects on normal B cells. EBV has surface glycoproteins that recognize and bind the complement receptor CD21, allowing the virus to attach to and infect B cells. This probably occurs in the tonsils following exposure to the virus in saliva. Viral infection of B cells is latent; that is, there is no viral replication, and the cells are not killed. However, EBV proteins are expressed in latently infected B cells that allow the cells to grow indefinitely (immortalization). The molecular basis of B-cell growth and immortalization is complex, but as with other viruses it involves the "hijacking" of several normal signaling pathways. One EBV gene, latent membrane protein-1 *(LMP-1),* is an oncogene capable of inducing B-cell lymphomas in mice. LMP-1 behaves like a constitutively active CD40 receptor, a key recipient of helper T-cell signals that stimulate B-cell growth (Chapter 6). LMP-1 activates the NF-κB and JAK/STAT signaling pathways and promotes B-cell survival and proliferation, all of which occur autonomously (i.e., without T cells or other outside signals) in EBV-infected B cells. Concurrently, LMP-1 prevents apoptosis by activating BCL2. Thus, the virus "borrows" normal B-cell activation pathways to expand the pool of latently infected cells. Another EBV gene, *EBNA-2,* encodes a nuclear protein that mimics a constitutively active Notch receptor. EBNA-2 transactivates several host genes, including cyclin D and the *SRC* family of proto-oncogenes. In addition, the EBV genome contains a gene encoding a homologue of IL-10 (vIL-10) that was "borrowed" from the host genome. vIL-10 suppresses the activation of T cells by macrophages and contributes to EBV-dependent transformation of B cells.

The EBV proteins that are required for B-cell immortalization and proliferation are highly immunogenic, and in normal individuals the EBV-driven polyclonal B-cell proliferation is readily controlled by a cytotoxic T-cell response. Depending on the timing and intensity of this response, the individual either remains asymptomatic or develops a self-limited episode of infectious mononucleosis (Chapter 8). If T-cell immunity is defective, however, EBV transformed B cells can produce a rapidly progressive, fatal lymphoma.

Burkitt lymphoma is a neoplasm of B lymphocytes that is endemic in central Africa and New Guinea, areas where it is the most common tumor of childhood. A morphologically identical lymphoma occurs sporadically throughout the world. The association between endemic Burkitt lymphoma and EBV is strong:

- More than 90% of endemic tumors carry the EBV genome.
- All affected patients have elevated antibody titers against viral capsid antigens.
- Serum antibody titers against viral capsid antigens are correlated with the risk of developing the tumor.

Although EBV is intimately involved in the causation of Burkitt lymphoma, several observations suggest that additional factors are involved. (1) EBV infection is not limited to the geographic locales where Burkitt lymphoma is found; in fact, it is a ubiquitous virus that infects almost all humans worldwide. (2) The EBV genome is found in only 15% to 20% of Burkitt lymphomas outside of endemic regions. (3) There are significant differences in the patterns of viral gene expression in EBV-transformed (but not tumorigenic) B-cell lines and Burkitt lymphoma cells. Most notably, Burkitt lymphoma cells do not express LMP-1, EBNA2, and other EBV proteins that drive B-cell growth and immortalization.

Given these observations, how then does EBV contribute to the genesis of endemic Burkitt lymphoma? One possibility is shown in Fig. 7.43. In regions where Burkitt lymphoma is endemic, concomitant infections such as malaria impair immune competence, allowing sustained B-cell proliferation. Eventually, T-cell immunity directed against EBV antigens such as EBNA2 and LMP-1 eliminates most of the EBV-infected B cells, but a small number of cells downregulate expression of these immunogenic antigens. These cells persist indefinitely, even in the face of normal immunity. Lymphoma cells may

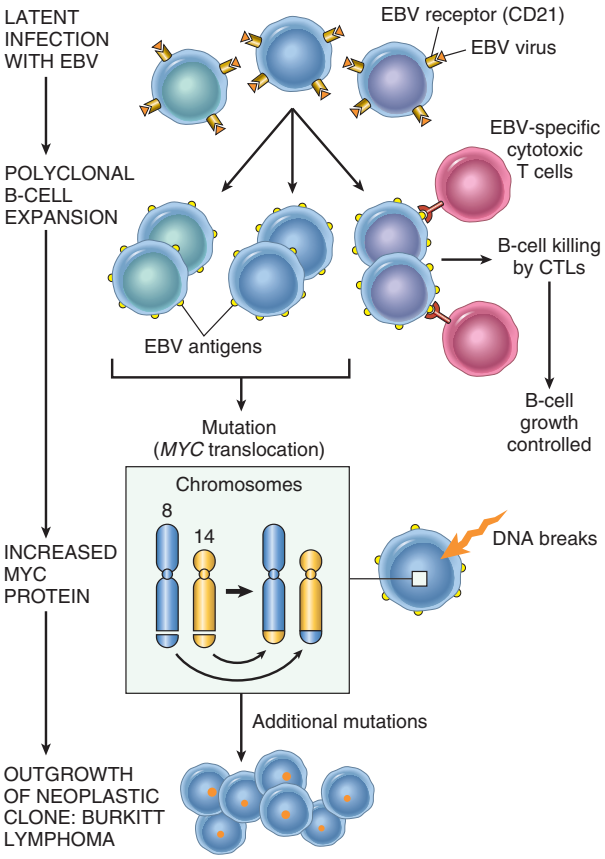

Figure 7.43 Pathogenesis of Epstein-Barr virus *(EBV)*–induced Burkitt lymphoma. *CTLs*, Cytotoxic T lymphocytes.

emerge from this population only with the acquisition of specific mutations, most notably translocations involving the *MYC* oncogene, as virtually all endemic and sporadic tumors possess the t(8;14) or other translocations that dysregulate *MYC*. Thus although sporadic Burkitt lymphomas are triggered by mechanisms other than EBV, they appear to develop through similar oncogenic pathways.

In summary, **in the case of Burkitt lymphoma, it seems that EBV is not directly oncogenic, but by acting as a polyclonal B-cell mitogen sets the stage for the acquisition of the (8;14) translocation and other mutations that ultimately produce a full-blown cancer.** In most individuals, EBV infection is readily controlled by effective immune responses, and lymphomagenesis is rare. By contrast, in regions where Burkitt lymphoma is endemic, cofactors such as chronic malaria may favor the acquisition of additional genetic events (e.g., t(8;14)) that lead to transformation.

The role played by EBV is more direct in B-cell lymphomas arising in immunosuppressed patients. Some persons with AIDS or who receive immunosuppressive therapy for preventing allograft rejection develop EBV-positive B-cell tumors, often at multiple sites and within extranodal tissues such as the gut or the central nervous system. These proliferations are polyclonal at the outset but can evolve into monoclonal neoplasms. In contrast to Burkitt lymphoma, tumors in immunosuppressed patients usually express LMP-1 and EBNA2, which are antigenic and would normally be recognized by cytotoxic T cells. Also, in contrast to Burkitt lymphoma, B-cell tumors in immunosuppressed individuals usually lack *MYC* translocations. These potentially lethal proliferations can be subdued if T-cell immunity can be restored, as may occur with withdrawal of immunosuppressive drugs in transplant recipients.

Nasopharyngeal carcinoma also is strongly associated with EBV. This tumor is endemic in southern China, parts of Africa, and the Inuit population of the Arctic. In contrast to Burkitt lymphoma, all nasopharyngeal carcinomas obtained from all parts of the world contain EBV, and antibody titers to viral capsid antigens are uniformly elevated in affected patients. The structure of the viral genome is identical (clonal) within individual tumors, indicating that EBV infection occurred before tumor development. EBV thus has a central role in the genesis of nasopharyngeal carcinoma, but (as with Burkitt lymphoma) its restricted geographic distribution indicates that genetic or environmental cofactors also contribute to its development. Unlike Burkitt lymphoma, LMP-1 is expressed in nasopharyngeal carcinoma cells and (as in B cells) activates the NF-κB pathway, which upregulates the expression of factors such as VEGF, FGF-2, MMP-9, and COX-2 that may contribute to oncogenesis. Nasopharyngeal carcinomas typically contain prominent infiltrates composed of T cells, which may be responding to viral antigens such as LMP-1, but this response is ineffective, suggesting that immune evasion mechanisms are likely to be important in this cancer. In line with this idea, nasopharyngeal carcinoma cells often express the immune checkpoint molecule PD-L1 and are responsive to PD-L1 inhibitors. Interestingly, EBV-positive carcinomas resembling nasopharyngeal carcinoma occasionally arise at other sites, such as the stomach and the thymus.

The relationship of EBV to the pathogenesis of Hodgkin lymphoma, yet another EBV-associated tumor, is discussed in Chapter 13.

Hepatitis B and C Viruses. **Worldwide, 70% to 85% of hepatocellular carcinomas are associated with infection with HBV or HCV.** HBV is endemic in countries of the Far East and Africa; correspondingly, these areas have the highest incidence of hepatocellular carcinoma. Despite compelling evidence incriminating HBV and HCV, the mode of action of these viruses in liver tumorigenesis is not fully elucidated. Oncogenes have yet to be identified in HBV or HCV genomes, and although the HBV DNA is integrated within the human genome, there is no consistent pattern of integration in liver cells. Indeed, while the oncogenic effects of HBV and HCV are multifactorial, the dominant effect seems to be immunologically mediated chronic inflammation and hepatocyte death leading to hepatocyte proliferation during regeneration and, over time, genomic damage.

As with any cause of hepatocellular injury, chronic viral infection leads to the compensatory proliferation of hepatocytes. This regenerative process is aided and abetted by a plethora of growth factors, cytokines, chemokines, and other bioactive substances. These are produced by activated immune cells and promote cell survival, tissue remodeling, and angiogenesis (Chapter 3). The activated immune cells

also produce other mediators, such as reactive oxygen species, that are genotoxic and mutagenic. One key molecular step may be activation of the NF-κB pathway, which blocks apoptosis, allowing the dividing hepatocytes to incur genotoxic stress and to accumulate mutations. Although this seems to be a dominant mechanism in the pathogenesis of virus-induced hepatocellular carcinoma, the HBV genome also contains genes that may directly promote the development of cancer. For example, an HBV gene known as *HBx* can activate a variety of transcription factors and several signal transduction pathways. In addition, viral integration can cause structural changes in chromosomes that dysregulate oncogenes and tumor suppressor genes.

Although not a DNA virus, HCV is also strongly linked to the pathogenesis of liver cancer. The molecular mechanisms used by HCV are less well defined than are those of HBV. In addition to chronic liver cell injury and compensatory regeneration, components of the HCV genome, such as the HCV core protein, may have a direct effect on tumorigenesis, possibly by activating a variety of growth-promoting signal transduction pathways.

Helicobacter pylori

First incriminated as a cause of peptic ulcers, *H. pylori* now has acquired the dubious distinction of being the first bacterium classified as a carcinogen. Indeed, *H. pylori* infection is implicated in the genesis of both gastric adenocarcinomas and gastric lymphomas.

The proposed scenario for the development of gastric adenocarcinoma in the setting of *H. pylori* infection is similar to that of HBV- and HCV-induced liver cancer, as it involves increased epithelial cell proliferation in a background of chronic inflammation. As in viral hepatitis, the inflammatory milieu contains numerous genotoxic agents, such as reactive oxygen species. The *H. pylori* genome also contains genes directly implicated in oncogenesis. Strains associated with gastric adenocarcinoma have been shown to contain a "pathogenicity island" that contains cytotoxin-associated A *(CagA)* gene. Although *H. pylori* is noninvasive, *CagA* penetrates into gastric epithelial cells, where it has a variety of effects including the initiation of a signaling cascade that mimics unregulated growth factor stimulation. The infection initially leads to development of chronic gastritis, followed by gastric atrophy, intestinal metaplasia of the lining cells, dysplasia, and cancer. This sequence takes decades to complete and occurs in only 3% of infected patients.

H. pylori is specifically associated with the development of gastric lymphomas of B-cell origin (also discussed in Chapters 13 and 17). Their molecular pathogenesis is incompletely understood but seems to involve strain-specific *H. pylori* factors as well as host genetic factors, such as polymorphisms in the promoters of inflammatory cytokines such as IL-1β and TNF. It is thought that *H. pylori* infection leads to the appearance of *H. pylori*–reactive T cells, which in turn stimulate a polyclonal B-cell proliferation. In chronic infections, currently unknown mutations may be acquired that give individual cells a growth advantage. These cells grow out into a monoclonal MALToma that nevertheless remains dependent on T-cell stimulation of B-cell pathways that activate the transcription factor NF-κB. At this stage, eradication of *H. pylori* by antibiotic therapy "cures" the lymphoma by removing the antigenic stimulus for T cells. At later stages, however, additional mutations may be acquired that cause constitutive NF-κB activation. At this point, the MALToma no longer requires the antigenic stimulus of the bacterium for growth and survival and develops the capacity to spread beyond the stomach to other tissues.

KEY CONCEPTS

VIRAL AND BACTERIAL ONCOGENESIS``

HTLV-1: a retrovirus that is endemic in Japan, the Caribbean, and parts of South America and Africa that causes adult T-cell leukemia/lymphoma.

- HTLV-I encodes two viral proteins, Tax and HBX, which are suspected to contribute to leukemogenesis through uncertain mechansisms.
- After a long latent period (decades), a small fraction of HTLV-I–infected individuals develop adult T-cell leukemia/lymphoma, a CD4+ tumor that arises from an HTLV-I–infected cell, presumably due to acquisition of additional mutations in the host cell genome.

HPV: an important cause of benign warts, cervical cancer, and oropharyngeal cancer.

- Oncogenic types of HPV encode the viral oncoproteins E6 and E7, which bind to p53 and Rb, respectively, with high affinity and neutralize their function.
- Development of cancer is associated with integration of HPV into the host genome and additional mutations needed for acquisition of cancer hallmarks.
- HPV cancers can be prevented by vaccination against high-risk HPV types.

EBV: ubiquitous herpesvirus implicated in the pathogenesis of Burkitt lymphomas, B-cell lymphomas in patients with T-cell immunosuppression (HIV infection, transplant recipients), and several other cancers.

- The EBV genome harbors several genes encoding proteins that trigger B-cell signaling pathways; in concert, these signals are potent inducers of B-cell growth and transformation.
- In the absence of T-cell immunity, EBV-infected B cells can rapidly "grow out" as aggressive B-cell tumors.
- In the presence of normal T-cell immunity, a small fraction of infected patients develop EBV-positive B-cell tumors (Burkitt lymphoma, Hodgkin lymphoma) or carcinomas (e.g., nasopharyngeal carcinoma).

HBV and HCV: cause of 70% to 85% of hepatocellular carcinomas worldwide.

- Oncogenic effects are multifactorial; dominant effect seems to be immunologically mediated chronic inflammation, hepatocellular injury, and reparative hepatocyte proliferation.
- HBx protein of HBV and the HCV core protein can activate signal transduction pathways that also may contribute to carcinogenesis.

H. pylori: implicated in gastric adenocarcinoma and MALToma.

- Pathogenesis of *H. pylori*–induced gastric cancers is multifactorial including chronic inflammation and reparative gastric cell proliferation.
- *H. pylori* pathogenicity genes, such as *CagA*, also may contribute by stimulating growth factor pathways.
- Chronic *H. pylori* infection leads to polyclonal B-cell proliferations that may give rise to a B-cell lymphoma (MALToma) of the stomach as a result of accumulation of mutations.

CLINICAL ASPECTS OF NEOPLASIA

Clinical Manifestations

Ultimately the importance of neoplasms lies in their effects on patients. Although malignant tumors are of course more threatening than benign tumors, any tumor, even a benign one, may cause morbidity and mortality.

Local and Hormonal Effects

Location is a critical determinant of the clinical effects of benign and malignant tumors. Tumors may impinge upon vital tissues and impair their function, cause death of involved tissues, and provide a nidus for infection. A small (1 cm) pituitary adenoma, although benign and possibly nonfunctional, can compress and destroy the surrounding normal gland and thus lead to serious hypopituitarism. Cancers arising within or metastatic to an endocrine gland may cause an endocrine insufficiency by destroying the gland. Neoplasms in the gut, both benign and malignant, may cause obstruction as they enlarge. Infrequently, peristaltic movement telescopes the neoplasm and its affected segment into the downstream segment, producing an obstructing intussusception (Chapter 17). Symptoms produced by a cancer due to its position can (ironically) be life-saving; for example, the few survivors of pancreatic cancer are those whose tumors "fortuitously" obstruct bile ducts early in their course, leading to the appearance of jaundice and other symptoms at a stage of the disease when surgical cure is possible.

Benign and malignant neoplasms arising in endocrine glands can cause clinical problems by producing hormones. Such functional activity is more typical of benign than of malignant tumors, which are more likely to be poorly differentiated and nonfunctional. A benign beta-cell adenoma of the pancreatic islets less than 1 cm in diameter may produce sufficient insulin to cause fatal hypoglycemia. In addition, nonendocrine tumors may elaborate hormones or hormone-like products and give rise to paraneoplastic syndromes (discussed later). The erosive and destructive growth of cancers or the expansile pressure of a benign tumor on any natural surface, such as the skin or mucosa of the gut, may cause ulcerations, secondary infections, and bleeding. Melena (blood in the stool) and hematuria, for example, are characteristic of neoplasms of the gut and urinary tract.

Cancer Cachexia

Cancer cachexia is a hypercatabolic state defined by a loss of muscle mass (with or without loss of fat) that cannot be explained by diminished food intake. It occurs in about 50% of cancer patients, most commonly in individuals with advanced gastrointestinal, pancreatic, and lung cancers, and is responsible for about 30% of cancer deaths. It is a highly debilitating condition characterized by extreme weight loss, fatigue, muscle atrophy, anemia, anorexia, and edema. Mortality is generally the consequence of atrophy of the diaphragm and other respiratory muscles.

The precise causes of cancer cachexia are not known, but inflammatory mediators, particularly TNF, IL-1, and IL-6, appear to have important roles. Administration of any of these cytokines to mice induces cachexia, whereas tumor-bearing mice are protected from cachexia by knockout of the TNF receptor. These observations are bolstered by clinical studies showing that cachexia in cancer patients is associated with higher levels of inflammatory cytokines. Evidence suggests that muscle loss occurs through a direct effect of inflammatory cytokines on skeletal muscle cells. Specifically, it appears that cytokines increase the degradation of major skeletal muscle structural proteins, such as myosin heavy chain, through signaling pathways that lead to ubiquitination of target proteins followed by proteolysis via the proteasome. It is also worth noting that similar cachetic states may be seen in patients without cancer, such as in those with chronic disseminated infections and AIDS, presumably at least in part due to the effects of inflammatory cytokines.

However, it must also be recognized that therapies directed against individual cytokines (e.g., TNF) in cancer patients have not been effective in reversing cachexia, suggesting that either a multiplicity of inflammatory cytokines, or other factors entirely, are also involved in its pathogenesis. In line with the latter possibility, while muscle wasting is the cardinal feature of cancer cachexia, many patients lose fat stores as well. One factor that may contribute to fat loss is a protein called lipid mobilizing factor, which has been detected in the sera and urine of patients with advanced cancer and which appears to sensitize adipocytes to lipolytic stimuli. It is likely that additional mechanisms underlying cancer cachexia await discovery.

Paraneoplastic Syndromes

Some cancer-bearing individuals develop signs and symptoms that cannot readily be explained by the anatomic distribution of the tumor or by the elaboration of hormones indigenous to the tissue from which the tumor arose; these are known as paraneoplastic syndromes. These occur in about 10% of persons with cancer. Paraneoplastic syndromes are important to recognize for several reasons:

- They may be the earliest manifestation of an occult neoplasm.
- In affected patients they can cause significant clinical problems and may even be lethal.
- They may mimic metastatic disease and therefore confound treatment.

A classification of paraneoplastic syndromes and their presumed origins is presented in Table 7.11. A few comments on some of the more common and interesting syndromes follow.

Endocrinopathies are frequently encountered paraneoplastic syndromes. By definition, the responsible cancers are not of endocrine origin and the secretory activity of such tumors is referred to as ectopic hormone production. *Cushing syndrome* is the most common endocrinopathy. Approximately 50% of affected individuals have carcinoma of the lung, chiefly the small-cell type. It is caused by excessive production of corticotropin (adrenocorticotropic hormone [ACTH]) or corticotropin-like peptides. The precursor of corticotropin is a large molecule known as pro-opiomelanocortin. Lung cancer patients with Cushing syndrome have elevated serum levels of both pro-opiomelanocortin and corticotropin. The former is not found in serum of patients with excess corticotropin produced by the pituitary.

Table 7.11 Paraneoplastic Syndromes

Clinical Syndromes	Major Forms of Underlying Cancer	Causal Mechanism
Endocrinopathies		
Cushing syndrome	Small cell carcinoma of lung Pancreatic carcinoma Neural tumors	ACTH or ACTH-like substance
Syndrome of inappropriate antidiuretic hormone secretion	Small cell carcinoma of lung Intracranial neoplasms	Antidiuretic hormone or atrial natriuretic hormones
Hypercalcemia	Squamous cell carcinoma of lung Breast carcinoma Renal carcinoma Adult T-cell leukemia/lymphoma	Parathyroid hormone-related protein (PTHRP), TGF-α, TNF, IL-1
Hypoglycemia	Ovarian carcinoma Fibrosarcoma Other mesenchymal sarcomas	Insulin or insulin-like substance
Polycythemia	Renal carcinoma Cerebellar hemangioma Hepatocellular carcinoma	Erythropoietin
Osteomalacia	Phosphaturic mesenchymal tumor	FGF-23
Nerve and Muscle Syndromes		
Myasthenia	Bronchogenic carcinoma Thymic neoplasms	Immunologic
Disorders of the central and peripheral nervous systems	Breast carcinoma	
Dermatologic Disorders		
Acanthosis nigricans	Gastric carcinoma Lung carcinoma Uterine carcinoma	Immunologic; secretion of epidermal growth factor
Dermatomyositis	Bronchogenic carcinoma Breast carcinoma	Immunologic
Osseous, Articular, and Soft Tissue Changes		
Hypertrophic osteoarthropathy and clubbing of the fingers	Bronchogenic carcinoma Thymic neoplasms	Unknown
Vascular and Hematologic Changes		
Venous thrombosis (Trousseau phenomenon)	Pancreatic carcinoma Bronchogenic carcinoma Other cancers	Tumor products (mucins that activate clotting)
Disseminated intravascular coagulation	Acute promyelocytic leukemia Prostatic carcinoma	Tumor products that activate clotting
Nonbacterial thrombotic endocarditis	Advanced cancers	Hypercoagulability
Red cell aplasia	Thymic neoplasms	Unknown
Others		
Nephrotic syndrome	Various cancers	Tumor antigens, immune complexes

ACTH, Adrenocorticotropic hormone; *IL*, interleukin; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *FGF-23*, fibroblast growth factor-23.

Hypercalcemia is probably the most common paraneoplastic syndrome; in fact, symptomatic hypercalcemia is more often related to cancer than to hyperparathyroidism. Two general processes are involved in cancer-associated hypercalcemia: (1) *osteolysis* induced by cancer, whether primary in bone, such as multiple myeloma, or metastatic to bone from any primary lesion, and (2) the production of *calcemic humoral substances* by extraosseous neoplasms. Only the second mechanism is considered to be paraneoplastic.

The humoral factor that is most commonly associated with paraneoplastic hypercalcemia is *parathyroid hormone–related protein* (PTHRP). As its name implies, PTHRP has partial structural homology to parathyroid hormone (PTH). PTHRP and PTH bind to the same G protein–coupled receptor, known as the PTH/PTHRP receptor (often referred to as PTH-R or PTHRP-R), and share some, but not all, biologic activities. Like PTH, PTHRP increases bone resorption and renal calcium uptake, while inhibiting renal phosphate transport, effects that raise serum calcium levels. In contrast to PTH, PTHRP is produced in small amounts by many normal tissues, including epithelial cell types such as keratinocytes, which may explain the relatively frequent