Chapter 6

Neoplasia

THOMAS P. STRICKER, MD, PhD
VINAY KUMAR, MD

Nomenclature
Characteristics of Benign and Malignant Neoplasms
Differentiation and Anaplasia
Rate of Growth
Local Invasion
Metastasis

Epidemiology
Cancer Incidence
Geographic and Environmental Variables
Age
Hereditary
Acquired Preneoplastic Disorders

Carcinogenesis: The Molecular Basis of Cancer
Self-Sufficiency in Growth Signals
Growth Factors
Growth Factor Receptors
Signal-Transducing Proteins
Nuclear Transcription Factors
Cyclins and Cyclin-Dependent Kinases (CDKs)

Inhibition of Growth-Inhibitory Signals
RB Gene and Cell Cycle
p53 Gene: Guardian of the Genome
Transforming Growth Factor-β Pathway
Adenomatous Polyposis Coli-β-Catenin Pathway

Evasion of Apoptosis
Limitless Replicative Potential
Development of Sustained Angiogenesis
Ability to Invade and Metastasize
Invasion of Extracellular Matrix (ECM)
Vascular Dissemination and Homing of Tumor Cells
Molecular Genetics of Metastasis
Genomic Instability—Enabler of Malignancy
Micro RNAs (miRNAs) and Carcinogenesis

Molecular Basis of Multistep Carcinogenesis
Karyotypic Changes in Tumors
Epigenetic Changes

Etiology of Cancer: Carcinogenic Agents
Chemical Carcinogens
Direct-Acting Agents
Indirect-Acting Agents
Mechanisms of Action of Chemical Carcinogens
Radiation Carcinogenesis
Viral and Microbial Oncogenesis
Oncogenic RNA Viruses
Oncogenic DNA Viruses
Helicobacter pylori

Host Defense Against Tumors: Tumor Immunity
Tumor Antigens
Antitumor Effector Mechanisms
Immune Surveillance

Clinical Aspects of Neoplasia
Effects of Tumor on Host
Cancer cachexia
Paraneoplastic Syndromes
Grading and Staging of Cancer
Laboratory Diagnosis of Cancer
Morphologic Methods
Tumor Markers
Molecular Diagnosis
Molecular Profiling of Tumors
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 neoplasms. Patients and the public often ask, “When will there be a cure for cancer?” The answer to this simple question is difficult because cancer is not one disease but many disorders that share a profound growth dysregulation. Some cancers, such as Hodgkin lymphomas, are curable, whereas others, such as cancer of the pancreas, have a high mortality. The only hope for controlling cancer lies in learning more about its pathogenesis, and great strides have been made in understanding the molecular basis of cancer. This chapter deals with the basic biology of neoplasia—the nature of benign and malignant neoplasms and the molecular basis of neoplastic transformation. The host response to tumors and the clinical features of neoplasia are also discussed.

**Nomenclature**

*Neoplasia* literally means “new growth.” A neoplasm, as defined by Willis, is “an abnormal mass of tissue the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after the cessation of the stimuli which evoked the change.” Fundamental to the origin of all neoplasms are heritable (genetic) changes that allow excessive and unregulated proliferation that is independent of physiologic growth-regulatory stimuli. Neoplastic cells are said to be transformed because they continue to replicate, apparently oblivious to the regulatory influences that control normal cell growth. Neoplasms therefore enjoy a certain degree of autonomy and more or less steadily increase in size regardless of their local environment and the nutritional status of the host. Their autonomy is by no means complete, however. Some neoplasms require endocrine support, and such dependencies sometimes can be exploited to the disadvantage of the neoplasm. All neoplasms depend on the host for their nutrition and blood supply.

In common medical usage, a neoplasm is often referred to as a tumor, and the study of tumors is called oncology (from oncos, “tumor,” and logos, “study of”). In oncology, the division of neoplasms into benign and malignant categories is important. This categorization is based on a judgment of a neoplasm’s potential clinical behavior.

A tumor is said to be benign when its microscopic and gross characteristics are considered to be relatively innocuous, implying that it will remain localized, it cannot spread to other sites, and is amenable to local surgical removal; the patient generally survives. It should be noted, however, that benign tumors can produce more than localized lumps, and sometimes they are responsible for serious disease, as pointed out later.

Benign tumors are collectively referred to as cancers, derived from the Latin word for crab—that is, they adhere to any part that they seize in an obstinate manner, similar to a crab’s behavior. Malignant, as applied to a neoplasm, implies that the lesion can invade and destroy adjacent structures and spread to distant sites (metastasize) to cause death. Not all cancers pursue so docile a course. Some are less aggressive and are treated successfully, but the designation malignant constitutes a red flag.

All tumors, benign and malignant, have two basic components: (1) the parenchyma, made up of transformed or neoplastic cells, and (2) the supporting, host-derived, non-neoplastic stroma, made up of connective tissue, blood vessels, and host-derived inflammatory cells. The parenchyma of the neoplasm largely determines its biologic behavior, and it is this component from which the tumor derives its name. The stroma is crucial to the growth of the neoplasm, since it carries the blood supply and provides support for the growth of parenchymal cells. As will be discussed later, stromal cells and neoplastic cells carry on a two-way conversation that influences the growth of the tumor.

**Benign Tumors.** In general, benign tumors are designated by attaching the suffix -oma to the cell type from which the tumor arises. A benign tumor arising in fibrous tissue is a fibroma; a benign cartilaginous tumor is a chondroma. The nomenclature of benign epithelial tumors is more complex. They are classified sometimes on the basis of their microscopic pattern and sometimes on the basis of their macroscopic pattern. Others are classified by their cells of origin.

For instance, the term adenoma is applied to benign epithelial neoplasms producing gland patterns and to neoplasms derived from glands but not necessarily exhibiting gland patterns. A benign epithelial neoplasm arising from renal tubule cells and growing in glandlike patterns would be termed an adenoma, as would a mass of benign epithelial cells that produces no glandular patterns but has its origin in the adrenal cortex. Papillomas are benign epithelial neoplasms, growing on any surface, that produce microscopic or macroscopic finger-like fronds. A poly is a mass that projects above a mucosal surface, as in the gut, to form a macroscopically visible structure (Fig. 6–1). Although this term is commonly used for benign tumors, some malignant tumors also may appear as polyps. Cystadenomas are hollow cystic masses; typically they are seen in the ovary.

**Malignant Tumors.** The nomenclature of malignant tumors essentially follows that of benign tumors, with certain additions and exceptions.

Malignant neoplasms arising in mesenchymal tissue or its derivatives are called sarcomas. A cancer of fibrous tissue origin is a fibrosarcoma, and a malignant neoplasm composed of chondrocytes is a chondrosarcoma. Sarcomas are designated by their histogenesis (i.e., the cell type of which they are composed). Malignant neoplasms of epithelial cell origin are called carcinomas. It must be remembered that the epithelia of the body are derived from all three germ-cell layers; a malignant neoplasm arising in the renal tubular epithelium (mesoderm) is a carcinoma, as are the cancers arising in the skin (ectoderm) and lining epithelium of the gut (endoderm). It is evident that mesoderm may give rise to carcinomas (epithelial) and sarcomas (mesenchymal). Carcinomas may be qualified further. Carcinomas that grow in a glandular pattern are called adenocarcinomas, and those that...
produce squamous cells are called squamous cell carcinomas. Sometimes the tissue or organ of origin can be identified, as in the designation of renal cell adenocarcinoma or cholangiocarcinoma, which implies an origin from bile ducts. Sometimes the tumor shows little or no differentiation and must be called poorly differentiated or undifferentiated carcinoma.

The parenchymal cells in a neoplasm, whether benign or malignant, resemble each other, as though all had been derived from a single progenitor. Indeed, neoplasms are of monoclonal origin, as is discussed later. In some instances, however, the tumor cells may undergo divergent differentiation, creating so-called mixed tumors. The best example is mixed tumor of salivary gland. These tumors have obvious epithelial components dispersed throughout a fibromyxoid stroma, sometimes harboring islands of cartilage or bone (Fig. 6–2). All of these diverse elements are thought to derive from epithelial cells, myoepithelial cells, or both in the salivary glands, and the preferred designation of these neoplasms is pleomorphic adenoma. Fibroadenoma of the female breast is another common mixed tumor. This benign tumor contains a mixture of proliferated ductal elements (adenoma) embedded in a loose fibrous tissue (fibroma). Although studies suggest that only the fibrous component is neoplastic, the term fibroadenoma remains in common usage.

The multifaceted mixed tumors should not be confused with a teratoma, which contains recognizable mature or immature cells or tissues representative of more than one germ-cell layer and sometimes all three. Teratomas originate from totipotential stem cells such as those normally present in the ovary and testis and sometimes abnormally present in sequestered midline embryonic rests. Such cells have the capacity to differentiate into any of the cell types found in the adult body and so, not surprisingly, may give rise to neoplasms that mimic, in a helter-skelter fashion, bits of bone, epithelium, muscle, fat, nerve, and other tissues.

The specific names of the more common forms of neoplasms are presented in Table 6–1. Some glaring inconsistencies may be noted. For example, the terms lymphoma, mesothelioma, melanoma, and seminoma are used for malignant neoplasms. These inappropriate usages are firmly entrenched in medical terminology.

There are other instances of confusing terminology. Hamartoma is a malformation that presents as a mass of disorganized tissue indigenous to the particular site. One may see a mass of mature but disorganized hepatic cells, blood vessels, and possibly bile ducts within the liver, or there may be a hamartomatous nodule in the lung containing islands of cartilage, bronchi, and blood vessels. Another misnomer is the term choristoma. This congenital anomaly is better described as a heterotopic rest of cells. For example, a small nodule of well-developed and normally organized pancreatic tissue may be found in the submucosa of the stomach, duodenum, or small intestine. This heterotopic rest may be replete with islets of Langerhans and exocrine glands. The term choristoma, connoting a neoplasm, imparts to the heterotopic rest a gravity far beyond its usual trivial significance. Although the terminology of neoplasms is regrettably not simple, it is important because it is the language by which the nature and significance of tumors are categorized.
### Table 6–1 Nomenclature of Tumors

<table>
<thead>
<tr>
<th>Tissue of Origin</th>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composed of One Parenchymal Cell Type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connective tissue and derivatives</td>
<td>Fibroma</td>
<td>Fibrosarcoma</td>
</tr>
<tr>
<td></td>
<td>Lipoma</td>
<td>Liposarcoma</td>
</tr>
<tr>
<td></td>
<td>Chondroma</td>
<td>Chondrosarcoma</td>
</tr>
<tr>
<td></td>
<td>Osteoma</td>
<td>Osteogenic sarcoma</td>
</tr>
<tr>
<td><strong>Endothelial and related tissues</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood vessels</td>
<td>Hemangioma</td>
<td>Angiosarcoma</td>
</tr>
<tr>
<td>Synovium</td>
<td>Lymphangioma</td>
<td>Lymphangiosarcoma</td>
</tr>
<tr>
<td>Mesothelium</td>
<td>Meningioma</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>Brain coverings</td>
<td></td>
<td>Invasive meningioma</td>
</tr>
<tr>
<td><strong>Blood cells and related cells</strong></td>
<td>Hemangioma</td>
<td>Leukemias</td>
</tr>
<tr>
<td></td>
<td>Lymphangioma</td>
<td>Lymphomas</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth</td>
<td>Leiomyoma</td>
<td>Leiomysosarcoma</td>
</tr>
<tr>
<td>Striated</td>
<td>Rhabdomyoma</td>
<td>Rhabdomyosarcoma</td>
</tr>
<tr>
<td><strong>Tumors of epithelial origin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratified squamous</td>
<td>Squamous cell papilloma</td>
<td>Squamous cell or epidermoid carcinoma</td>
</tr>
<tr>
<td>Basal cells of skin or adnexa</td>
<td>Adenoma</td>
<td>Basal cell carcinoma</td>
</tr>
<tr>
<td>Epithelial lining of glands or ducts</td>
<td>Papilloma</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td></td>
<td>Cystadenoma</td>
<td>Papillary carcinomas</td>
</tr>
<tr>
<td></td>
<td>Bronchial adenoma</td>
<td>Cystadenocarcinoma</td>
</tr>
<tr>
<td>Respiratory passages</td>
<td>Renal tubular adenoma</td>
<td>Bronchogenic carcinoma</td>
</tr>
<tr>
<td>Renal epithelium</td>
<td>Liver cell adenoma</td>
<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>Liver cells</td>
<td>Urinary tract epithelium</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>(transitional)</td>
<td>(germ cells)</td>
<td>Urothelial carcinoma</td>
</tr>
<tr>
<td>Placental epithelium</td>
<td>Hydatidiform mole</td>
<td>Choriocarcinoma</td>
</tr>
<tr>
<td>Testicular epithelium (germ cells)</td>
<td></td>
<td>Seminoma</td>
</tr>
<tr>
<td><strong>Tumors of melanocytes</strong></td>
<td>Nevus</td>
<td>Embryonal carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malignant melanoma</td>
</tr>
</tbody>
</table>

**More Than One Neoplastic Cell Type—Mixed Tumors, Usually Derived from One Germ Cell Layer**

<table>
<thead>
<tr>
<th>Salivary glands</th>
<th>Pleomorphic adenoma (mixed tumor of salivary gland)</th>
<th>Malignant mixed tumor of salivary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal anlage</td>
<td></td>
<td>Wilms tumor</td>
</tr>
</tbody>
</table>

**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 |

### CHARACTERISTICS OF BENIGN AND MALIGNANT NEOPLASMS

Nothing is more important to the patient with a tumor than being told “It is benign.” In most instances such a prediction can be made with remarkable accuracy based on long-established clinical and anatomic criteria, but some neoplasms defy easy characterization. Certain features may indicate innocence, and others may indicate malignancy. These problems are not the rule, however, and there are four fundamental features by which benign and malignant tumors can be distinguished. These are differentiation and anaplasia, rate of growth, local invasion, and metastasis.

**Differentiation and Anaplasia**

Differentiation and anaplasia refer only to the parenchymal cells that constitute the transformed elements of neoplasms. The differentiation of parenchymal cells refers to the extent to which they resemble their normal forebears morphologically and functionally. The stroma carrying the blood supply is crucial to the growth of tumors but does not aid in the separation of benign from malignant ones. The amount of stromal connective tissue does determine, however, the consistency of a neoplasm. Certain cancers induce a dense, abundant fibrous stroma (desmoplasia), making them hard, so-called scirrhous tumors.

Benign neoplasms are composed of well-differentiated cells that closely resemble their normal counterparts. A lipoma is made up of mature fat cells laden with cytoplasmic lipid vacuoles, and a chondroma is made up of mature cartilage cells that synthesize their usual cartilaginous matrix—evidence of morphologic and functional differentiation. In well-differentiated benign tumors, mitoses are extremely scant in number and are of normal configuration.

Malignant neoplasms are characterized by a wide range of parenchymal cell differentiation, from sur-
prisingly well differentiated (Fig. 6–3) to completely undifferentiated. For example, well-differentiated adenocarcinomas of the thyroid may contain normal-appearing follicles. Such tumors sometimes may be difficult to distinguish from benign proliferations. Between the two extremes lie tumors loosely referred to as moderately well differentiated.

The better the differentiation of the cell, the more completely it retains the functional capabilities found in its normal counterparts. Benign neoplasms and even well-differentiated cancers of endocrine glands frequently elaborate the hormones characteristic of their origin. Well-differentiated squamous cell carcinomas elaborate keratin (see Fig. 6–3), just as well-differentiated hepatocellular carcinomas elaborate bile. In other instances unanticipated functions emerge. Some cancers may elaborate fetal proteins not produced by comparable cells in the adult. Cancers of nonendocrine origin may produce so-called ectopic hormones. For example, certain lung carcinomas may produce adrenocorticotropic hormone (ACTH), parathyroid-like hormone, insulin, glucagon, and others. More is said about these phenomena later. Despite exceptions, the more rapidly growing and the more anaplastic a tumor, the less likely it is to have specialized functional activity.

Malignant neoplasms that are composed of undifferentiated cells are said to be anaplastic. Lack of differentiation, or anaplasia, is considered a hallmark of malignancy. The term anaplasia literally means “to form backward.” It implies dedifferentiation, or loss of the structural and functional differentiation of normal cells. It is now known, however, that at least some cancers arise from stem cells in tissues; in these tumors failure of differentiation, rather than dedifferentiation of specialized cells, accounts for undifferentiated tumors. Recent studies also indicate that, in some cases dedifferentiation of apparently mature cells does occur during carcinogenesis.

Anaplastic cells display marked pleomorphism (i.e., marked variation in size and shape) (Fig. 6–4). Characteristically the nuclei are extremely hyperchromatic (darkly stained) and large. The nuclear-to-cytoplasmic ratio may approach 1:1 instead of the normal 1:4 or 1:6. Giant cells that are considerably larger than their neighbors may be formed and possess either one enormous nucleus or several nuclei. Anaplastic nuclei are variable and bizarre in size and shape. The chromatin is coarse and clumped, and nucleoli may be of astounding size. More important, mitoses are often numerous and distinctly atypical; anarchic multiple spindles may be seen and sometimes appear as tripolar or quadrupolar forms (Fig. 6–5). Also, anaplastic cells usually fail to develop recognizable patterns of orientation to one another (i.e., they lose normal polarity). They may grow in sheets, with total loss of communal structures, such as gland formations or stratified squamous architecture. Anaplasia is the most extreme disturbance in cell growth encountered in the spectrum of cellular proliferations.

Before we leave the subject of differentiation and anaplasia, we should discuss dysplasia, a term used to describe disorderly but non-neoplastic proliferation. Dysplasia is encountered principally in the epithelium. It is a loss in the uniformity of individual cells and in their architectural orientation. Dysplastic cells exhibit considerable pleomorphism and often possess hyperchromatic nuclei that are abnormally large for the size of the cell. Mitotic figures are more abundant than usual. Frequently the mitoses appear in abnormal locations within the epithelium. In dysplastic stratified squamous epithelium, mitoses are not confined to the basal layers, where they normally occur, but may appear at all levels and even in surface cells. There is considerable architectural anarchy. For example, the usual progressive maturation of tall cells in the basal layer to flattened squames on the surface may be lost and replaced by a disordered scrambling of dark basal-appearing cells (Fig. 6–6). When dysplastic changes are marked and involve the entire thickness of the epithelium, the lesion is referred to as carcinoma in situ, a pre-invasive stage of cancer (Chapter 19). Although
dysplastic changes are often found adjacent to foci of malignant transformation, and long-term studies of cigarette smokers show that epithelial dysplasia almost invariably antedates the appearance of cancer, the term dysplasia without qualifications does not indicate cancer, and dysplasias do not necessarily progress to cancer. Mild-to-moderate changes that do not involve the entire thickness of epithelium may be reversible, and with removal of the putative inciting causes, the epithelium may revert to normal.

**Rate of Growth**

Most benign tumors grow slowly, and most cancers grow much faster, eventually spreading locally and to distant sites (metastasizing) and causing death. There are many exceptions to this generalization, however, and some benign tumors grow more rapidly than some cancers. For example, the rate of growth of leiomyomas (benign smooth muscle tumors) of the uterus is influenced by the circulating levels of estrogens. They may increase rapidly in size during pregnancy then cease growing, becoming largely fibrocalcific, after menopause. Other influences, such as adequacy of blood supply or pressure constraints, also may affect the growth rate of benign tumors. Adenomas of the pituitary gland locked into the sella turcica have been observed to shrink suddenly. Presumably, they undergo a wave of necrosis as progressive enlargement compresses their blood supply. Despite these caveats and the variation in growth rate from one neoplasm to another, it is generally true that most benign tumors increase in size slowly over the span of months to years.

The rate of growth of malignant tumors correlates in general with their level of differentiation. In other words, rapidly growing tumors tend to be poorly differentiated. However, there is wide variation in the rate of growth. Some grow slowly for years, then enter a phase of rapid growth, signifying the emergence of an aggressive subclone of transformed cells. Others grow relatively slowly and steadily, and there are exceptional instances when growth comes almost to a standstill. Even more exceptionally, some cancers (particularly choriocarcinomas) have disappeared spontaneously as they have become totally necrotic, leaving only secondary metastatic implants. Despite these rarities, most cancers progressively enlarge over time, some slowly, others rapidly, but the notion that they “emerge out of the blue” is not true. Many lines of experimental and clinical evidence document that most if not all cancers take years and sometimes decades to evolve into clinically overt lesions. Rapidly growing malignant tumors often contain central areas of ischemic necrosis because the tumor blood supply, derived from the host, fails to keep pace with the oxygen needs of the expanding mass of cells.

**Cancer Stem Cells and Lineages.** A clinically detectable tumor contains a heterogeneous population of cells, which originated from the clonal growth of a single cell. It has been hypothesized that this population contains...
cancer stem cells, which, in analogy to tissue stem cells, have the capacity to initiate and sustain the tumor. Recently, cancer stem cells, sometimes called tumor-initiating cells, were identified in breast cancer, glioblastoma multiforme (a brain tumor), and acute myeloid leukemia. Cancer stem cells constitute fewer than 2% of the cells in breast tumors and 0.1% to 1.0% of cells in acute myeloid leukemia. These findings have important implications for cancer treatment. Therapies that may efficiently kill the progeny of cancer stem cells would leave in place the cells capable of regenerating the tumor. Whether cancer stem cells exist in all tumors is not yet clear.

Local Invasion

A benign neoplasm remains localized at its site of origin. It does not have the capacity to infiltrate, invade, or metastasize to distant sites, as do malignant neoplasms. For example, as fibromas and adenomas slowly expand, most develop an enclosing fibrous capsule that separates them from the host tissue. This capsule probably is derived from the stroma of the host tissue as the parenchymal cells atrophy under the pressure of the expanding tumor. The stroma of the tumor itself also may contribute to the capsule (Figs. 6–7 and 6–8). It should be emphasized, however, that not all benign neoplasms are encapsulated. For example, the leiomyoma of the uterus is discretely demarcated from the surrounding smooth muscle by a zone of compressed and attenuated normal myometrium, but there is no well-developed capsule. Nonetheless, a well-defined cleavage plane exists around these lesions. A few benign tumors are neither encapsulated nor discretely defined; this is particularly true of some vascular benign neoplasms of the dermis. These exceptions are pointed out only to emphasize that although encapsulation is the rule in benign tumors, the lack of a capsule does not imply that a tumor is malignant.

Cancers grow by progressive infiltration, invasion, destruction, and penetration of the surrounding tissue (Figs. 6–9 and 6–10). They do not develop well-defined capsules. There are, however, occasional instances in which a slowly growing malignant tumor deceptively appears to be encased by the stroma of the surrounding host tissue, but microscopic examination usually reveals tiny crablike feet penetrating the margin and infiltrating adjacent structures. The infiltrative mode of growth makes it necessary to remove a wide margin of surrounding normal tissue when surgical excision of a malignant tumor is attempted. Surgical pathologists carefully examine the margins of resected tumors to ensure that they are devoid of cancer cells (clean margins). Next to the development of metastases, local invasiveness is the most reliable feature that distinguishes malignant from benign tumors.

Metastasis

The term metastasis connotes the development of secondary implants (metastases) discontinuous with the primary tumor, in remote tissues (Fig. 6–11). The properties of invasiveness and, even more so, metastasis, more unequivocally identify a neoplasm as malignant than any of the other attributes of a tumor. Not all cancers have equivalent ability to metastasize, however. At one extreme are basal cell carcinomas of the skin and most primary tumors of the central nervous system that are highly invasive in their primary sites of origin but rarely metastasize. At the other extreme are osteogenic (bone) sarcomas, which usually have metastasized to the lungs at the time of initial discovery.

Approximately 30% of newly diagnosed patients with solid tumors (excluding skin cancers other than melanomas) present with clinically evident metastases. An additional 20% have occult (hidden) metastases at the time of diagnosis.

In general, the more anaplastic and the larger the primary neoplasm, the more likely is metastatic spread; however, exceptions abound. Extremely small cancers have been known to metastasize, and, conversely, some large and ominous-looking lesions may not spread. Dissemination strongly prejudices, if it does not preclude, the possibility of cure of the disease, so it is obvious that,
short of prevention of cancer, no achievement would confer greater benefit on patients than methods to prevent metastasis.

Malignant neoplasms disseminate by one of three pathways: (1) seeding within body cavities, (2) lymphatic spread, or (3) hematogenous spread.

Spread by seeding occurs when neoplasms invade a natural body cavity. This mode of dissemination is particularly characteristic of cancers of the ovary, which often cover the peritoneal surfaces widely. The implants literally may glaze all peritoneal surfaces and yet not invade the underlying parenchyma of the abdominal organs. Here is an instance of the ability to reimplant elsewhere that seems to be separable from the capacity to invade. Neoplasms of the central nervous system, such as a medulloblastoma or ependymoma, may penetrate the cerebral ventricles and be carried by the cerebrospinal fluid to reimplant on the meningeal surfaces, either within the brain or in the spinal cord.

Lymphatic spread is more typical of carcinomas, whereas hematogenous spread is favored by sarcomas. There are numerous interconnections, however, between the lymphatic and vascular systems, and so all forms of cancer may disseminate through either or both systems. The pattern of lymph node involvement depends principally on the site of the primary neoplasm and the natural pathways of lymphatic drainage of the site. Lung carcinomas arising in the respiratory passages metastasize first to the regional bronchial lymph nodes, then to the tracheobronchial and hilar nodes. Carcinoma of the breast usually arises in the upper outer quadrant and first spreads to the axillary nodes. However, medial breast lesions may drain through the chest wall to the nodes along the internal mammary artery. Thereafter, in both instances, the supraclavicular and infraclavicular nodes may be seeded. In some cases, the cancer cells seem to traverse all of the lymph nodes ultimately to reach the vascular compartment via the thoracic duct.

A “sentinal lymph node” is defined as the first lymph node in a regional lymphatic basin that receives lymph flow from a primary tumor. It can be delineated by injection of blue dyes or radiolabelled tracers. Biopsy of sentinel lymph nodes allows determination of the extent of spread of tumor, and can be used to plan treatment.

It should be noted that although enlargement of nodes near a primary neoplasm should arouse strong suspicions of metastatic spread, it does not always imply cancerous involvement. The necrotic products of the neoplasm and tumor antigens often evoke reactive changes in the nodes, such as enlargement and hyperplasia of the follicles (lymphadenitis) and proliferation of macrophages in the subcapsular sinuses (sinus histiocytosis).

Hematogenous spread is the most feared consequence of a cancer. It is the favored pathway for sarcomas, but carcinomas use it as well. As might be expected, arteries are penetrated less readily than are veins. With venous invasion, the blood-borne cells follow the venous flow draining the site of the neoplasm, with tumor cells often...
stopping in the first capillary bed they encounter. Since all portal area drainage flows to the liver, and all caval blood flows to the lungs, the liver and lungs are the most frequently involved secondary sites in hematogenous dissemination. Cancers arising near the vertebral column often embolize through the paravertebral plexus; this pathway probably is involved in the frequent vertebral metastases of carcinomas of the thyroid and prostate.

Certain carcinomas have a propensity to invade veins. Renal cell carcinoma often invades the renal vein to grow in a snakelike fashion up the inferior vena cava, sometimes reaching the right side of the heart. Hepatocellular carcinomas often penetrate portal and hepatic radicles to grow within them into the main venous channels. Remarkably, such intravenous growth may not be accompanied by widespread dissemination.

Many observations suggest that mere anatomic localization of the neoplasm and natural pathways of venous drainage do not wholly explain the systemic distributions of metastases. For example, prostatic carcinoma preferentially spreads to bone, bronchogenic carcinomas tend to involve the adrenals and the brain, and neuroblastomas spread to the liver and bones. Conversely, skeletal muscles, although rich in capillaries, are rarely the site of secondary deposits. The molecular basis of such tissue-specific homing of tumor cells is discussed later.

In conclusion, the various features discussed in the preceding sections, as summarized below and in Figure 6–12, usually permit the differentiation of benign and malignant neoplasms. Against this background of the structure and behavior of neoplasms, we can turn to some considerations of their nature and origins.

**SUMMARY**

**Characteristics of Benign and Malignant Tumors**
- Benign and malignant tumors can be distinguished on the basis of the degree of differentiation, rate of growth, local invasiveness, and distant spread.
- Benign tumors resemble the tissue of origin and are well differentiated; malignant tumors are poorly or completely undifferentiated (anaplastic).
- Benign tumors are slow growing, whereas malignant tumors generally grow faster.
- Benign tumors are well circumscribed and have a capsule; malignant tumors are poorly circumscribed and invade the surrounding normal tissues.
- Benign tumors remain localized to the site of origin, whereas malignant tumors are locally invasive and they metastasize to distant sites.

**EPIDEMIOLOGY**

Because cancer is a disorder of cell growth and behavior, its ultimate cause must be defined at the cellular and molecular levels. Cancer epidemiology can contribute substantially to knowledge about the origin of cancer. The now well-established concept that cigarette smoking is causally associated with lung cancer arose primarily from epidemiologic studies. A comparison of the incidence of colon cancer and dietary patterns in the western world...
and Africa led to the recognition that dietary fat and fiber content may figure importantly in the causation of this cancer. Major insights into the causes of cancer can be obtained by epidemiologic studies that relate particular environmental, racial (possibly hereditary), and cultural influences to the occurrence of specific neoplasms. Certain diseases associated with an increased risk of developing cancer (preneoplastic disorders) also provide clues to the pathogenesis of cancer. In the following discussion we first summarize the overall incidence of cancer to gain an insight into the magnitude of the cancer problem, then we review some issues relating to the patient and environment that influence the predisposition to cancer.

**Cancer Incidence**

Some perspective on the likelihood of developing a specific form of cancer can be gained from national incidence and mortality data. Overall, it is estimated that about 1.4 million new cancer cases will occur in 2006, and 565,000 people will die of cancer in the United States. The incidence of the most common forms of cancer and the major killers is presented in Figure 6–13.

Over several decades, the death rates of many forms of malignant neoplasia have changed. Particularly notable is the significant increase in the overall cancer death rate among men that was attributable largely to lung cancer, but this has finally begun to drop. In contrast, the overall death rate among women has fallen slightly, mostly as a result of the decline in death rates from cancers of the uterine cervix, stomach, and large bowel. These welcome trends have more than counterbalanced the striking climb in the rate of lung cancer among women, which not long ago was a relatively uncommon form of neoplasia in this sex. The declining death rate from cervical cancer is directly related to widespread use of cytologic smear studies for early detection of this tumor while it is still curable. The causes of decline in death rates for cancers of the stomach are obscure; however, there have been speculations about decreasing exposure to dietary carcinogens.

**Geographic and Environmental Variables**

Although many impressive advances in understanding the molecular pathogenesis of cancer have been made by analyzing hereditary cancers, it is fair to state that environmental factors that give rise to somatic mutations are the predominant cause of the most common sporadic cancers. This notion is supported by the geographic differences in death rates from specific forms of cancer. For example, death rates from breast cancer are about fourfold to fivefold higher in the United States and Europe compared with Japan. Conversely, the death rate for stomach carcinoma in men and women is about seven times higher in Japan than in the United States. Liver cell carcinoma is relatively infrequent in the United States but is the most lethal cancer among many African populations. Nearly all the evidence indicates that these geographic differences are environmental rather than genetic in origin. Nisei (second-generation Japanese living in the United States) have mortality rates for certain forms of cancer that are intermediate between those of natives of Japan and of Americans who have lived in the United States for many generations. The two rates come closer with each passing generation.

There is no paucity of environmental carcinogens. They lurk in the ambient environment, in the workplace, in food, and in personal practices. They can be as un-
versal as sunlight, can be found particularly in urban settings (e.g., asbestos), or can be limited to a certain occupation (Table 6–2). Certain features of diet have been implicated as possible predisposing influences. Among the possible environmental influences, the most distressing are those incurred in personal practices, notably cigarette smoking and chronic alcohol consumption. The risk of cervical cancer is linked to age at first intercourse and the number of sex partners (pointing to a causal role for venereal transmission of an oncogenic virus). There is no escape: It seems that everything one does to earn a livelihood, to subsist, or to enjoy life turns out to be illegal, immoral, or fattening, or—most disturbing—possibly carcinogenic.

### Age

In general, the frequency of cancer increases with age. Most cancer mortality occurs between ages 55 and 75; the rate declines, along with the population base, after age 75. The rising incidence with age may be explained by the accumulation of somatic mutations associated with the emergence of malignant neoplasms (discussed later). The decline in immune competence that accompanies aging also may be a factor.

Cancer causes slightly more than 10% of all deaths among children younger than 15 years (Chapter 7). The major lethal cancers in children are leukemia, tumors of the central nervous system, lymphomas, soft tissue sarcomas, and bone sarcomas. As discussed later, study of several childhood tumors, particularly retinoblastoma and Wilms tumor, has provided novel insights into the pathogenesis of malignant transformation.

### Heredity

The evidence now indicates that for many types of cancer, including the most common forms, there exist not only environmental influences but also hereditary predispositions. Hereditary forms of cancer can be divided into three categories (Table 6–3).

#### Inherited Cancer Syndromes

Inherited cancer syndromes include several well-defined cancers in which inheritance...
Virtually all the common types of Familial Cancers.

Chapter 23). Not the target of transformation (e.g., Lisch nodules.

Sometimes, there are abnormalities in tissue that are polyposis of the colon and in multiple endocrine neoplasia.

Tumors within this group often are associated with a familial pattern of inheritance.

Childhood retinoblastoma is the most striking example of this category. Approximately 40% of retinoblastomas are familial. As is discussed later, a tumor suppressor gene has been implicated in the pathogenesis of this tumor. Carriers of this gene have a 10,000-fold increased risk of developing retinoblastoma, usually bilaterally. They also have a greatly increased risk of developing a second cancer, particularly osteogenic sarcoma. Familial adenomatous polyposis is another hereditary disorder marked by an extraordinarily high risk of cancer. Individuals who inherit the autosomal dominant mutation have, at birth or soon thereafter, innumerable polyoid adenomas of the colon, and virtually 100% of patients develop a carcinoma of the colon by age 50 (see Table 6–3).

Tumors within this group often are associated with a specific marker phenotype. There may be multiple benign tumors in the affected tissue, as occurs in familial polyposis of the colon and in multiple endocrine neoplasia. Sometimes, there are abnormalities in tissue that are not the target of transformation (e.g., Lisch nodules and café-au-lait spots in neurofibromatosis type 1; Chapter 23).

Familial Cancers. Virtually all the common types of cancers that occur sporadically have been reported to occur in familial forms. Examples include carcinomas of colon, breast, ovary, and brain. Features that characterize familial cancers include early age at onset, tumors arising in two or more close relatives of the index case, and sometimes multiple or bilateral tumors. Familial cancers are not associated with specific marker phenotypes. For example, in contrast to the familial adenomatous polyposis syndrome, familial colon cancers do not arise in preexisting benign polyps. The transmission pattern of familial cancers is not clear. In general, siblings have a relative risk between 2 and 3. Segregation analysis of large families usually reveals that predisposition to the tumors is dominant, but multifactorial inheritance cannot be easily ruled out. As discussed later, certain familial cancers can be linked to the inheritance of mutant genes.

Autosomal Recessive Syndromes of Defective DNA Repair. Besides the dominantly inherited precancerous conditions, a small group of autosomal recessive disorders is collectively characterized by chromosomal or DNA instability. One of the best-studied examples is xeroderma pigmentosum, in which DNA repair is defective. This and other familial disorders of DNA instability are described later.

In summary, no more than 5% to 10% of all human cancers fall into one of the three aforementioned categories. What can be said about the influence of heredity in the large preponderance of malignant tumors? There is emerging evidence that the influence of hereditary factors is subtle and indirect. The genotype may influence the likelihood of one’s developing environmentally induced cancers. For example, polymorphisms in drug-metabolizing enzymes confer genetic predisposition to lung cancers in cigarette smokers. A striking genetic predisposition to developing mesotheliomas (an asbestos-associated tumor) also has been noted, but the relevant gene is not yet known.

Acquired Preneoplastic Disorders

In addition to the genetic influences described earlier, certain clinical conditions are well-recognized predispositions to the development of malignant neoplasia and are referred to as preneoplastic disorders. This designation is unfortunate because it implies a certain inevitability, but in fact, although such conditions may increase the likelihood, in most instances cancer does not develop. A brief listing of the chief conditions follows:

- Persistent regenerative cell replication (e.g., squamous cell carcinoma in the margins of a chronic skin fistula or in a long-unhealed skin wound; hepatocellular carcinoma in cirrhosis of the liver)
- Hyperplastic and dysplastic proliferations (e.g., endometrial carcinoma in atypical endometrial hyperplasia; bronchogenic carcinoma in the dysplastic bronchial mucosa of habitual cigarette smokers)
- Chronic atrophic gastritis (e.g., gastric carcinoma in pernicious anemia or following long-standing Helicobacter pylori infection)
- Chronic ulcerative colitis (e.g., an increased incidence of colorectal carcinoma in long-standing disease)
**Neoplasia**

- Leukoplakia of the oral cavity, vulva, or penis (e.g., increased risk of squamous cell carcinoma)
- Villous adenomas of the colon (e.g., high risk of transformation to colorectal carcinoma)

In this context it may be asked, “What is the risk of malignant change in a benign neoplasm?” or, stated differently, “Are benign tumors precancerous?” In general the answer is no, but inevitably there are exceptions, and perhaps it is better to say that each type of benign tumor is associated with a particular level of risk, ranging from high to virtually nonexistent. For example, adenomas of the colon as they enlarge can undergo malignant transformation in 30% of cases; in contrast, malignant change is extremely rare in leiomyomas of the uterus.

---

**SUMMARY**

**Epidemiology of Cancer**
- The incidence of cancer varies with age, race, geographic factors, and genetic backgrounds. Cancers are most common at the two extremes of age. The geographic variation results mostly from different environmental exposures.
- Most cancers are sporadic, but some are familial. Predisposition to hereditary cancers may be autosomal dominant or autosomal recessive. The former are usually linked to inheritance of a germ-line mutation of cancer suppressor genes, whereas the latter are typically associated with inherited defects in DNA repair.
- Familial cancers tend to be bilateral and arise earlier in life than their sporadic counterparts.

---

**CARCINOGENESIS: THE MOLECULAR BASIS OF CANCER**

It could be argued that the proliferation of literature on the molecular basis of cancer has outpaced the growth of even the most malignant of tumors. It is easy to get lost in the growing forest of information. First, we list some fundamental principles before delving into the details of the genetic basis of cancer.

*Nonlethal genetic damage lies at the heart of carcinogenesis.* Such genetic damage (or mutation) may be acquired by the action of environmental agents, such as chemicals, radiation, or viruses, or it may be inherited in the germ line. The genetic hypothesis of cancer implies that a tumor mass results from the clonal expansion of a single progenitor cell that has incurred genetic damage (i.e., tumors are monoclonal). This expectation has been realized in most tumors that have been analyzed. Clonality of tumors is assessed readily in women who are heterozygous for polymorphic X-linked markers, such as the enzyme glucose-6-phosphate dehydrogenase or X-linked restriction-fragment-length polymorphisms. The principle underlying such an analysis is illustrated in Figure 6–14.

**Figure 6–14**

Diagram depicting the use of X-linked isoenzyme cell markers as evidence of the monoclonality of neoplasms. Because of random X inactivation, all females are mosaics with two cell populations (with glucose-6-phosphate dehydrogenase isoenzyme A or B in this case). When neoplasms that arise in women who are heterozygous for X-linked markers are analyzed, they are made up of cells that contain the active maternal (Xa) or the paternal (Xb) X chromosome, but not both. Currently, X-linked molecular markers are used more commonly than isoenzyme variants.

**Four classes of normal regulatory genes**—growth-promoting proto-oncogenes, growth-inhibiting tumor suppressor genes, genes that regulate programmed cell death (i.e., apoptosis), and genes involved in DNA repair—are the principal targets of genetic damage. Collectively the genetic alterations in tumor cells confer upon them growth and survival advantages over normal cells, as will be evident from the discussion that follows.

Mutant alleles of proto-oncogenes are called oncogenes. They are considered dominant because mutation of a single allele can lead to cellular transformation. In contrast, typically both normal alleles of tumor suppressor genes must be damaged for transformation to occur, so this family of genes is sometimes referred to as recessive oncogenes. However, recent work has clearly shown that, in some cases, loss of a single allele of a tumor suppressor gene can promote transformation (haploinsufficiency). Genes that regulate apoptosis may be dominant,
as are proto-oncogenes, or they may behave as tumor suppressor genes. Tumor suppressor genes are usefully placed into two general groups, promoters and caretakers. Promoters are the traditional tumor suppressor genes, such as RB or p53, where mutation of the gene leads to transformation by releasing the brakes on cellular proliferation. Caretaker genes are responsible for processes that ensure the integrity of the genome, such as DNA repair. Mutation of caretaker genes does not directly transform cells by affecting proliferation or apoptosis. Instead, DNA repair genes affect cell proliferation or survival indirectly by influencing the ability of the organism to repair nonlethal damage in other genes, including proto-oncogenes, tumor suppressor genes, and genes that regulate apoptosis. A disability in the DNA repair genes can predispose cells to widespread mutations in the genome and thus to neoplastic transformation. Cells with mutations in caretaker genes are said to have developed a mutator phenotype.

Carcinogenesis is a multistep process at both the phenotypic and the genetic levels, resulting from the accumulation of multiple mutations. As discussed earlier, malignant neoplasms have several phenotypic attributes, such as excessive growth, local invasiveness, and the ability to form distant metastases. Furthermore, it is well established that over a period of time, many tumors become more aggressive and acquire greater malignant potential. This phenomenon is referred to as tumor progression and is not simply represented by an increase in tumor size. Careful clinical and experimental studies reveal that increasing malignancy is often acquired in an incremental fashion. At the molecular level, tumor progression and associated heterogeneity most likely result from multiple mutations that accumulate independently in different cells, generating subclones with different characteristics (Fig. 6–15) such as ability to invade, rate of growth, metastatic ability, karyotype, hormonal responsiveness, and susceptibility to anti-neoplastic drugs. Some of the mutations may be lethal; others may spur cell growth by affecting proto-oncogenes or cancer suppressor genes. Even though most malignant tumors are monoclonal in origin, by the time they become clinically evident, their constituent cells are extremely heterogeneous. During progression, tumor cells are subjected to immune and nonimmune selection pressures. For example, cells that are highly antigenic are destroyed by host defenses, whereas those with reduced growth factor requirements are positively selected. A growing tumor, therefore, tends to be enriched for subclones that “beat the odds” and are adept at survival, growth, invasion, and metastasis.

**SUMMARY**

**Overview of Carcinogenesis**

- Tumors arise from clonal growth of cells that have incurred mutations in four classes of genes. These include genes that regulate cell growth (proto-oncogenes and tumor suppressor genes) and those that regulate apoptosis and DNA repair.
- Mutation in no single gene is sufficient to cause cancer. Typically, the phenotypic attributes characteristic of malignancy develop when multiple mutations involving multiple genes accumulate. The stepwise accumulation of mutations and increasing malignancy is referred to as tumor progression.

---

**Figure 6–15**

Tumor progression and generation of heterogeneity. New subclones arise from the descendants of the original transformed cell by multiple mutations. With progression the tumor mass becomes enriched for variants that are more adept at evading host defenses and are likely to be more aggressive.
CHAPTER 6 Neoplasia

With this overview (Fig. 6–16), we can now address in detail the molecular pathogenesis of cancer and discuss the carcinogenic agents that inflict genetic damage. In the past 20 years hundreds of cancer-associated genes have been discovered. Some, such as \( p53 \), are commonly mutated; others, such as \( c-ABL \), are affected only in certain leukemias. Each cancer gene has a specific function, the dysregulation of which contributes to the origin or progression of malignancy. It is best therefore to consider cancer-related genes in the context of seven fundamental changes in cell physiology that together dictate the malignant phenotype. All except the mutator phenotype are illustrated in Figure 6–17:

1. Self-sufficiency in growth signals
2. Insensitivity to growth-inhibitory signals
3. Evasion of apoptosis
4. Limitless replicative potential (i.e., overcoming cellular senescence and avoiding mitotic catastrophe)
5. Development of sustained angiogenesis
6. Ability to invade and metastasize
7. Genomic instability resulting from defects in DNA repair

Mutations in genes that regulate some or all of these cellular traits are seen in every cancer, and hence these will form the basis of our discussion of the molecular origins of cancer. In the ensuing discussion it should be noted that gene symbols are italicized but their protein products are not (e.g., \( RB \) gene and \( RB \) protein).

**Self-Sufficiency in Growth Signals**

Genes that promote autonomous cell growth in cancer cells are called *oncogenes*. They are derived by mutations in proto-oncogenes and are characterized by the ability to promote cell growth in the absence of normal growth-promoting signals. Their products, called *oncoproteins*, resemble the normal products of proto-oncogenes except that oncoproteins are devoid of important regulatory elements, and their production in the transformed cells does not depend on growth factors or other external signals. To aid in the understanding of the nature and functions of oncoproteins, it is necessary to review briefly the sequence of events that characterize normal cell proliferation; these were introduced in Chapter 3. Under physiologic conditions, cell proliferation can be readily resolved into the following steps:

- The binding of a growth factor to its specific receptor on the cell membrane

**Figure 6–16**

Flow chart depicting a simplified scheme of the molecular basis of cancer.
Transient and limited activation of the growth factor receptor, which in turn activates several signal-transducing proteins on the inner leaflet of the plasma membrane.

Transmission of the transduced signal across the cytosol to the nucleus via second messengers or a cascade of signal transduction molecules.

Induction and activation of nuclear regulatory factors that initiate DNA transcription.

Entry and progression of the cell into the cell cycle, resulting ultimately in cell division.

With this background we can identify the strategies used by cancer cells to acquire self-sufficiency in growth signals. They can be grouped on the basis of their role in the signal transduction cascade and cell cycle regulation. Indeed, each one of the steps above is susceptible to corruption by cancer cells.

Growth Factors

All normal cells require stimulation by growth factors to undergo proliferation. Most soluble growth factors are made by one cell type and act on a neighboring cell to stimulate proliferation (paracrine action). Many cancer cells acquire growth self-sufficiency, however, by acquiring the ability to synthesize the same growth factors to which they are responsive. For example, many glioblastomas secrete platelet-derived growth factor (PDGF) and express the PDGF receptor, and many sarcomas make both transforming growth factor-α (TGF-α) and its receptor. Similar autocrine loops are fairly common in many types of cancer. Genes that encode homologues of fibroblast growth factors (e.g., bst-1 and FGF3) have been detected in several gastrointestinal and breast tumors; FGF-2 is expressed in human melanomas but not normal melanocytes. Hepatocyte growth factor (HGF) and its receptor c-Met are both overexpressed in follicular carcinomas of the thyroid. In many instances the growth factor gene itself is not altered or mutated, but the products of other oncogenes (e.g., RAS) stimulate overexpression of growth factor genes and the subsequent development of an autocrine loop.

Growth Factor Receptors

The next group in the sequence of signal transduction is growth factor receptors, and several oncogenes that result from the overexpression or mutation of growth factor receptors have been identified. Mutant receptor proteins deliver continuous mitogenic signals to cells, even in the absence of the growth factor in the environment. More common than mutations is overexpression of growth factor receptors, which can render cancer cells hypersensitive to levels of the growth factor that would not normally trigger proliferation. The best-documented examples of overexpression involve the epidermal growth factor (EGF) receptor family. ERBB1, the EGF receptor, is overexpressed in 80% of squamous cell carcinomas of the lung, 50% or more of glioblastomas, and 80 to 100% of epithelial tumors of the head and neck. A related receptor, called HER2/NEU (ERBB2), is amplified in 25% to 30% of breast cancers and adenocarcinomas of the lung, ovary, and salivary glands. These tumors are exquisitely sensitive to the mitogenic effects of small amounts of growth factors, and a high level of HER2/NEU protein in breast cancer cells is a harbinger of poor prognosis. The significance of HER2/NEU in the pathogenesis of breast cancers is illustrated dramatically by the clinical benefit derived from blocking the extracellular domain of this receptor with anti-HER2/NEU antibodies. Treatment of breast cancer with anti-HER2/NEU antibody is an elegant example of “bench to bedside” medicine.

Signal-Transducing Proteins

A relatively common mechanism by which cancer cells acquire growth autonomy is mutations in genes that encode various components of the signaling pathways downstream of growth factor receptors. These signaling molecules couple growth factor receptors to their nuclear targets. Many such signaling proteins are associated with the inner leaflet of the plasma membrane, where they receive signals from activated growth factor receptors and transmit them to the nucleus, either through second messengers or through a cascade of phosphorylation and activation of signal transduction molecules. Two important members in this category are RAS and ABL. Each of these is discussed briefly.
RAS is the most commonly mutated proto-oncogene in human tumors. Indeed, approximately 30% of all human tumors contain mutated versions of the RAS gene, and the incidence is even higher in some specific cancers (e.g., colon and pancreatic adenocarcinomas). RAS is a member of a family of small G proteins that bind guanosine nucleotides (guanosine triphosphate [GTP] and guanosine diphosphate [GDP]), similar to the larger trimeric G proteins. Normal RAS proteins flip back and forth between an excited signal-transmitting state and a quiescent state. RAS proteins are inactive when bound to GDP; stimulation of cells by growth factors leads to exchange of GDP for GTP and subsequent conformational changes that generates active RAS (Fig. 6–18). The activated RAS in turn stimulates down-stream regulators of proliferation, such as the RAF–mitogen-activated protein (MAP) kinase mitogenic cascade, which floods the nucleus with signals for cell proliferation. The excited signal-emitting stage of the normal RAS protein is short-lived, however, because its intrinsic guanosine triphosphatase (GTPase) activity hydrolyzes GTP to GDP, releasing a phosphate group and returning the protein to its quiescent inactive state. The GTPase activity of activated RAS protein is magnified dramatically by a family of GTPase-activating proteins (GAPs), which act as molecular brakes that prevent uncontrolled RAS activation by favoring hydrolysis of GTP to GDP.

The RAS gene is most commonly activated by point mutations. Molecular analyses of RAS mutations have revealed three hot spots, which encode residues either within the GTP-binding pocket or the enzymatic region essential for GTP hydrolysis. Mutations at these locations interfere with GTP hydrolysis that is essential to convert RAS into an inactive form. RAS is thus trapped in its activated GTP-bound form, and the cell is forced into a continuously proliferating state. It follows from this scenario that the consequences of mutations in RAS protein would be mimicked by mutations in the GAPs that fail to restrain normal RAS proteins. Indeed, disabling mutation of neurofibromin 1, a GAP, is associated with familial neurofibromatosis type 1 (Chapter 23).

In addition to RAS, several non–receptor-associated tyrosine kinases function as signal transduction molecules. In this group, ABL is the most well defined with respect to carcinogenesis. The ABL proto-oncogene has tyrosine kinase activity that is dampened by internal negative regulatory domains. In chronic myeloid leukemia and certain acute leukemias, this activity is unleashed

![Figure 6–18](image-url)

**Figure 6–18**

Model for action of RAS genes. When a normal cell is stimulated through a growth factor receptor, inactive (GDP-bound) RAS is activated to a GTP-bound state. Activated RAS recruits RAF-1 and stimulates the MAP-kinase pathway to transmit growth-promoting signals to the nucleus. MYC gene is one of several targets of the activated RAS pathway. The mutant RAS protein is permanently activated because of inability to hydrolyze GTP, leading to continuous stimulation of cells without any external trigger. The anchoring of RAS to the cell membrane by the farnesyl moiety is essential for its action, and drugs that inhibit farnesylation can inhibit RAS action.
because the ABL gene is translocated from its normal abode on chromosome 9 to chromosome 22, where it fuses with part of the breakpoint cluster region (BCR) gene. The BCR-ABL hybrid protein has potent, unregulated tyrosine kinase activity, which activates several pathways, including the RAS-RAF cascade. Other studies have revealed a completely novel function of ABL in oncogenesis. Normal ABL protein localizes in the nucleus, where its role is to promote apoptosis of cells that suffer DNA damage. This is analogous to the role of the p53 gene (discussed later). The BCR-ABL gene cannot perform this function, because it is retained in the cytoplasm as a result of abnormal tyrosine kinase activity. Thus, a cell with BCR-ABL fusion gene is dysregulated in two ways: inappropriate tyrosine kinase activity leads to growth autonomy, while simultaneously apoptosis is impaired.

The crucial role of BCR-ABL in transformation has been confirmed by the dramatic clinical response of patients with chronic myeloid leukemia after therapy with an inhibitor of the BCR-ABL fusion kinase called imatinib mesylate (Gleevec); this is another example of rational drug design emerging from an understanding of the molecular basis of cancer.

**Nuclear Transcription Factors**

Ultimately, all signal transduction pathways enter the nucleus and have an impact on a large bank of responder genes that orchestrate the cells’ orderly advance through the mitotic cycle. Indeed, the ultimate consequence of signaling through oncogenes like RAS or ABL is inappropriate and continuous stimulation of nuclear transcription factors that drive growth-promoting genes. Growth autonomy may thus occur as a consequence of mutations affecting genes that regulate transcription of DNA. A host of oncogenes, including products of the MYC, MYB, JUN, FOS, and REL oncogenes, function as transcription factors that regulate the expression of growth-promoting genes, such as cyclins. Of these, the MYC gene is involved most commonly in human tumors. The MYC proto-oncogene is expressed in virtually all tumors, and the MYC protein is induced rapidly when quiescent cells receive a signal to divide. In normal cells, MYC levels decline to near basal level when the cell cycle begins. In contrast, oncogenic versions of the MYC gene are associated with persistent expression or overexpression, contributing to sustained proliferation.

The MYC protein can either activate or repress the transcription of other genes. Those activated by MYC include several growth-promoting genes, including cyclin-dependent kinases (CDKs), whose products drive cells into the cell cycle (discussed next). Genes repressed by MYC include the CDK inhibitors (CDKIs). Thus, MYC promotes tumorigenesis by increasing expression of genes that promote progression through the cell cycle and repressing genes that slow or prevent progression through the cell cycle. Dysregulation of the MYC gene resulting from a t(8;14) translocation occurs in Burkitt lymphoma, a B-cell tumor. MYC is also amplified in breast, colon, lung, and many other cancers; the related N-MYC and L-MYC genes are amplified in neuroblastomas and small-cell cancers of lung.

**Cyclins and Cyclin-Dependent Kinases (CDKs)**

The ultimate outcome of all growth-promoting stimuli is the entry of quiescent cells into the cell cycle. Cancers may become autonomous if the genes that drive the cell cycle become dysregulated by mutations or amplification. As alluded to in Chapter 3, the orderly progression of cells through the various phases of the cell cycle is orchestrated by 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 the cell through the cell cycle. On completion of this task, cyclin levels decline rapidly. More than 15 cyclins have been identified; cyclins D, E, A, and B appear sequentially during the cell cycle and bind to one or more CDK. The cell cycle may thus be seen as a relay race in which each lap is regulated by a distinct set of cyclins, and as one set of cyclins leaves the track, the next set takes over (Fig. 6–19).

With this background it is easy to appreciate that mutations that dysregulate the activity of cyclins and CDKs would favor cell proliferation. Mishaps affecting the expression of cyclin D or CDK4 seem to be a common event in neoplastic transformation. The cyclin D genes are overexpressed in many cancers, including those affecting the breast, esophagus, liver, and a subset of lymphomas. Amplification of the CDK4 gene occurs in melanomas, sarcomas, and glioblastomas. Mutations affecting cyclin B and cyclin E and other CDKs also occur, but they are much less frequent than those affecting cyclin D/CDK4.

While cyclins arouse the CDKs, their inhibitors (CDKIs), of which there are many, silence the CDKs and exert negative control over the cell cycle. One family of CDKIs, composed of three proteins, called p21 [CDKN1A], p27 [CDKN1B], and p57 [CDKN1C], inhibits the CDKs broadly, whereas the other family of CDKIs has selective effects on cyclin D/CDK4 and cyclin D/CDK6. The four members of this family (p15 [CDKN2B], p16 [CDKN2A], p18 [CDKN2C], and p19 [CDKN2D]) are sometimes called INK4 (A–D) proteins. Expression of these inhibitors is down-regulated by mitogenic signaling pathways, thus promoting the progression of the cell cycle. For example, p27 [CDKN1B], a CDKI that inhibits cyclin E, is expressed throughout G1. Mitogenic signals obtund p27 in a variety of ways, relieving inhibition of cyclin E–CDK2 and thus allowing the cell cycle to proceed. Interestingly, the CDKN2A gene locus, also called INK4a/ARF, encodes two protein products: the p16 INK4a and p14ARF. Both block cell cycle progression but have different targets. p16 [CDKN2A] inhibits RB phosphorylation by blocking cyclin D–CDK4 complex, whereas p14ARF activates the p53 pathway by inhibiting MDM2 (discussed below). Thus, both proteins function as tumor suppressors, and deletion of this locus, frequent in many tumors, impacts both the RB and p53 pathways. The CDKIs are frequently mutated or otherwise silenced in many human malignancies. Germ-line mutations of CDKN2A are associated with 25% of melanoma-prone kindreds. Somatically acquired deletion or inactivation of CDKN2A is seen in 75% of pancreatic carcinomas, 40% to 70% of glioblastomas, 50% of esophageal cancers, and 20% of non-small-cell lung carcinomas, soft tissue sarcomas, and bladder cancers.
Figure 6–19

Schematic illustration of the role of cyclins, CDKs, and CDKIs in regulating the cell cycle. The shaded arrows represent the phases of the cell cycle during which specific cyclin-CDK complexes are active. As illustrated, cyclin D–CDK4, cyclin D–CDK6, and cyclin E–CDK2 regulate the G1-to-S transition by phosphorylation of the RB protein (pRB). Cyclin A–CDK2 and cyclin A–CDK1 are active in the S phase. Cyclin B–CDK1 is essential for the G2-to-M transition. Two families of CDK inhibitors can block activity of CDKs and progression through the cell cycle. The so-called INK4 inhibitors composed of p16, p15, p18, and p19, act on cyclin D–CDK4 and cyclin D–CDK6. The other family of three inhibitors, p21, p27, and p57, can inhibit all CDKs.

**SUMMARY**

**Oncogenes that Promote Unregulated Proliferation (Self-sufficiency in Growth Signals)**

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

Oncogenes: mutant versions of proto-oncogenes that function autonomously without a requirement for normal growth-promoting signals

Oncogenes can promote uncontrolled cell proliferation by several mechanisms:

- Stimulus-independent expression of growth factor and its receptor, setting up an autocrine loop of cell proliferation
- PDGF-PDGF-receptor in brain tumors
- Mutations in genes encoding growth factor receptors, leading to overexpression or constitutive signaling by the receptor (e.g., EGF receptors)
- EGF-receptor family members, including HER2/NEU (breast, lung, and other tumors)
- Mutations in genes encoding signaling molecules
- RAS is commonly mutated in human cancers; normally flips between resting GDP-bound state and active GTP-bound state; mutations block hydrolysis of GTP to GDP, leading to unchecked signaling
- Fusion of ABL tyrosine kinase with BCR protein in certain leukemias generates a hybrid protein with constitutive kinase activity
- Overproduction or unregulated activity of transcription factors
- Translocation of MYC in some lymphomas leads to overexpression and unregulated expression of its target genes controlling cell cycling and survival
- Mutations that activate cyclin genes or inactivate normal regulators of cyclins and cyclin-dependent kinases
Retinoblastoma also are at greatly increased risk of developing osteosarcomas and some soft tissue sarcomas.

At this point, we should clarify some terminology. A cell heterozygous at the \( RB \) locus is not neoplastic. Tumors develop when the cell becomes homozygous for the mutant allele or, in other words, loses heterozygosity of the normal \( RB \) gene.

The signals and signal-transducing pathways for growth inhibition are much less well understood than are those for growth promotion. Nevertheless, it is reasonable to assume that, similar to mitogenic signals, growth-inhibitory signals may originate outside the cell and use receptors, signal transducers, and nuclear transcription regulators to accomplish their effects. The tumor suppressor genes seem to encode various components of this growth-inhibitory pathway.

In principle, antigrowth signals can prevent cell proliferation by two complementary mechanisms. The signal may cause dividing cells to go into \( G_0 \) (quiescence), where they remain until external cues prod their reentry into the proliferative pool. Alternatively the cells may enter a postmitotic, differentiated pool and lose replicative potential. It is useful to begin our discussion of growth-inhibitory mechanisms and their evasion by focusing initially on the \( RB \) gene, the prototypic tumor suppressor gene.

### SUMMARY

**Insensitivity to Growth-Inhibitory Signals**

- Tumor suppressor genes encode proteins that inhibit cellular proliferation by regulating the cell cycle. Unlike oncogenes, both copies of the gene must be lost for tumor development, leading to loss of heterozygosity at the gene locus.
- In cases with familial predisposition to develop tumors, the affected individuals inherit one defective (nonfunctional) copy of a tumor suppressor gene and lose the second one through somatic mutation. In sporadic cases both copies are lost through somatic mutations.

**RB Gene and Cell Cycle**

Much is known about the \( RB \) gene, because this was the first tumor suppressor gene discovered. The \( RB \) gene product is a DNA-binding protein that is expressed in every cell type examined, where it exists in an active hypophosphorylated and an inactive hyperphosphorylated state. The importance of \( RB \) lies in its enforcement of \( G_1 \), or the gap between mitosis (M) and DNA replication (S). In embryos, cell divisions proceed at an amazing clip, with DNA replication beginning immediately after mitosis ends. However, as development proceeds, two gaps are incorporated into the cell cycle: Gap 1 (\( G_1 \)) between mitosis (M) and DNA replication (S), and Gap 2 (\( G_2 \)) between DNA replication (S) and mitosis (M) (see Fig. 6–19). Although each phase of the cell cycle circuitry is monitored carefully, the transition from \( G_1 \) to S is believed to be an extremely important checkpoint in the
Once cells cross the G1 checkpoint, they can pause the cell cycle for a time but are obligated to complete mitosis. In G1, however, cells can exit the cell cycle, either temporarily, called quiescence, or permanently, called senescence. In G1, therefore, diverse signals are integrated to determine whether the cell should enter the cell cycle, exit the cell cycle and differentiate, or die. RB is a key node in this decision process. To understand why RB is such a crucial player, we must review the mechanisms that enforce the G1 phase.

The initiation of DNA replication requires the activity of cyclin E/CDK2 complexes, and expression of cyclin E is dependent on the E2F family of transcription factors. Early in G1, RB is in its hypophosphorylated active form, and it binds to and inhibits the E2F family of transcription factors, preventing transcription of cyclin E. Hypophosphorylated RB blocks E2F-mediated transcription in at least two ways (Fig. 6–21). First, it sequesters E2F, preventing it from interacting with other transcriptional activators. Second, RB recruits chromatin remodeling proteins, such as histone deacetylases and histone methyltransferases, which bind to the promoters of E2F-responsive genes such as cyclin E. These enzymes modify chromatin at the promoters to make DNA insensitive to transcription factors. This situation is changed upon mitogenic signaling. Growth factor signaling leads to cyclin D expression and activation of cyclin D–CDK4/6 complexes. These complexes phosphorylate RB, inactivating the protein and releasing E2F to induce target genes such as cyclin E. Expression of cyclin E then stimulates DNA replication and progression through the cell cycle. When the cells enter S phase, they are committed to divide without additional growth factor stimulation. During the ensuing M phase, the phosphate groups are removed from RB by cellular phosphatases, regenerating the hypophosphorylated form of RB.

E2F is not the sole target of RB. The versatile RB protein has been shown to bind to a variety of other transcription factors that regulate cell differentiation. For example, RB stimulates myocyte-, adipocyte-,
The role of RB in regulating the G1–S checkpoint of the cell cycle. Hypophosphorylated RB in complex with the E2F transcription factors binds to DNA, recruits chromatin remodeling 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. The phosphorylation of RB is inhibited by CDKIs, because they inactivate cyclin-CDK complexes. Virtually all cancer cells show dysregulation of the G1–S checkpoint as a result of mutation in one of four genes that regulate the phosphorylation of RB; these genes are RB, cyclin D, CDK4, and CDKN2A (p16). EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

**SUMMARY**

**RB Gene and Cell Cycle**

- RB exerts antiproliferative effects by controlling the G1-to-S transition of the cell cycle. In its active form RB is hypophosphorylated and binds to E2F transcription factor. This interaction prevents transcription of genes like cyclin E that are needed for DNA replication, and so the cells are arrested in G1.
- Growth factor signaling leads to cyclin D expression, activation of the cyclin D–CDK4/6 complexes, inactivation of RB by phosphorylation, and thus release of E2F.
- Loss of cell cycle control is fundamental to malignant transformation. Almost all cancers will have disabled the G1 checkpoint, by mutation of either RB or genes that affect RB function, like cyclin D, CDK4, and CDKIs.
- Many oncogenic DNA viruses, like HPV, encode proteins (e.g., E7) that bind to RB and render it nonfunctional.

The emerging paradigm is that loss of normal cell cycle control is central to malignant transformation and that at least one of the four key regulators of the cell cycle (CDKN2A, cyclin D, CDK4, RB) is mutated in most human cancers. Furthermore, the transforming proteins of several oncogenic animal and human DNA viruses seem to act, in part, by neutralizing the growth-inhibitory activities of RB. Simian virus 40 and polyomavirus large-T antigens, adenovirus E1A protein, and human papillomavirus (HPV) E7 protein all bind to the hypophosphorylated form of RB. The RB protein, unable to bind to the E2F transcription factors, is functionally deleted, and the cells lose the ability to be inhibited by antigrowth signals that funnel through the RB nexus.
\textbf{p53 Gene: Guardian of the Genome}

The \textit{p53} tumor suppressor gene is one of the most commonly mutated genes in human cancers. \textit{p53} thwarts neoplastic transformation by three interlocking mechanisms: activation of temporary cell cycle arrest (termed quiescence), induction of permanent cell cycle arrest (termed senescence), or triggering of programmed cell death (termed apoptosis). Fundamentally, \textit{p53} can be viewed as a central monitor of stress, directing the stressed cells toward an appropriate response. A variety of stresses can trigger the \textit{p53} response pathways, including anoxia, inappropriate oncogene expression (e.g., MYC or RAS), and damage to the integrity of DNA. By managing the DNA-damage response, \textit{p53} plays a central role in maintaining the integrity of the genome, as will be evident from the following discussion.

In nonstressed, healthy cells, \textit{p53} has a short half-life (20 minutes) because of its association with MDM2, a protein that targets it for destruction. When the cell is stressed, for example by an assault on its DNA, \textit{p53} undergoes post-transcriptional modifications that release it from MDM2 and increase its half-life. During the process of being unshackled from MDM2, \textit{p53} also becomes activated as a transcription factor. Dozens of genes whose transcription is triggered by \textit{p53} have been found. They can be grouped into two broad categories: those that cause cell cycle arrest and those that cause apoptosis. If DNA damage can be repaired during cell cycle arrest, the cell reverts to a normal state; if the repair fails, \textit{p53} induces apoptosis or senescence. These actions are discussed next.

The manner in which \textit{p53} senses DNA damage and determines the adequacy of DNA repair are not completely understood. The key initiators of the DNA-damage pathway are two related protein kinases: ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia mutated related (ATR). As the name implies, the ATM gene was originally identified as the germ-line mutation in patients with ataxia-telangiectasia. Patients with this disease, which is characterized by an inability to repair certain kinds of DNA damage, suffer from an increased incidence of cancer. The types of damage sensed by ATM and ATR are different, but the downstream pathways they activate are similar. Once triggered, both ATM and ATR phosphorylate a variety of targets, including \textit{p53} and DNA repair proteins. Phosphorylation of these two targets leads to a pause in the cell cycle and stimulation of DNA repair pathways respectively.

\textit{p53}-mediated cell cycle arrest may be considered the primordial response to DNA damage (Fig. 6–22). It occurs late in the G1 phase and is caused mainly by \textit{p53}-dependent transcription of the CDKI \textit{CDKN1A} (p21). The \textit{CDKN1A} gene, as described earlier, inhibits cyclin/CDK complexes and prevents phosphorylation of RB essential for cells to enter G1 phase. Such a pause in cell cycling is welcome, because it gives the cells “breathing time” to repair DNA damage. \textit{p53} also helps the process by inducing certain proteins, such as GADD45 (growth arrest and DNA damage), that help in DNA repair. \textit{p53} can stimulate DNA repair pathways by transcription-independent mechanisms as well. If DNA damage is repaired successfully, \textit{p53} up-regulates transcription of MDM2, leading to destruction of \textit{p53} and relief of the cell cycle block. If the damage cannot be repaired, the cell may enter \textit{p53}-induced senescence or undergo \textit{p53}-directed apoptosis.

\textit{p53}-induced senescence is a permanent cell cycle arrest characterized by specific changes in morphology and gene expression that differentiate it from quiescence or reversible cell cycle arrest. Senescence requires activation of \textit{p53} and/or RB and expression of their mediators, such as the CDKIs. Such cell cycle arrest is generally irreversible, although it may require the continued expression of \textit{p53}. The mechanisms of senescence are unclear but seem to involve global chromatin changes, which drastically and permanently alter gene expression.

\textit{p53}-induced apoptosis of cells with irreversible DNA damage is the ultimate protective mechanism against neoplastic transformation. It is mediated by several pro-apoptotic genes such as BAX and PUMA (described later).

To summarize, \textit{p53} senses DNA damage and assists in DNA repair by causing G1 arrest and inducing DNA repair genes. A cell with damaged DNA that cannot be repaired is directed by \textit{p53} to either enter senescence or undergo apoptosis (see Fig. 6–22). In view of these activities, \textit{p53} has been rightfully called a “guardian of the genome.” With homozygous loss of \textit{p53}, DNA damage goes unrepaired, mutations become fixed in dividing cells, and the cell turns onto a one-way street leading to malignant transformation.

Confirming the importance of \textit{p53} in controlling carcinogenesis, more than 70% of human cancers have a defect in this gene, and the remaining malignant neoplasms have defects in genes up-stream or down-stream of \textit{p53}. Homozygous loss of the \textit{p53} gene is found in virtually every type of cancer, including carcinomas of the lung, colon, and breast—the three leading causes of cancer deaths. In most cases, inactivating mutations affecting both \textit{p53} alleles are acquired in somatic cells. Less commonly, some individuals inherit a mutant \textit{p53} allele; this disease is called the \textit{Li-Fraumeni syndrome}. As with the RB gene, inheritance of one mutant allele predisposes individuals to develop malignant tumors because only one additional hit is needed to inactivate the second, normal allele. Patients with the \textit{Li-Fraumeni syndrome} have a 25-fold greater chance of developing a malignant tumor by age 50 compared with the general population. In contrast to patients who inherit a mutant \textit{RB} allele, the spectrum of tumors that develop in patients with the \textit{Li-Fraumeni syndrome} is varied; the most common types of tumors are sarcomas, breast cancer, leukemia, brain tumors, and carcinomas of the adrenal cortex. Compared with sporadic tumors, patients with \textit{Li-Fraumeni syndrome} develop tumors at a younger age and may develop multiple primary tumors.

As with RB protein, normal \textit{p53} also can be rendered nonfunctional by certain DNA viruses. Proteins encoded by oncogenic HPVs, hepatitis B virus (HBV), and possibly Epstein-Barr virus (EBV) can bind to normal \textit{p53} and nullify its protective function. Thus, DNA viruses can subvert two of the best-understood tumor suppressor genes, \textit{RB} and \textit{p53}. 
Neoplasia

Ionizing radiation  
Carcinogens  
Mutagens

Normal cell  
(p53 normal)

Cell with 
mutations or 
loss of p53

Hypoxia  
DNA damage

DNA damage

p53 activated and 
binds to DNA

Transcription dependent and independent effects on targets

Sensescence  
p21  
GADD45  
BAX

G1 arrest  
Successful repair  
Repair fails

Normal cells  
Apoptosis  
Malignant tumor

Figure 6–22

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 G1 and induction of DNA repair, by transcriptional up-regulation of the cyclin-dependent kinase inhibitor CDKN1A (p21) and the 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 p53, DNA damage does not induce cell cycle arrest or DNA repair, and genetically damaged cells proliferate, giving rise eventually to malignant neoplasms.

SUMMARY

**p53 Gene: Guardian of the Genome**

- p53 is the central monitor of stress in the cell and can be activated by anoxia, inappropriate oncogene signaling, or DNA damage. Activated p53 controls the expression and activity of genes involved in cell cycle arrest, DNA repair, cellular senescence, and apoptosis.
- DNA damage leads to activation of p53 by phosphorylation. Activated p53 drives transcription of CDKN1A (p21) that prevents RB phosphorylation and therefore causes a G1-S block in the cell cycle. This pause allows the cells to repair DNA damage.
- If DNA damage cannot be repaired, p53 induces cellular senescence or apoptosis.
- Of human tumors, 70% have homozygous loss of p53. Patients with the rare Li-Fraumeni syndrome inherit one defective copy in the germ line and lose the second one in somatic tissues; such individuals develop a variety of tumors.
- As with RB, p53 can be incapacitated by binding to proteins encoded by oncogenic DNA viruses like HPV, and possibly EBV and HBV.
Transforming Growth Factor-β Pathway

Although much is known about the circuitry that applies brakes to the cell cycle, the molecules that transmit antiproliferative signals to cells are less well characterized. Best known is TGF-β, a member of a family of dimeric growth factors that includes bone morphogenetic proteins and activins. In most normal epithelial, endothelial, and hematopoietic cells, TGF-β is a potent inhibitor of proliferation. It regulates cellular processes by binding to a complex composed of TGF-β receptors I and II. Dimerization of the receptor upon ligand binding leads to a cascade of events that result in the transcriptional activation of CDKIs with growth-suppressing activity, as well as repression of growth-promoting genes such as c-MYC, CDK2, CDK4, and cyclins A and E.

In many forms of cancer, the growth-inhibiting effects of TGF-β pathways are impaired by mutations in the TGF-β signaling pathway. These mutations may affect the type II TGF-β receptor or SMAD molecules that serve to transduce antiproliferative signals from the receptor to the nucleus. Mutations affecting the type II receptor are seen in cancers of the colon, stomach, and endometrium. Mutational inactivation of SMAD4, one of 10 proteins involved in TGF-β signaling, is common in pancreatic cancers. In 100% of pancreatic cancers and 83% of colon cancers, at least one component of the TGF-β pathway is mutated.

Adenomatous Polyposis Coli–β-Catenin Pathway

In the rare hereditary disease called adenomatous polyposis coli (APC), patients develop numerous adenomatous polyps in the colon that have a very high incidence of transformation into colonic cancers. These patients consistently show loss of a tumor suppressor gene called APC (named for the disease). The APC gene exerts antiproliferative effects in an unusual manner. It is a cytoplasmic protein whose dominant function is to regulate the intracellular levels of β-catenin, a protein with many functions. On the one hand, β-catenin binds to the cytoplasmic portion of E-cadherin, a cell surface protein that mediates intercellular interactions; on the other hand, it can translocate to the nucleus and activate cell proliferation. Here the focus is on the latter function of this protein. β-catenin is an important component of the so-called WNT signaling pathway that regulates cell proliferation (illustrated in Fig. 6–23). WNT is a soluble factor that can induce cellular proliferation. It does so by binding to its receptor and transmitting signals that prevent the degradation of β-catenin, allowing it to translocate to the nucleus, where it acts as a transcriptional activator in conjunction with another molecule, called TcF (see Fig. 6–23B). In quiescent cells, which are not exposed to WNT, cytoplasmic β-catenin is degraded by a destruction complex, of which APC is an integral part. In the presence of APC, β-catenin is degraded by the destruction complex, preventing it from accumulating in the cytoplasm and allowing it to translocate to the nucleus and activate cell proliferation.
part (see Fig. 6–23A). With loss of APC (in malignant cells), β-catenin degradation is prevented, and the WNT signaling response is inappropriately activated in the absence of WNT (see Fig. 6–23C). This leads to transcription of growth-promoting genes, such as cyclin D1 and MYC.

APC behaves as a typical tumor suppressor gene. Individuals born with one mutant allele develop hundreds to thousands of adenomatous polyps in the colon during their teens or 20s, which show loss of the other APC allele. Almost invariably, one or more polyps undergo malignant transformation upon accumulation of other mutations in the cells within the polyp, as discussed later. APC mutations are seen in 70% to 80% of sporadic colon cancers. Colonic cancers that have normal APC genes show activating mutations of β-catenin that render them refractory to the degrading action of APC.

Evasion of Apoptosis

Accumulation of neoplastic cells may result not only from activation of growth-promoting oncogenes or inactivation of growth-suppressing tumor suppressor genes, but also from mutations in the genes that regulate apoptosis. A large family of genes that regulate apoptosis has been identified. Before we can understand how tumor cells evade apoptosis, it is essential to review briefly the biochemical pathways to apoptosis. As discussed in Chapter 1, there are two distinct programs that activate apoptosis, the extrinsic and intrinsic pathways. Figure 6–24 shows, in simplified form, the sequence of events that lead to apoptosis by signaling through the death receptor CD95/Fas (extrinsic pathway) and by DNA damage (intrinsic pathway). The extrinsic pathway is initiated when CD95 is bound to its ligand, CD95L, leading to trimerization of the receptor and thus its cytoplasmic death domains, which attract the intracellular adaptor protein FADD. This protein recruits procaspase 8 to form the death-inducing signaling complex. Procaspase 8 is activated by cleavage into smaller subunits, generating caspase 8. Caspase 8 then activates downstream caspases such as caspase 3, a typical executioner caspase that cleaves DNA and other substrates to cause cell death. The intrinsic pathway of apoptosis is triggered by a variety of stimuli, including withdrawal of survival factors, stress, and injury. Activation of this pathway leads to permeabilization of mitochondrial outer membrane, with resultant release of molecules, such as cytochrome c, that initiate apoptosis. The integrity of the mitochondrial outer membrane is regulated by pro-apoptotic and anti-apoptotic members of the BCL2 family of proteins. The

SUMMARY

**Transforming Growth Factor-β and Adenomatous Polyposis Coli-β-Catenin Pathways**

- TGF-β inhibits proliferation of many cell types by activation of growth-inhibiting genes like CDKIs and suppression of growth-promoting genes like MYC and cyclins.
- TGF-β function is compromised in many tumors by mutations in its receptors (colon, stomach, endometrium) or by mutational inactivation of SMAD genes that transduce TGF-β signaling (pancreas).
- APC gene exerts antiproliferative actions by regulating the destruction of the cytoplasmic protein β-catenin. With a loss of APC, β-catenin is not destroyed and it translocates to the nucleus, where it acts as a growth-promoting transcription factor.
- In familial adenomatous polyposis syndrome inheritance of a germ-line mutation in the APC gene causes the development of hundreds of colonic polyps at a young age. One or more of these polyps evolves into a colonic cancer with loss of heterozygosity at the APC locus. Somatic loss of both alleles of the APC gene is seen in approximately 70% of sporadic colon cancers.

**Figure 6–24**

Simplified schema of CD95 receptor-induced and DNA damage-triggered pathways of apoptosis and mechanisms used by tumor cells to evade cell death. (1) Reduced CD95 level. (2) Inactivation of death-induced signaling complex by FLICE protein. (3) Reduced egress of cytochrome c from mitochondrion as a result of up-regulation of BCL2. (4) Reduced levels of pro-apoptotic BAX resulting from loss of p53. (5) Loss of APAF-1. (6) Up-regulation of inhibitors of apoptosis.
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 exemplified by BCL2 and BCL-XL. A third set of proteins (so-called BH3-only proteins) including BAD, BID, and PUMA, regulate the balance between the pro- and anti-apoptotic members of the BCL2 family. The BH3-only proteins promote apoptosis by neutralizing the actions of anti-apoptotic proteins like BCL2 and BCL-XL. 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. Cytochrome c leaks into the cytosol, where it binds to APAF-1, activating caspase 9. Like caspase 8 of the extrinsic pathway, caspase 9 can cleave and activate the executioner caspases. Because of the pro-apoptotic effect of BH3 only proteins, efforts are underway to develop of BH3 mimetic drugs.

Within this framework, it is possible to illustrate the multiple sites at which apoptosis is frustrated by cancer cells (see Fig. 6–24). Starting from the surface, reduced levels of CD95 may render the tumor cells less susceptible to apoptosis by Fas ligand (FasL). Some tumors have high levels of FLIP, a protein that can bind death-inducing signaling complex and prevent activation of caspase 8. Of these genes, perhaps best established is the role of BCL2 in protecting tumor cells from apoptosis. As discussed later, approximately 85% of B-cell lymphomas of the follicular type (Chapter 12) carry a characteristic t(14;18) (q32;q21) translocation. Recall that 14q32, the site where immunoglobulin heavy-chain genes are found, is also involved in the pathogenesis of Burkitt lymphoma. Juxtaposition of this transcriptionally active locus with BCL2 (located at 18q21) causes overexpression of the BCL2 protein. This in turn increases the BCL2/BCL-XL buffer, protecting lymphocytes from apoptosis and allowing them to survive for long periods; there is therefore a steady accumulation of B lymphocytes, resulting in lymphadenopathy and marrow infiltration. Because BCL2-overexpressing lymphomas arise in large part from reduced cell death rather than explosive cell proliferation, they tend to be indolent (slow growing) compared with many other lymphomas.

As mentioned before, p53 is an important pro-apoptotic gene that induces apoptosis in cells that are unable to repair DNA damage. The actions of p53 are mediated in part by transcriptional activation of BAX, but there are other connections as well between p53 and the apoptotic machinery.

**SUMMARY**

**Evasion of Apoptosis**
- Apoptosis can be initiated through the extrinsic or intrinsic pathways.
- Both pathways result in the activation of a proteolytic cascade of caspases that destroys the cell.
- Mitochondrial outer membrane permeabilization is regulated by the balance between pro-apoptotic (e.g., BAX, BAK) and anti-apoptotic molecules (BCL2, BCL-XL). BH3-only molecules activate apoptosis by tilting the balance in favor of the pro-apoptotic molecules.
- In 85% of follicular B-cell lymphomas the anti-apoptotic gene *BCL2* is activated by the t(8;14) translocation.

**Limitless Replicative Potential**

As was discussed in the section on cellular aging (Chapter 1), most normal human cells have a capacity of 60 to 70 doublings. After this, the cells lose the capacity to divide and enter senescence. This phenomenon has been ascribed to progressive shortening of telomeres at the ends of chromosomes. Indeed, short telomeres seem to be recognized by the DNA repair machinery as double-stranded DNA breaks, and this leads to cell cycle arrest mediated by p53 and RB. Cells in which the checkpoints are disabled by p53 or RB mutations, the nonhomologous end-joining pathway is activated as a last-ditch effort to save the cell, joining the shortened ends of two chromosomes. This inappropriately activated repair system results in dicentric chromosomes that are pulled apart at anaphase, resulting in new double-stranded DNA breaks. The resulting genomic instability from the repeated bridge-fusion-breakage cycles eventually produces mitotic catastrophe, characterized by massive cell death. It follows that for tumors to grow indefinitely, as they often do, loss of growth restraints is not enough. Tumor cells must also develop ways to avoid both cellular senescence and mitotic catastrophe (Fig. 6–25). If during crisis a cell manages to reactivate telomerase, the bridge-fusion-breakage cycles cease and the cell is able to avoid death. However, during this period of genomic instability that precedes telomerase activation, numerous mutations could accumulate, helping the cell march toward malignancy. Passage through a period of genomic instability probably explains the complex karyotypes frequently seen in human carcinomas. Telomerase, active in normal stem cells, is normally absent from, or at very low levels in, most somatic cells. By contrast, telomere maintenance is seen in virtually all types of cancers. In 85% to 95% of cancers, this is due to up-regulation of the enzyme telomerase. A few tumors use other mechanisms, termed alternative lengthening of telomeres, which probably depend on DNA recombination. Interestingly, in the progression from colonic adenoma to colonic adenocarcinoma, early lesions had a high degree of genomic instability with low telomerase expression, whereas malignant lesions had complex karyotypes with high levels of telomerase activity, consistent with a model of telomere-driven tumorigenesis in human cancer. Several other mechanisms of genomic instability are discussed later.
CHAPTER 6 Neoplasia

Development of Sustained Angiogenesis

Even with all the genetic abnormalities discussed above, tumors cannot enlarge beyond 1 to 2 mm in diameter unless they are vascularized. Like normal tissues, tumors require delivery of oxygen and nutrients and removal of waste products; presumably the 1- to 2-mm zone represents the maximal distance across which oxygen, nutrients, and waste can diffuse from blood vessels. Cancer cells can stimulate neo-angiogenesis, during which new vessels sprout from previously existing capillaries, or, in some cases, vasculogenesis, in which endothelial cells are recruited from the bone marrow (Chapter 3). Tumor vasculature is abnormal, however. The vessels are leaky, dilated, and have a haphazard pattern of connection. Neovascularization has a dual effect on tumor growth: Perfusion supplies needed nutrients and oxygen, and newly formed endothelial cells stimulate the growth of adjacent tumor cells by secreting growth factors, such as insulin-like growth factors, PDGF, and granulocyte-macrophage colony-stimulating factor. Angiogenesis is required not only for continued tumor growth but also for access to the vasculature and hence for metastasis. Angiogenesis is thus a necessary biologic correlate of malignancy.

How do growing tumors develop a blood supply? The emerging paradigm is that tumor angiogenesis is controlled by the balance between angiogenic factors and factors that inhibit angiogenesis. Early in their growth, most human tumors do not induce angiogenesis. They remain small or in situ for years until the angiogenic switch terminates this stage of vascular quiescence. The molecular basis of the angiogenic switch involves increased production of angiogenic factors and/or loss of angiogenesis inhibitors. These factors may be produced directly by the tumor cells themselves or by inflammatory...
cells (e.g., macrophages) or other stromal cells associated with the tumors. The angiogenic switch is controlled by several physiologic stimuli, such as hypoxia. Relative lack of oxygen stimulates production of a variety of pro-angiogenic cytokines, such as vascular endothelial growth factor (VEGF), through activation of hypoxia-induced factor-1α (HIF1α), an oxygen-sensitive transcription factor. HIF1α is continuously produced, but in normoxic settings the von Hippel–Lindau protein (VHL) binds to HIF1α, leading to ubiquitination and destruction of HIF1α. In hypoxic conditions, such as a tumor that has reached a critical size, the lack of oxygen prevents HIF1α recognition by VHL, and it is not destroyed. HIF1α translocates to the nucleus and activates transcription of its target genes, such as VEGF. Because of these activities, VHL acts as a tumor suppressor gene, and germ-line mutations of the VHL gene are associated with hereditary renal cell cancers, pheochromocytomas, hemangiomas of the central nervous system, retinal angiomas, and renal cysts (VHL syndrome). Both pro- and anti-angiogenic factors are regulated by many other genes frequently mutated in cancer. For example, in normal cells, p53 can stimulate expression of anti-angiogenic molecules, such as thrombospondin-1, and repress expression of pro-angiogenic molecules, such as VEGF. Thus, loss of p53 in tumor cells not only removes the cell cycle checkpoints listed above, but also provides a more permissive environment for angiogenesis. The transcription of VEGF is also influenced by signals from the RAS-MAP kinase pathway, and mutations of RAS or MYC up-regulate the production of VEGF.

Proteases, either elaborated by the tumor cells directly or from stromal cells in response to the tumor, are also involved in regulating the balance between angiogenic and anti-angiogenic factors. Many proteases can release the angiogenic basic FGF stored in the extracellular matrix (ECM); conversely, three potent angiogenesis inhibitors—angiostatin, endostatin, and vascuostatin—are produced by proteolytic cleavage of plasminogen, collagen, and transthyretin, respectively. Because of the crucial role of angiogenesis in tumor growth, much interest is focused on anti-angiogenesis therapy. Indeed, anti-VEGF antibody is now approved for the treatment of several types of cancers.

**Summary**

**Development of Sustained 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. Because of its ability to degrade HIF1α and thus prevent angiogenesis, VHL acts as a tumor suppressor gene. Inheritance of germ-line mutations of this gene causes VHL syndrome, characterized by the development of a variety of tumors.
- Many other factors regulate angiogenesis; for example, p53 induces synthesis of the angiogenesis inhibitor thrombospondin-1.

**Ability to Invade and Metastasize**

The spread of tumors is a complex process involving a series of sequential steps, diagrammed in Figure 6–26. Predictably, this sequence of steps may be interrupted at any stage by either host-related or tumor-related factors. For the purpose of discussion, the metastatic cascade can be subdivided into two phases: invasion of ECM and vascular dissemination, and homing of tumor cells.
Invasion of Extracellular Matrix (ECM)

As is well known, human tissues are organized into a series of compartments separated from each other by two types of ECM: basement membranes and interstitial connective tissue. Though organized differently, each of these components of ECM is composed of collagens, glycoproteins, and proteoglycans. A review of Figure 6–26 reveals that tumor cells must interact with the ECM at several stages in the metastatic cascade. A carcinoma first must breach the underlying basement membrane, then traverse the interstitial connective tissue, and ultimately gain access to the circulation by penetrating the vascular basement membrane. This cycle is repeated when tumor cell emboli extravasate at a distant site. Thus, to metastasize, a tumor cell must cross several different basement membranes, as well as negotiate through at least two interstitial matrices. Invasion of the ECM is an active process that requires four steps (see Fig. 6–27):

1. Detachment of tumor cells from each other
2. Degradation of ECM
3. Attachment to novel ECM components
4. Migration of tumor cells

The first step in the metastatic cascade is a loosening of tumor cells. As mentioned earlier, E-cadherins act as intercellular glues, and their cytoplasmic portions bind to β-catenin (see Fig. 6–23). Adjacent E-cadherin molecules keep the cells together; in addition, as discussed earlier, E-cadherin can transmit antigrowth signals by sequestering β-catenin. **E-cadherin function is lost in almost all epithelial cancers, either by mutational inactivation of E-cadherin genes, by activation of β-catenin genes, or by inappropriate expression of the SNAIL and TWIST transcription factors, which suppress E-cadherin expression.**

The second step in invasion is local degradation of the basement membrane and interstitial connective tissue. Tumor cells may either secrete proteolytic enzymes themselves or induce stromal cells (e.g., fibroblasts and inflammatory cells) to elaborate proteases. Multiple different families of proteases, such as matrix metalloproteinases (MMPs), cathepsin D, and urokinase plasminogen activator, have been implicated in tumor cell invasion. MMPs regulate tumor invasion not only by remodeling insoluble components of the basement membrane and interstitial matrix but also by releasing ECM-sequestered growth factors. Indeed, cleavage products of collagen and proteoglycans also have chemotactic, angiogenic, and growth-promoting effects. For example, MMP-9 is a gelatinase that cleaves type IV collagen of the epithelial and vascular basement membrane and also stimulates release of VEGF from ECM-sequestered pools. Benign tumors of the breast, colon, and stomach show little type

---

**Figure 6–27**

A–D, Schematic illustration of the sequence of events in the invasion of epithelial basement membranes by tumor cells. Tumor cells detach from each other because of reduced adhesiveness, then secrete proteolytic enzymes, degrading the basement membrane. Binding to proteolytically generated binding sites and tumor cell migration follow.

---

**A. LOOSENING OF INTERCELLULAR JUNCTIONS**

Laminin

Type IV collagen

Basement membrane

Cadherins

**B. DEGRADATION**

Type IV collagenase

Plasminogen activator

Type IV collagen cleavage

**C. ATTACHMENT**

Type IV collagen

Fibronectin receptor

Laminin receptor

Laminin

**D. MIGRATION**

Autocrine motility factor

Fibronectin
IV collagenase activity, whereas their malignant counterparts overexpress this enzyme. Concurrently, the levels of metalloproteinase inhibitors are reduced so that the balance is tilted greatly toward tissue degradation. Indeed, overexpression of MMPs and other proteases have been reported for many tumors. Because of these observations, attempts are being made to use protease inhibitors as therapeutic agents.

The third step in invasion involves changes in attachment of tumor cells to ECM proteins. Normal epithelial cells have receptors, such as integrins, for basement membrane laminin and collagens that are polarized at their basal surface; these receptors help to maintain the cells in a resting, differentiated state. Loss of adhesion in normal cells leads to induction of apoptosis, while, not surprisingly, tumor cells are resistant to this form of cell death. 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 complex, multistep process that involves many families of receptors and signaling proteins that eventually impinge on the actin cytoskeleton. Such movement seems to be potentiated and directed by tumor cell–derived cytokines, such as autocrine motility factors. In addition, cleavage products of matrix components (e.g., collagen, laminin) and some growth factors (e.g., insulin-like growth factors I and II) have chemotactic activity for tumor cells. Stromal cells also produce paracrine effectors of cell motility, such as hepatocyte growth factor/scatter factor (HGF/SCF), which bind to receptors on tumor cells. Concentrations of HGF/SCF are elevated at the advancing edges of the highly invasive brain tumor glioblastoma multiforme, supporting their role in motility.

It has become clear in recent years, however, that the ECM and stromal cells surrounding tumor cells do not merely represent a static barrier for tumor cells to traverse but rather represent a variable environment in which reciprocal signaling between tumor cells and stromal cells may either promote or prevent tumorigenesis and/or tumor progression. Stromal cells that interact with tumors include innate and adaptive immune cells (discussed later), as well as fibroblasts. A variety of studies have demonstrated that tumor-associated fibroblasts exhibit altered expression of genes that encode ECM molecules, proteases, protease inhibitors, and various growth factors. Thus, tumor cells live in a complex and ever-changing milieu composed of ECM, growth factors, fibroblasts, and immune cells, with significant cross-talk among all the components. The most successful tumors may be those that can co-opt and adapt this environment to their own nefarious ends.

**Vascular Dissemination and Homing of Tumor Cells**

When in the circulation, tumor cells are vulnerable to destruction by host immune cells (discussed later). In the bloodstream, some tumor cells form emboli by aggregating and adhering to circulating leukocytes, particularly platelets; aggregated tumor cells are thus afforded some protection from the antitumor host effector cells. Most tumor cells, however, circulate as single cells. Extravasation of free tumor cells or tumor emboli involves adhesion to the vascular endothelium, followed by egress through the basement membrane into the organ parenchyma by mechanisms similar to those involved in invasion.

The site of extravasation and the organ distribution of metastases generally can be predicted by the location of the primary tumor and its vascular or lymphatic drainage. Many tumors metastasize to the organ that represents the first capillary bed they encounter after entering the circulation. However, in many cases the natural pathways of drainage do not readily explain the distribution of metastases. As pointed out earlier, some tumors (e.g., lung cancers) tend to involve the adrenal glands with some regularity but almost never spread to skeletal muscle. Such organ tropism may be related to the expression of adhesion molecules by tumor cells whose ligands are expressed preferentially on the endothelium of target organs. Another mechanism of site-specific homing involves chemokines and their receptors. As discussed in Chapter 2, chemokines participate in directed movement (chemotaxis) of leukocytes, and it seems that cancer cells use similar tricks to home in on specific tissues. Human breast cancer cells express high levels of the chemokine receptors CXCR4 and CCR7. The ligands for these receptors (i.e., chemokines CXCL12 and CCL21) are highly expressed only in those organs where breast cancer cells metastasize. On the basis of this observation, it is speculated that blockade of chemokine receptors may limit metastases. After extravasation, tumor cells are dependent on a receptive stroma for growth. Thus, tumors may fail to metastasize to certain target tissues because they present a nonpermissive growth environment. Despite the foregoing considerations, the precise localization of metastases cannot be predicted with any form of cancer. Evidently many tumors have not read the relevant chapters of the pathology textbooks!

**Molecular Genetics of Metastasis**

A long-held theory of tumor progression suggests that, as tumors grow, individual cells randomly accumulate mutations, creating subclones with distinct combinations of mutations. According to this hypothesis only a small subpopulation of the tumor cells contains all the mutations necessary for metastasis. However, recent experiments, in which gene profiling of primary tumors and metastatic deposits has been compared, challenge this hypothesis. For example, a subset of breast cancers has a gene expression signature similar to that found in metastases, although no clinical evidence for metastasis is apparent. In these tumors it seems that most if not all cells develop a predilection for metastatic spread early, during primary carcinogenesis. Metastases, according to this view, are not dependent on the stochastic generation of metastatic subclones postulated above. It should be noted, however, that gene expression analyses like those described above would not detect a small subset of metastatic subclones within a
large tumor. Perhaps both mechanisms are operative, with aggressive tumors acquiring a metastases-permissive gene expression pattern early in tumorigenesis that requires some additional random mutations to complete the metastatic phenotype.

One open question in the field is, are there genes whose principal or sole contribution to tumorigenesis is to control metastases? This question is of more than academic interest, because if altered forms of certain genes promote or suppress the metastatic phenotype, their detection in a primary tumor would have both prognostic and therapeutic implications. Metastasis is a complex phenomenon involving a variety of steps and pathways described above. It is thought therefore that, unlike transformation, in which a subset of proteins like p53 and RB seem to play a key role, genes that function as “metastasis oncogenes” or “metastatic suppressors” are rare. Among candidates for such metastasis oncogenes are SNAIL and TWIST, which encode transcription factors whose primary function is to promote a process called epithelial-to-mesenchymal transition (EMT). In EMT, carcinoma cells down-regulate certain epithelial markers (e.g., E-cadherin) and up-regulate certain mesenchymal markers (e.g., vimentin and smooth muscle actin). These changes are believed to favor the development of a migratory phenotype that is essential for metastasis. Loss of E-cadherin expression seems to be a key event in EMT, and SNAIL and TWIST are transcriptional repressors that promote EMT by down-regulating E-cadherin expression. EMT has been documented mainly in breast tumors. EMT has been documented mainly in breast carcinoma cells. The phenomenon of epithelial-to-mesenchymal transition (EMT) is central to understanding how primary cancer cells detach from the primary tumor, invade and colonize distant organs. EMT is a complex cellular process that involves the reorganization of the cytoskeleton, the remodeling of extracellular matrix (ECM), and the acquisition of a mesenchymal phenotype characterized by increased cell motility and invasion.

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 membranes 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 the metastatic site.

Genomic Instability—Enabler of Malignancy

In the preceding section we discussed six defining features of malignancy and the genetic alterations that are responsible for the phenotypic attributes of cancer cells. How do these mutations arise? Although humans literally swim in environmental agents that are mutagenic (e.g., chemicals, radiation, sunlight), cancers are relatively rare outcomes of these encounters. This state of affairs results from the ability of normal cells to repair DNA damage. The importance of DNA repair in maintaining the integrity of the genome is highlighted by several inherited disorders in which genes that encode proteins involved in DNA repair are defective. Individuals born with such inherited defects in DNA repair proteins are at a greatly increased risk of developing cancer. Typically, genomic instability occurs when both copies of the gene are lost; however, recent work has suggested that at least a subset of these genes may promote cancer in a haploinsufficient manner. Defects in three types of DNA repair systems—mismatch repair, nucleotide excision repair, and recombination repair—are presented next.

Hereditary Nonpolyposis Colon Cancer Syndrome. The role of DNA repair genes in predisposition to cancer is illustrated dramatically by hereditary nonpolyposis colon carcinoma (HNPCC) syndrome. This disorder, characterized by familial carcinomas of the colon affecting predominantly the cecum and proximal colon (Chapter 15), results from defects in genes involved in DNA mismatch repair. When a strand of DNA is being repaired, these genes act as “spell checkers.” For example, if there is an erroneous pairing of G with T rather than the normal A with T, the mismatch repair genes correct the defect. Without these “proofreaders,” errors gradually accumulate in several genes, including proto-oncogenes and cancer suppressor genes. Mutations in at least four mismatch repair genes have been found to underlie HNPCC (Chapter 15). Each affected individual inherits one defective copy of one of several DNA mismatch repair genes and acquires the second hit in colonic epithelial cells. Thus, DNA repair genes behave like tumor suppressor genes in their mode of inheritance, but in contrast to tumor suppressor genes (and oncogenes), they affect cell growth only indirectly—by allowing mutations in other genes during the process of normal cell division. One of the hallmarks of patients with mismatch repair defects is microsatellite instability ( MSI ). Microsatellites are tandem repeats of one to six nucleotides found throughout the genome. In normal people, the length of these microsatellites remains constant. However, in patients with HNPCC, these satellites are unstable and increase or decrease in length. Although HNPCC accounts only for 2% to 4% of all colonic cancers, MSI can be detected in about 15% of sporadic cancers. The growth-regulating genes that are mutated in HNPCC patients have not yet been fully characterized.

Xeroderma Pigmentosum. Patients with another inherited disorder, xeroderma pigmentosum, are at increased risk for the development of cancers of the skin exposed to the ultraviolet (UV) light contained in sun rays. The
basis of this disorder is defective DNA repair. UV light causes cross-linking of pyrimidine residues, preventing normal DNA replication. Such DNA damage is repaired by the nucleotide excision repair system. Several proteins are involved in nucleotide excision repair, and an inherited loss of any one can give rise to xeroderma pigmentosum.

Diseases with Defects in DNA Repair by Homologous Recombination. A group of autosomal recessive disorders comprising Bloom syndrome, ataxia-telangiectasia, and Fanconi anemia is characterized by hypersensitivity to other DNA-damaging agents, such as ionizing radiation (Bloom syndrome and ataxia-telangiectasia), or DNA cross-linking agents, such as nitrogen mustard (Fanconi anemia). Their phenotype is complex and includes, in addition to predisposition to cancer, features such as neural symptoms (ataxia-telangiectasia), anemia (Fanconi anemia), and developmental defects (Bloom syndrome). As mentioned earlier, the gene mutated in ataxia-telangiectasia is ATM, which seems to be important in recognizing and responding to DNA damage caused by ionizing radiation. Evidence for the role of DNA repair genes in the origin of cancer also comes from the study of hereditary breast cancer. Mutations in two genes, BRCA1 and BRCA2, account for 80% of cases of familial breast cancer. 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. Likewise, mutations in the BRCA2 gene increase the risk of breast cancer in both men and women as well as cancer of the ovary, prostate, pancreas, bile ducts, stomach, and melanocytes. Although the functions of these genes have not been elucidated fully, cells that lack these genes develop chromosomal breaks and severe aneuploidy. Indeed, both genes seem to function, at least in part, in the homologous recombination DNA repair pathway. For example, BRCA1 forms a complex with other proteins in the homologous recombination pathway and is also linked to the ATM checkpoint pathway. BRCA2 was identified as one of several genes mutated in Fanconi anemia and the BRCA2 protein has been shown to bind to RAD51, a protein required for catalysis of the primary reaction of homologous recombination. Similar to other tumor suppressor genes, both copies of BRCA1 and BRCA2 must be inactivated for cancer to develop. Although linkage of BRCA1 and BRCA2 to familial breast cancers is established, these genes are rarely inactivated in sporadic cases of breast cancer. In this regard, BRCA1 and BRCA2 are different from other tumor suppressor genes, such as APC and p53, which are inactivated in both familial and sporadic cancers.

SUMMARY

Genomic Instability—Enabler of Malignancy
• Individuals with inherited mutations of genes involved in DNA repair systems are at a greatly increased risk of developing cancer.

Patients with HNPPC syndrome have defects in the mismatch repair system and develop carcinomas of the colon. These patients show microsatellite instability (MSI), in which short repeats throughout the genome change in length.

• Syndromes involving defects in the homologous recombination DNA repair system compose a group of disorders (Bloom syndrome, ataxia-telangiectasia, and Fanconi anemia) that are characterized by hypersensitivity to DNA-damaging agents, such as ionizing radiation. BRCA1 and BRCA2, which are mutated in familial breast cancers, are involved in DNA repair.

MicroRNAs (MiRNAs) and Cancer

As discussed in Chapter 7, miRNAs are non-coding, single-stranded RNAs, approximately 22 nucleotides in length, that function as negative regulators of genes. They inhibit gene expression post-transcriptionally by repressing translation, or in some cases, by mRNA cleavage. Given that miRNAs control cell growth, differentiation, and cell survival, it is not surprising that there is accumulating evidence to support their role in carcinogenesis. As illustrated by Figure 6–28, miRNAs can participate in neoplastic transformation either by increasing the expression of oncogenes or reducing the expression of tumor suppressor genes. If an miRNA inhibits the translation of an oncogene, a reduction in the quantity or function of that miRNA will lead to overproduction of the oncogene product. Conversely, if the target of an miRNA is a tumor suppressor gene, then overactivity of the miRNA can reduce the tumor suppressor protein. Such relationships have already been established by miRNA profiling of several human tumors. For example, downregulation or deletion of certain miRNAs in some leukemias and lymphomas results in increased expression of BCL2, the antiapoptotic gene. Thus, by negatively regulating BCL2, such miRNAs behave as tumor suppressor genes. Similar miRNA-mediated upregulation of RAS, and MYC oncogenes has also been detected in lung tumors and in certain B cell leukemias respectively. In some brain and breast tumors there is 5–100 fold greater expression of certain miRNAs. Although the targets of these miRNAs have not been identified, presumably they are unidentified tumor suppressor genes, whose activities are reduced by the overexpressed miRNA.

These findings not only provide novel insights into carcinogenesis, they also have practical implications. For instance, drugs that inhibit or augment the functions of miRNAs could be useful in chemotherapy. Since miRNAs regulate normal cellular differentiation, the patterns of miRNA expression (“miRNA profiling”) can provide
clues to the cell of origin and classification of tumors. Much remains to be learned about these oncogenic miRNAs, or so called “oncomirs.”

**Molecular Basis of Multistep Carcinogenesis**

Given that malignant tumors must develop several fundamental abnormalities, discussed above, it follows that each cancer must result from accumulation of multiple mutations. Indeed, recently completed genome-wide analysis of breast and colon cancers has revealed that individual tumors accumulate an average of 90 mutant genes. A much smaller subset of these (~11/tumor) were mutated at significant frequency. Included among these are some known oncogenes and tumor suppressor genes, while others were not previously known to be tumor-associated. Each of these alterations represents crucial steps in the progression from a normal cell to a malignant tumor. Furthermore, it seems that evolution has installed a variety of “intrinsic tumor-suppressive mechanisms” such as apoptosis and senescence that thwart the actions of growth-promoting mutations. Indeed, in cells with competent checkpoints, oncogenic signaling through genes like \( \text{RAS} \) leads not to transformation, but to senescence or apoptosis. Thus, emergence of malignant tumors requires mutational loss of many genes including those that regulate apoptosis and senescence. A dramatic example of incremental acquisition of the malignant phenotype is documented by the study of colon carcinoma. These lesions are believed to evolve through a series of morphologically identifiable stages: colon epithelial hyperplasia followed by formation of adenomas that progressively enlarge and ultimately undergo malignant transformation (Chapter 15). The proposed molecular correlates of this adenoma-carcinoma sequence are illus-

**Figure 6–28**

Role of miRNAs in tumorigenesis. A. Reduced activity of a miRNA that inhibits translation of an oncogene gives rise to an excess of oncoproteins. B. Overactivity of a miRNA that targets a tumor suppression gene reduces the production of the tumor suppressor protein. Question marks in A and B are meant to indicate that the mechanisms by which changes in the level or activity of miRNA are not entirely known.
trated in Figure 6–29. According to this scheme, inactivation of the APC tumor suppressor gene occurs first, followed by activation of RAS and, ultimately, loss of a tumor suppressor gene on 18q and loss of p53. The precise temporal sequence of mutations may be different in each organ and tumor type.

Karyotypic Changes in Tumors
The genetic damage that activates oncogenes or inactivates tumor suppressor genes may be subtle (e.g., point mutations) or large enough to be detected in a karyotype. As previously discussed, the RAS oncogene represents the best example of activation by point mutation. In certain neoplasms, karyotypic abnormalities are nonrandom and common. Specific abnormalities have been identified in most leukemias and lymphomas, and in an increasing number of nonhematopoietic tumors. The common types of nonrandom structural abnormalities in tumor cells are (1) balanced translocations, (2) deletions, and (3) cytogenetic manifestations of gene amplification. In addition, whole chromosomes may be gained or lost, termed aneuploidy.

Balanced Translocations. Balanced translocations are extremely common, especially in hematopoietic neoplasms. Translocations can activate proto-oncogenes in two ways. First, specific translocations can result in overexpression of proto-oncogenes by removing them from their normal regulatory elements and placing them under control of an inappropriate promoter. Second, translocations can result in fusion genes, combining the DNA sequence of two unrelated genes in new ways. This results in the expression of growth-promoting chimeric proteins. Most notable is the Philadelphia (Ph) chromosome in chronic myeloid leukemia, comprising a reciprocal and balanced translocation between chromosomes 22 and, usually, 9 (Fig. 6–30). As a consequence, chromosome 22 appears abbreviated. This cytogenetic change, seen in more than 90% of cases of chronic myeloid leukemia, is a reliable marker of the disease. The few Ph chromosome-negative cases of chronic myeloid leukemia show molecular evidence of the BCR-ABL rearrangement, the crucial consequence of Ph translocation. As mentioned earlier, such changes give rise to the BCR-ABL fusion gene with potent tyrosine kinase activity. In more than 90% of cases of Burkitt lymphoma the cells have a translocation, usually between chromosomes 8 and 14. This leads to overexpression of MYC gene on chromosome 8 by juxtaposition with immunoglobulin heavy chain gene on chromosome 14. In follicular B-cell lymphomas, a reciprocal translocation between chromosomes 14 and 18 leads to overexpression of the BCL2 gene on chromosome 18.

Hematopoietic cells are most commonly the targets of such translocations, probably because these cells purposefully make DNA breaks during the processes of antibody or T-cell receptor recombination. However, several solid tumors have also been shown to possess a recurrent translocation, such as the t(11;22)(q24;12) translocation in Ewing sarcoma that results in fusion of the EWS transcription factor with Fli-1. Recently, a subset of prostate cancers has been shown to possess a fusion protein between a prostate-expressed protein and members of the ETS family of transcription factors.

Deletions. Chromosomal deletions are the second most prevalent structural abnormality in tumor cells. Compared with translocations, deletions are more common in nonhematopoietic solid tumors. As discussed, deletions of chromosome 13q band 14 are associated with retinoblastoma. Deletions of 17p, 5q, and 18q have all been noted in colorectal cancers; these regions harbor three tumor suppressor genes. Deletion of 3p, noted in several tumors, is extremely common in small-cell lung carcinomas, and the hunt is on for one or more cancer suppressor genes at this locale.
ETIOLOGY OF CANCER: CARCINOGENIC AGENTS

Genetic damage lies at the heart of carcinogenesis. What agents inflict such damage? Three classes of carcinogenic agents can be identified: (1) chemicals, (2) radiant energy, and (3) microbial agents. Chemicals and radiant energy are documented causes of cancer in humans, and onco-genic viruses are involved in the pathogenesis of tumors in several animal models and at least in some human tumors. In the following discussion, each class of agents is considered separately, but it is important to note that several may act in concert or sequentially to produce the multiple genetic abnormalities characteristic of neoplastic cells.

Chemical Carcinogens

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, the Danish Chimney Sweeps Guild ruled that its members must bathe daily. No public health measure since that time has achieved so much in the control of a form of cancer. Subsequently, hundreds of chemicals have been shown to be carcinogenic in animals. Some of the major agents are presented in Table 6–4. A few comments are offered on a handful of these.

Direct-Acting Agents

Direct-acting agents require no metabolic conversion to become carcinogenic. They are in general weak carcinogens but are important because some of them are cancer chemotherapeutic drugs (e.g., alkylating agents) that have successfully cured, controlled, or delayed recurrence of...
certain types of cancer (e.g., leukemia, lymphoma, Hodgkin lymphoma, and ovarian carcinoma), only to evoke later a second form of cancer, usually leukemia. This situation is even more tragic when their initial use has been for non-neoplastic disorders, such as rheumatoid arthritis or Wegener granulomatosis. The risk of induced cancer is low, but its existence dictates judicious use of such agents.

### Indirect-Acting Agents

The designation *indirect-acting agent* refers to chemicals that require metabolic conversion to an *ultimate carcinogen* before they become active. Some of the most potent indirect chemical carcinogens—the polycyclic hydrocarbons—are present in fossil fuels. For example, benzo[a]pyrene and other carcinogens are formed in the high-temperature combustion of tobacco in cigarette smoking. These products are implicated in the causation of lung cancer in cigarette smokers. Polycyclic hydrocarbons may also be produced from animal fats during the process of broiling meats and fish. The principal active products in many hydrocarbons are epoxides, which form covalent adducts (addition products) with molecules in the cell, principally DNA, but also with RNA and proteins.

The aromatic amines and azo dyes are another class of indirect-acting carcinogens. Before its carcinogenicity was recognized, β-naphthylamine was responsible for a 50-fold increased incidence of bladder cancers in heavily exposed workers in the aniline dye and rubber industries. Many other occupational carcinogens were listed in Table 6–2. Because indirect-acting carcinogens require metabolic activation for their conversion to DNA-damaging agents, much interest is focused on the enzymatic pathways that are involved, such as the cytochrome P-450-dependent monooxygenases. The genes that encode these enzymes are polymorphic, and enzyme activity varies among different individuals. It is widely believed that the susceptibility to chemical carcinogenesis depends at least in part on the specific allelic form of the enzyme inherited. Thus, it may be possible in the future to assess cancer risk in a given individual by genetic analysis of such enzyme polymorphisms.

A few other agents merit brief mention. Aflatoxin B₁ is of interest because it is a naturally occurring agent produced by some strains of *Aspergillus*, a mold that grows on improperly stored grains and nuts. There is a strong correlation between the dietary level of this food contaminant and the incidence of hepatocellular carcinoma in some parts of Africa and the Far East. Additionally, vinyl chloride, arsenic, nickel, chromium, insecticides, fungicides, and polychlorinated biphenyls are potential carcinogens in the workplace and about the house. Finally, nitrites used as food preservatives have caused concern, since they cause nitrosylation of amines contained in the food. The nitrosoamines so formed are suspected to be carcinogenic.

### Mechanisms of Action of Chemical Carcinogens

Because malignant transformation results from mutations, it should come as no surprise that most chemical carcinogens are mutagenic. Indeed, all direct and ultimate carcinogens contain highly reactive electrophile groups that form chemical adducts with DNA, as well as with proteins and RNA. Although any gene may be the target of chemical carcinogens, the commonly mutated oncogenes and tumor suppressors, such as RAS and p53, are important targets of chemical carcinogens. Indeed, specific chemical carcinogens, such as aflatoxin B₁, produce characteristic mutations in the p53 gene, such that detection of the “signature mutation” within the p53 gene establishes aflatoxin as the causative agent. These associations are proving useful tools in epidemiologic studies of chemical carcinogenesis.

Carcinogenicity of some chemicals is augmented by subsequent administration of *promoters* (e.g., phorbol esters, hormones, phenols, and drugs) that by themselves are nontumorigenic. To be effective, repeated or sustained exposure to the promoter must follow the application of the mutagenic chemical, or *initiator*. The initiation-promotion sequence of chemical carcinogenesis raises an
important question: Since promoters are not mutagenic, how do they contribute to tumorigenesis? Although the effects of tumor promoters are pleiotropic, induction of cell proliferation is a sine qua non of tumor promotion. It seems most likely that while the application of an initiator may cause the mutational activation of an oncogene such as RAS, subsequent application of promoters leads to clonal expansion of initiated (mutated) cells. Forced to proliferate, the initiated clone of cells accumulates additional mutations, developing eventually into a malignant tumor. Indeed, the concept that sustained cell proliferation increases the risk of mutagenesis, and hence neoplastic transformation, is also applicable to human carcinogenesis. For example, pathologic hyperplasia of the endometrium (Chapter 19) and increased regenerative activity that accompanies chronic liver cell injury are associated with the development of cancer in these organs. Were it not for the DNA repair mechanisms discussed earlier, the incidence of chemically induced cancers in all likelihood would be much higher. As mentioned above, the rare hereditary disorders of DNA repair, including xeroderma pigmentosum, are associated with greatly increased risk of cancers induced by UV light and certain chemicals.

**SUMMARY**

**Chemical Carcinogens**

- 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 like cytochrome P-450 may influence carcinogenesis.
- Following 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 include direct-acting (e.g., alkylating agents used for chemotherapy), indirect-acting (e.g., benzopyrene, azo dyes, and aflatoxin), and promoters/agents that cause pathologic hyperplasias of liver, endometrium.

**Radiation Carcinogenesis**

Radiation, whatever its source (UV rays of sunlight, x-rays, nuclear fission, radionuclides) is an established carcinogen. Unprotected miners of radioactive elements have a 10-fold increased incidence of lung cancers. Follow-up of survivors of the atomic bombs dropped on Hiroshima and Nagasaki disclosed a markedly increased incidence of leukemia—principally acute and chronic myeloid leukemia—after an average latent period of about 7 years, as well as an increased mortality rate from thyroid, breast, colon, and lung carcinomas. The nuclear power accident at Chernobyl in the former Soviet Union continues to exact its toll in the form of high cancer incidence in the surrounding areas. Therapeutic irradiation of the head and neck can give rise to papillary thyroid cancers years later. The oncogenic properties of ionizing radiation are related to its mutagenic effects; it causes chromosome breakage, translocations, and, less frequently, point mutations. Biologically, double-stranded DNA breaks seem to be the most important form of DNA damage caused by radiation. There is also some evidence that nonlethal doses of radiation may induce genomic instability, favoring carcinogenesis.

The oncogenic effect of UV rays merits special mention because it highlights the importance of DNA repair in carcinogenesis. Natural UV radiation derived from the sun can cause skin cancers (melanomas, squamous cell carcinomas, and basal cell carcinomas). At greatest risk are fair-skinned people who live in locales such as Australia and New Zealand that receive a great deal of sunlight. Nonmelanoma skin cancers are associated with total cumulative exposure to UV radiation, whereas melanomas are associated with intense intermittent exposure—as occurs with sunbathing. UV light has several biologic effects on cells. Of particular relevance to carcinogenesis is the ability to damage DNA by forming pyrimidine dimers. This type of DNA damage is repaired by the nucleotide excision repair pathway. With extensive exposure to UV light, the repair systems may be overwhelmed, and skin cancer results. As mentioned above, patients with the inherited disease xeroderma pigmentosum have a defect in the nucleotide excision repair pathway. As expected, there is a greatly increased predisposition to skin cancers in this disorder.

**SUMMARY**

**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 squamous cell carcinomas and melanomas of the skin.

**Viral and Microbial Oncogenesis**

Many DNA and RNA 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. Also discussed is the emerging role of the bacterium *H. pylori* in gastric cancer.

**Oncogenic RNA Viruses**

The study of oncogenic retroviruses in animals has provided spectacular insights into the genetic basis of cancer. However, human T-cell leukemia virus-1 (HTLV-1) is the only retrovirus that has been demonstrated to cause cancer in humans. HTLV-1 is associated with a form of T-cell leukemia/lymphoma that is endemic in certain parts of Japan and the Caribbean basin but is found sporadically elsewhere, including the United States. Similar to the human immunodeficiency virus (HIV), HTLV-1 has tropism for CD4+ T cells, and 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 only in about 3% to 5% of infected individuals after a long latent period of 20 to 50 years.

There is little doubt that HTLV-1 infection of T lymphocytes is necessary for leukemogenesis, but the molecular mechanisms of transformation are not clear. HTLV-1 does not contain a *viral oncogene*, and in contrast to certain animal retroviruses, no consistent integration site next to a cellular oncogene has been discovered. Indeed, the long latency period between initial infection and development of disease suggests a multistep process, during which many oncogenic mutations are accumulated.

The genome of HTLV-1 contains, in addition to the usual retroviral genes, a unique region called *pX*. This region encodes several genes, including one called *TAX*. The TAX protein has been shown to be necessary and sufficient for cellular transformation. By interacting with several transcription factors, such as NF-κB, the TAX protein can transactivate the expression of genes that encode cytokines, cytokine receptors, and costimulatory molecules. This inappropriate gene expression leads to autocrine signaling loops and increased activation of pro-mitogenic signaling cascades. Furthermore, TAX can drive progression through the cell cycle by directly binding to and activating cyclins. In addition, TAX can repress the function of several tumor suppressor genes that control the cell cycle, including *CDKN2A/p16* and *p53*. From these and other observations the following scenario is emerging (Fig. 6–32): The TAX gene turns on several cytokine genes and their receptors (IL-2 and IL-2R, IL-15, and IL-15R), setting up an autocrine system that drives T-cell proliferation. Of these cytokines, IL-15 seems to be more important, but much remains to be defined. Additionally, a parallel paracrine pathway is activated by increased production of granulocyte-macrophage colony-stimulating factor, which stimulates neighboring macrophages to produce other T-cell mitogens. Initially the T-cell proliferation is polyclonal because the virus infects many cells, but, because of TAX-based inactivation of tumor suppressor genes such as *p53*, the proliferating T cells are at increased risk of secondary transforming events (mutations), which lead ultimately to the outgrowth of a monoclonal neoplastic T-cell population.

**SUMMARY**

**Oncogenic RNA Viruses**

- HTLV-1 causes a T-cell leukemia that is endemic in Japan and the Caribbean.
- HTLV-1 encodes a viral TAX protein, which turns on genes for cytokines and their receptors in infected T cells. This sets up autocrine and paracrine signaling loops that stimulate T-cell proliferation. Although this proliferation is initially polyclonal, the proliferating T cells are at increased risk of secondary mutations that lead to the outgrowth of a monoclonal leukemia.

**Oncogenic DNA Viruses**

As with RNA viruses, several oncogenic DNA viruses that cause tumors in animals have been identified. Four DNA viruses—human papillomavirus (HPV), Epstein-Barr virus (EBV), Kaposi sarcoma herpesvirus (KSHV, also called human herpesvirus 8), and hepatitis B virus (HBV)—are of special interest, because they are strongly...
associated with human cancer. KSHV and Kaposi sarcoma were discussed in Chapter 5. The others are presented here.

**Human Papillomavirus**

Scores of genetically distinct types of HPV have been identified. Some types (e.g., 1, 2, 4, and 7) definitely cause benign squamous papillomas (warts) in humans (Chapters 19 and 22). By contrast, high-risk HPVs (e.g., 16 and 18) have been implicated in the genesis of several cancers, particularly squamous cell carcinoma of the cervix and anogenital region. In addition, at least 20% of oropharyngeal cancers are associated with HPV. In contrast to cervical cancers, genital warts have low malignant potential and are associated with low-risk HPVs predominantly HPV-6 and HPV-11.

The oncogenic potential of HPV can be related to products of two viral genes, E6 and E7. Together, they interact with a variety of growth-regulating proteins encoded by protooncogenes and tumor suppressor genes. The E7 protein binds to the retinoblastoma protein and displaces the E2F transcription factors that are normally sequestered by RB, promoting progression through the cell cycle. Interestingly, E7 protein from high-risk HPV types has a higher affinity for RB than does E7 from low-risk HPV types. E7 also inactivates the CDKIs CDKN1A/p21 and CDKN1B/p27. E7 proteins from high-risk HPV types (types 16, 18, and 31) also bind and presumably activate cyclins E and A. The E6 protein has complementary effects. It binds to and mediates the degradation of p53 and BAX, a pro-apoptotic member of the BCL2 family, and it activates telomerase. In analogy with E7, E6 from high-risk HPV types has a higher affinity for p53 than E6 from low-risk HPV types. Interestingly, in benign warts the HPV genome is maintained in a nonintegrated episomal form, while in cancers the HPV genome is randomly integrated into the host genome. Integration interrupts the viral DNA, resulting in over-expression of the oncoproteins E6 and E7. Furthermore, cells in which the viral genome has integrated show significantly more genomic instability.

To summarize, infection with high-risk HPV types simulates the loss of tumor suppressor genes, activates cyclins, inhibits apoptosis, and combats cellular senescence. Thus, it is evident that many of the hallmarks of cancer discussed earlier are driven by HPV proteins. However, infection with HPV itself is not sufficient for carcinogenesis. For example, when human keratinocytes are transfected with DNA from HPV 16, 18, or 31 in vitro, they are immortalized, but they do not form tumors in experimental animals. Cotransfection with a mutated RAS gene results in full malignant transformation. These data strongly suggest that HPV, in all likelihood, acts in concert with other environmental factors (Chapter 19). However, the primacy of HPV infection in the causation of cervical cancer is attested to by the near complete protection from this cancer by anti-HPV vaccines.

**Epstein-Barr Virus**

EBV has been implicated in the pathogenesis of several human tumors: Burkitt lymphoma, B-cell lymphomas in patients with acquired immunodeficiency syndrome and other causes of immunosuppression, a subset of Hodgkin lymphoma, and nasopharyngeal carcinoma. Except for nasopharyngeal carcinoma, all others are B-cell tumors. A subset of T-cell lymphomas and the rare NK-cell lymphomas may also be related to EBV.

Burkitt lymphoma is endemic in certain parts of Africa and is sporadic elsewhere. In endemic areas, tumor cells in virtually all patients carry the EBV genome. The molecular basis of B-cell proliferations induced by EBV is complex. EBV uses the complement receptor, CD21, to attach to and infect B cells. In vitro such infection leads to polyclonal B-cell proliferation and generation of B-lymphoblastoid cell lines. One of the EBV-encoded genes, called LMP-1, acts as an oncogene, and its expression in transgenic mice induces B-cell lymphomas. LMP-1 promotes B-cell proliferation by activating signaling pathways, such as NF-kB and JAK/STAT, which mimic B-cell activation via the B-cell surface molecule CD40. Concurrently, LMP-1 prevents apoptosis by activating BCL2. Thus, the virus “borrows” a normal B-cell activation pathway to promote its own replication by expanding the pool of cells susceptible to infection. Another EBV-encoded gene, EBNA-2, transactivates several host genes, including cyclin D and the src family genes. In addition, the EBV genome contains a viral cytokine, viL-10, that was pirated from the host genome. This viral cytokine can prevent macrophages and monocytes from activating T cells and is required for EBV-dependent transformation of B cells.

In immunologically normal individuals, EBV-driven polyclonal B-cell proliferation in vivo is readily controlled, and the individual either remains asymptomatic or develops a self-limited episode of infectious mononucleosis (Chapter 12). Evasion of the immune system seems to be a key step in EBV-related oncogenesis. In regions of the world where Burkitt lymphoma is endemic, concomitant (endemic) malaria (or other infections) impair immune competence, allowing sustained B-cell proliferation. Interestingly, although LMP-1 is the primary transforming oncogene in the EBV genome, it is not expressed in EBV-derived Burkitt lymphoma, because it is also one of the major viral antigens recognized by the immune system. Presumably, infected cells expressing viral antigens such as LMP-1 are kept in check by the immune system. Lymphoma cells emerge only when additional mutations, such as the t(8;14) translocation, a consistent feature of this tumor, activate the MYC oncogene. MYC activation may substitute for LMP-1 signaling, allowing the tumor cells to down-regulate LMP-1 and evade the immune system. In keeping with this scenario, EBV-derived B-cell lymphomas from immunocompromised patients, discussed below, retain expression of LMP-1. It should be noted that in nonendemic areas, 80% of tumors do not harbor the EBV genome, but all tumors possess the specific t(8;14) translocation. This observation suggests that, although non-African Burkitt lymphomas are triggered by mechanisms other than EBV, they develop cancer by similar pathways.

In immunosuppressed patients, including those with HIV disease and organ transplant recipients, EBV-infected B cells undergo polyclonal expansion, producing lymphoblastoid-like cells. In contrast to Burkitt lym-
phoma, the B lymphoblasts in immunosuppressed patients do express viral antigens, such as LMP-1, that are recognized by T cells. These potentially lethal proliferations can be subdued if the immunologic status of the host improves, as may occur with withdrawal of immunosuppressive drugs in transplant recipients.

Nasopharyngeal carcinoma is endemic in southern China and some other locales, and the EBV genome is found in all tumors. LMP-1 is expressed in epithelial cells as well. In these cells, as in B cells, LMP-1 activates the NF-kB pathway. Furthermore, LMP-1 induces the expression of pro-angiogenic factors such as VEGF, FGF-2, MMP-9, and COX2, which may contribute to oncogenesis. As in Burkitt lymphoma, EBV acts in concert with other, unidentified, factors (Chapter 13).

**SUMMARY**

**Oncogenic DNA Viruses**
- HPV has been associated with benign warts, as well as cervical cancer.
- The oncogenic ability of HPV is related to the expression of two viral oncoproteins, E6 and E7; they bind to RB and p53, respectively, neutralizing their function; they also activate cyclins.
- E6 and E7 from high-risk HPV (that give rise to cancers) have higher affinity for their targets than E6 and E7 from low-risk HPV (that give rise to low-grade tumors).
- EBV has been implicated in the pathogenesis of Burkitt lymphomas, lymphomas in immunosuppressed individuals with HIV infection or organ transplantation, some forms of Hodgkin lymphoma, and nasopharyngeal carcinoma. All except the nasopharyngeal cancers are B-cell tumors.
- Certain EBV gene products contribute to oncogenesis by stimulating a normal B-cell proliferation pathway. Concomitant compromise of immune competence allows sustained B-cell proliferation and eventually development of lymphoma with occurrence of additional mutations such as t(8;14), leading to activation of the MYC gene.

**Hepatitis B and Hepatitis C Viruses**

The epidemiologic evidence linking chronic HBV and hepatitis C virus (HCV) infection with hepatocellular carcinoma is strong (Chapter 16). It is estimated that 70% to 85% of hepatocellular carcinomas worldwide are due to infection with HBV or HCV. However, the mode of action of these viruses in tumorigenesis is not fully elucidated. The HBV and HCV genomes do not encode any viral oncoproteins, and although the HBV DNA is integrated within the human genome, there is no consistent pattern of integration in liver cells. Indeed, the oncogenic effects of HBV and HCV are multifactorial, but the dominant effect seems to be immunologically mediated.

Hepatitis B and Hepatitis C Viruses
- Between 70% and 85% of hepatocellular carcinomas worldwide are due to infection with HBV or HCV.
- The oncogenic effects of HBV and HCV are multifactorial, but the dominant effect seems to be immunologically mediated chronic inflammation with hepatocyte death leading to regeneration, and genomic damage. Although the immune system is generally thought to be protective, recent work has demonstrated that in the setting of unresolved chronic inflammation, as occurs in viral hepatitis or chronic gastritis cause by *H. pylori* (see below), the immune response may become maladaptive, promoting tumorigenesis.

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 produced by activated immune cells that promote cell survival, tissue remodeling, and angiogenesis. The activated immune cells also produce other mediators, such as reactive oxygen species, that are genotoxic and mutagenic. One key molecular step seems to be activation of the NF-kB pathway in hepatocytes caused by mediators derived from the activated immune cells. Activation of the NF-kB pathway within hepatocytes blocks apoptosis, allowing the dividing hepatocytes to incur genotoxic stress and to accumulate mutations. Although this seems to be the dominant mechanism in the pathogenesis of viral-induced hepatocellular carcinoma, both HBV and HCV also contain proteins within their genomes that may more directly promote the development of cancer. The HBV genome contains a gene known as HBx, and mice transgenic for this gene develop hepatocellular cancers. HBx can directly or indirectly activate a variety of transcription factors and several signal transduction pathways. In addition, viral integration can cause secondary rearrangements of chromosomes, including multiple deletions that may harbor unknown tumor suppressor genes.

Though 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 that 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 scenario for the development of gastric adenocarcinoma is similar to that of HBV- and HCV-induced liver cancer. 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. There is an initial development of chronic inflammation/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. Like HBV and HCV, 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 is injected into gastric epithelial cells, where it has a variety of effects, including the initiation of a signaling cascade that mimics unregulated growth factor stimulation.

As mentioned above, H. pylori is associated with an increased risk for the development of gastric lymphomas as well. The gastric lymphomas are of B-cell origin, and because the transformed B cells normally reside in the marginal zones of lymphoid follicles, these tumors are also called MALT lymphomas (marginal zone–associated lymphomas; Chapter 12). 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 tumor necrosis factor (TNF). It is thought that H. pylori infection leads to the formation of H. pylori–reactive T cells, which in turn cause polyclonal B-cell proliferations. In time, a monoclonal B-cell tumor emerges in the proliferating B cells, perhaps as a result of accumulation of mutations in growth-regulatory genes. In keeping with this, early in the course of disease, eradication of H. pylori “cures” the lymphoma by removing antigenic stimulus for T cells.

SUMMARY

Helicobacter pylori

- H. pylori infection has been implicated in both gastric adenocarcinoma and MALT lymphoma.
- The mechanism of H. pylori–induced gastric cancers is multifactorial, including immunologically mediated chronic inflammation, stimulation of gastric cell proliferation, and production of reactive oxygen species that damage DNA. H. pylori pathogenicity genes, such as CagA, may also contribute by stimulating growth factor pathways.

- It is thought that H. pylori infection leads to polyclonal B-cell proliferations and that eventually a monoclonal B-cell tumor (MALT lymphoma) emerges as a result of accumulation of mutations.

HOST DEFENSE AGAINST TUMORS: TUMOR IMMUNITY

The idea that tumors are not entirely self was conceived by Ehrlich, who proposed that immune-mediated recognition of autologous tumor cells may be a “positive mechanism” capable of eliminating transformed cells. Subsequently, Lewis Thomas and McFarlane Burnet formalized this concept by coining the term immune surveillance to refer to recognition and destruction of non-self tumor cells on their appearance. That cancers occur implies that immune surveillance is imperfect; however, that some tumors escape such policing does not preclude the possibility that others may have been aborted. Here we address certain questions about tumor immunity: What is the nature of tumor antigens? What host effector systems may recognize tumor cells? Is tumor immunity effective against spontaneous neoplasms?

Tumor Antigens

Antigens that elicit an immune response have been demonstrated in many experimentally induced tumors and in some human cancers. Initially, they were broadly classified into two categories based on their patterns of expression: tumor-specific antigens, which are present only on tumor cells and not on any normal cells, and tumor-associated antigens, which are present on tumor cells and also on some normal cells. This classification, however, is imperfect, because many antigens thought to be tumor specific turned out to be expressed by some normal cells as well. The modern classification of tumor antigens is based on their molecular structure and source. An important advance in the field of tumor immunology was the development of techniques for identifying tumor antigens that were recognized by cytotoxic T lymphocytes (CTLs), because CTLs are the major immune defense mechanism against tumors. Recall that CTLs recognize peptides derived from cytoplasmic proteins that are displayed bound to class I major histocompatibility complex (MHC) molecules (Chapter 5). Below we describe the main classes of tumor antigens (Fig. 6–33).

Products of Mutated Oncogenes and Tumor Suppressor Genes. Neoplastic transformation, as we have discussed, results from genetic alterations, some of which may result in the expression of cell surface antigens that are seen as non-self by the immune system. Antigens in this category are derived from mutant oncoproteins and cancer suppressor proteins. Unique tumor antigens arise from products of β-catenin, RAS, p53, and CDK4 genes, which frequently are mutated in tumors. Because the mutant
proteins are present only in tumors, their peptides are expressed only in tumor cells. Since many tumors may carry the same mutation, such antigens are shared by different tumors. Although CTLs can be induced against such antigens, they do not appear to elicit protective responses in vivo.

**Products of Other Mutated Genes.** Because of the genetic instability of tumor cells, many genes are mutated in these cells, including genes whose products are not related to the transformed phenotype and have no known function. Products of these mutated genes are potential tumor antigens. These antigens are extremely diverse, because the carcinogens that induce the tumors may randomly mutagenize virtually any host gene. Mutated cellular proteins are found more frequently in chemical carcinogen- or radiation-induced animal tumors than in spontaneous human cancers. They can be targeted by the immune system, since there is no self-tolerance against them.

**Overexpressed or Aberrantly Expressed Cellular Proteins.** Tumor antigens may be normal cellular proteins that are abnormally expressed in tumor cells and elicit immune responses. In a subset of human melanomas some tumor antigens are structurally normal proteins that are produced at low levels in normal cells and overexpressed in tumor cells. One such antigen is tyrosinase, an enzyme involved in melanin biosynthesis that is expressed only in normal melanocytes and melanomas. T cells from melanoma patients recognize peptides derived from tyrosinase, raising the possibility that tyrosinase vaccines may stimulate such responses to melanomas; clinical trials with these vaccines are ongoing. It may be surprising that these patients are able to respond to a normal self-antigen. The probable explanation is that tyrosinase is normally produced in such small amounts and in so few cells that it is not recognized by the immune system and fails to induce tolerance.

Another group, the so called “cancer-testis” antigens, are encoded by genes that are silent in all adult tissues except the testis—hence their name. 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 MHC class I antigens. Thus, for all practical purposes, these antigens are tumor specific. Prototypic of this group is the MAGE family of genes. Although they are tumor specific, MAGE antigens are not unique for individual tumors. MAGE-1 is expressed on 37% of melanomas and a variable number of lung, liver, stomach, and esophageal carcinomas. Similar antigens called GAGE, BAGE, and RAGE have been detected in other tumors.
Tumor Antigens Produced by Oncogenic Viruses. As we have discussed, some viruses are associated with cancers. Not surprisingly, these viruses produce proteins that are recognized as foreign by the immune system. The most potent of these antigens are proteins produced by latent DNA viruses; examples in humans include HPV and EBV. There is abundant evidence that CTLs recognize antigens of these viruses and that a competent immune system plays a role in surveillance against virus-induced tumors because of its ability to recognize and kill virus-infected cells. Indeed, vaccines against HPV antigens have been found effective in prevention of cervical cancers in young females.

Oncofetal Antigens. Oncofetal antigens or embryonic antigens, such as carcinoembryonic antigen (CEA) and α-fetoprotein, are expressed during embryogenesis but not in normal adult tissues. Derepression of the genes that encode these antigens causes their reexpression in colon and liver cancers. Antibodies can be raised against these, and they are useful for detection of oncofetal antigens. Although, as discussed later, they are not entirely tumor specific, they can serve as serum markers for cancer.

Altered Cell Surface Glycolipids and Glycoproteins. Most human and experimental tumors express higher than normal levels and/or abnormal forms of surface glycoproteins and glycolipids, which may be diagnostic markers and targets for therapy. These altered molecules include gangliosides, blood group antigens, and mucins. Although most of the epitopes recognized by antibodies raised against such antigens are not specifically expressed on tumors, they are present at higher levels on cancer cells than on normal cells. This class of antigens is a target for cancer therapy with specific antibodies.

Several mucins are of special interest and have been the focus of diagnostic and therapeutic studies. These include CA-125 and CA-19-9, expressed on ovarian carcinomas, and MUC-1, expressed on breast carcinomas. Unlike many other types of mucins, MUC-1 is an integral membrane protein that is normally expressed only on the apical surface of breast ductal epithelium, a site that is relatively sequestered from the immune system. In ductal carcinomas of the breast, however, the molecule is expressed in an unpolarized fashion and contains new, tumor-specific carbohydrate and peptide epitopes. These epitopes induce both antibody and T-cell responses in cancer patients and are therefore being considered as candidates for tumor vaccines.

Cell Type–Specific Differentiation Antigens. Tumors express molecules that are normally present on the cells of origin. These antigens are called differentiation antigens, because they are specific for particular lineages or differentiation stages of various cell types. Their importance is as potential targets for immunotherapy and for identifying the tissue of origin of tumors. For example, lymphomas may be diagnosed as B-cell–derived tumors by the detection of surface markers characteristic of this lineage, such as CD10 and CD20. Antibodies against these molecules are also used for tumor immunotherapy. These differentiation antigens are typically normal self-antigens, and therefore they do not induce immune responses in tumor-bearing hosts.

Antitumor Effector Mechanisms

Cell-mediated immunity is the dominant anti-tumor mechanism in vivo. Although antibodies can be made against tumors, there is no evidence that they play a protective role under physiologic conditions. The cellular effectors that mediate immunity were described in Chapter 5, so it is necessary here only to characterize them briefly.

Cytotoxic T Lymphocytes. The role of specifically sensitized CTLs in experimentally induced tumors is well established. In humans, they seem to play a protective role, chiefly against virus-associated neoplasms (e.g., EBV-induced Burkitt lymphoma and HPV-induced tumors). The presence of MHC-restricted CD8+ cells that can kill autologous tumor cells within human tumors suggests that the role of T cells in immunity against human tumors may be broader than previously suspected. In some cases, such CD8+ T cells do not develop spontaneously in vivo but can be generated by immunization with tumor antigen-pulsed dendritic cells.

Natural Killer Cells. NK cells are lymphocytes that are capable of destroying tumor cells without prior sensitization; they may provide the first line of defense against tumor cells. After activation with IL-2, NK cells can lyse a wide range of human tumors, including many that seem to be nonimmunogenic for T cells. T cells and NK cells seem to provide complementary antitumor mechanisms. Tumors that fail to express MHC class I antigens cannot be recognized by T cells, but these tumors may trigger NK cells because the latter are inhibited by recognition of normal autologous class I molecules (Chapter 5). The triggering receptors on NK cells are extremely diverse and belong to several gene families. NKG2D proteins expressed on NK cells and some T cells are important activating receptors. They recognize stress-induced antigens that are expressed on tumor cells and cells that have incurred DNA damage and are at risk for neoplastic transformation.

Macrophages. Activated macrophages exhibit cytotoxicity against tumor cells in vitro. T cells, NK cells, and macrophages may collaborate in antitumor reactivity, because interferon-γ, a cytokine secreted by T cells and NK cells, is a potent activator of macrophages. Activated macrophages may kill tumors by mechanisms similar to those used to kill microbes (e.g., production of reactive oxygen metabolites; Chapter 2) or by secretion of tumor necrosis factor (TNF).

Humoral Mechanisms. Although there is no evidence for the protective effects of anti-tumor antibodies against spontaneous tumors, administration of monoclonal antibodies against tumor cells can be therapeutically effective. A monoclonal antibody against CD20, a B cell surface antigen, is widely used for treatment of certain non-Hodgkin lymphomas.

Immune Surveillance

Given the host of possible and potential antitumor mechanisms, is there any evidence that they operate in vivo to...
prevent the emergence of neoplasms? The strongest argument for the existence of immune surveillance is the increased frequency of cancers in immunodeficient hosts. About 5% of individuals with congenital immunodeficiencies develop cancers, a rate that is about 200 times that for individuals without such immunodeficiencies. Analogously, immunosuppressed transplant recipients and patients with acquired immunodeficiency syndrome have increased numbers of malignancies. It should be noted that most (but not all) of these neoplasms are lymphomas, often lymphomas of activated B cells. Particularly illustrative is X-linked lymphoproliferative disorder. When affected boys develop an EBV infection, such infection does not take the usual self-limited form of infectious mononucleosis but instead evolves into a chronic or sometimes fatal form of infectious mononucleosis or, even worse, malignant lymphoma.

Most cancers occur in individuals who do not suffer from any overt immunodeficiency. If immune surveillance exists, how do cancers evade the immune system in immunocompetent hosts? Several escape mechanisms have been proposed:

- **Selective outgrowth of antigen-negative variants.** During tumor progression, strongly immunogenic subclones may be eliminated.
- **Loss or reduced expression of histocompatibility molecules.** Tumor cells may fail to express normal levels of HLA class I, escaping attack by CTLs. Such cells, however, may trigger NK cells.
- **Immunosuppression.** Many oncogenic agents (e.g., chemicals and ionizing radiation) suppress host immune responses. Tumors or tumor products also may be immunosuppressive. For example, TGF-β, secreted in large quantities by many tumors, is a potent immunosuppressant. In some cases, the immune response induced by the tumor may inhibit tumor immunity. Several mechanisms of such inhibition have been described. For instance, recognition of tumor cells may lead to engagement of the T-cell inhibitory receptor, CTLA-4, or activation of regulatory T cells that suppress immune responses.

It is worth mentioning that although much of the focus in the field of tumor immunity has been on the mechanisms by which the host immune system defends against tumors, there is some recent evidence that, paradoxically, the immune system may promote the growth of tumors. It is possible that activated lymphocytes and macrophages produce growth factors for tumor cells. Enzymes, such as MMPs, that enhance tumor invasion, may also be produced. Harnessing the protective actions of the immune system and abolishing its ability to increase tumor growth is obviously an important goal of immunologists and oncologists.

**CLINICAL ASPECTS OF NEOPLASIA**

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. Indeed, both malignant and benign tumors may cause problems because of (1) location and impingement on adjacent structures, (2) functional activity such as hormone synthesis or the development of paraneoplastic syndromes, (3) bleeding and infections when the tumor ulcerates through adjacent surfaces, (4) symptoms that result from rupture or infarction, and (5) cachexia or wasting. The following discussion considers the effects of a tumor on the host, the grading and clinical staging of cancer, and the laboratory diagnosis of neoplasms.

**Effects of Tumor on Host**

Location is crucial in both benign and malignant tumors. A small (1-cm) pituitary adenoma can compress and destroy the surrounding normal gland and give rise to hypopituitarism. A 0.5-cm leiomyoma in the wall of the renal artery may lead to renal ischemia and serious hypertension. A comparably small carcinoma within the common bile duct may induce fatal biliary tract obstruction.

Hormone production is seen with benign and malignant neoplasms arising in endocrine glands. Adenomas and carcinomas arising in the β-cells of the islets of the pancreas can produce hyperinsulinism, sometimes fatal. Analogously, some adenomas and carcinomas of the adrenal cortex elaborate corticosteroids that affect the patient (e.g., aldosterone, which induces sodium retention, hypertension, and hypokalemia). Such hormonal activity is more likely with a well-differentiated benign tumor than with a corresponding carcinoma.

**SUMMARY**

**Immune Surveillance**

- Tumor cells can be recognized by the immune system as non-self 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, tumor antigens produced by oncogenic viruses, oncofetal antigens, altered glycolipids and glycoproteins, and cell type-specific differentiation antigens.
- Immunosuppressed patients have an increased risk of cancer.
- 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 secretion of factors (e.g., TGF-β) from the tumor.
A tumor may ulcerate through a surface, with consequent bleeding or secondary infection. Benign or malignant neoplasms that protrude into the gut lumen may become caught in the peristaltic pull of the gut, causing intussusception (Chapter 15) and intestinal obstruction or infarction.

**Cancer Cachexia**

Many cancer patients suffer progressive loss of body fat and lean body mass, accompanied by profound weakness, anorexia, and anemia, referred to as cachexia. There is some correlation between the size and extent of spread of the cancer and the severity of the cachexia. However, cachexia is not caused by the nutritional demands of the tumor. Although patients with cancer are often anorexic, current evidence indicates that cachexia results from the action of soluble factors such as cytokines produced by the tumor and the host rather than reduced food intake. In patients with cancer, calorie expenditure remains high, and basal metabolic rate is increased, despite reduced food intake. This is in contrast to the lower metabolic rate that occurs as an adaptational response in starvation. The basis of these metabolic abnormalities is not fully understood. It is suspected that TNF produced by macrophages in response to tumor cells or by the tumor cells themselves mediates cachexia. TNF suppresses appetite and inhibits the action of lipoprotein lipase, inhibiting the release of free fatty acids from lipoproteins. Additionally, a protein-mobilizing factor called proteolysis-inducing factor, which causes breakdown of skeletal muscle proteins by the ubiquitin-proteosome pathway, has been detected in the serum of cancer patients. Other molecules with lipolytic action also have been found. There is no satisfactory treatment for cancer cachexia other than removal of the underlying cause, the tumor.

**Paraneoplastic Syndromes**

Symptom complexes that occur in patients with cancer and that cannot be readily explained by local or distant spread of the tumor or by the elaboration of hormones indigenous to the tissue of origin of the tumor are referred to as paraneoplastic syndromes. They appear in 10% to 15% of patients with cancer, and it is important to recognize them for several reasons:

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

The paraneoplastic syndromes are diverse and are associated with many different tumors (Table 6–5). The most common syndromes are hypercalcemia, Cushing syndrome, and nonbacterial thrombotic endocarditis; the neoplasms most often associated with these and other syndromes are lung and breast cancers and hematologic malignancies. Hypercalcemia in cancer patients is multifactorial, but the most important mechanism is the synthesis of a parathyroid hormone–related protein (PTHrP) by tumor cells. Also implicated are other tumor-derived factors, such as TGF-α, a polypeptide factor that activates osteoclasts, and the active form of vitamin D. Another possible mechanism for hypercalcemia is widespread osteolytic metastatic disease of bone, but it should be noted that hypercalcemia resulting from skeletal metastases is not a paraneoplastic syndrome. Cushing syndrome as a paraneoplastic phenomenon is usually related to ectopic production of ACTH or ACTH-like polypeptides by cancer cells, as occurs in small-cell cancers of the lung. Sometimes one tumor induces several syndromes concurrently. For example, bronchogenic carcinomas may elaborate products identical to or having the effects of ACTH, antiuretic hormone, parathyroid hormone, serotonin, human chorionic gonadotropin, and other bioactive substances.

Paraneoplastic syndromes may also manifest as hypercoagulability leading to venous thrombosis and nonbacterial thrombotic endocarditis (Chapter 11). Other manifestations are clubbing of the fingers and hypertrophic osteoarthropathy in patients with lung carcinomas (Chapter 13). Still others are discussed in the consideration of cancers of the various organs of the body.

**Grading and Staging of Cancer**

Methods to quantify the probable clinical aggressiveness of a given neoplasm and its apparent extent and spread in the individual patient are necessary for making accurate prognosis and for comparing end results of various treatment protocols. For instance, the results of treating extremely small, highly differentiated thyroid adenocarcinomas that are localized to the thyroid gland are likely to be different from those obtained from treating highly anaplastic thyroid cancers that have invaded the neck organs.

The grading of a cancer attempts to establish some estimate of its aggressiveness or level of malignancy based on the cytologic differentiation of tumor cells and the number of mitoses within the tumor. The cancer may be classified as grade I, II, III, or IV, in order of increasing anaplasia. Criteria for the individual grades vary with each form of neoplasia and so are not detailed here. Difficulties in establishing clear-cut criteria have led in some instances to descriptive characterizations (e.g., “well-differentiated adenocarcinoma with no evidence of vascular or lymphatic invasion” or “highly anaplastic sarcoma with extensive vascular invasion”).

Staging of cancers is based on the size of the primary lesion, its extent of spread to regional lymph nodes, and the presence or absence of metastases. This assessment is usually based on clinical and radiographic examination (computed tomography and magnetic resonance imaging) and in some cases surgical exploration. Two methods of staging are currently in use: the TNM system (T, primary tumor; N, regional lymph node involvement; M, metastases) and the AJC (American Joint Committee) system. In the TNM system, T1, T2, T3, and T4 describe the increasing size of the primary lesion; N0, N1, N2, and
CHAPTER 6 Neoplasia

N3 indicate progressively advancing node involvement; and M0 and M1 reflect the absence or presence of distant metastases. In the AJC method, the cancers are divided into stages 0 to IV, incorporating the size of primary lesions and the presence of nodal spread and of distant metastases. Examples of the application of these two staging systems are cited in subsequent chapters. It is worth noting that when compared with grading, staging has proved to be of greater clinical value.

Table 6–5 Paraneoplastic Syndromes

<table>
<thead>
<tr>
<th>Clinical Syndromes</th>
<th>Major Forms of Underlying Cancer</th>
<th>Causal Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrinopathies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cushing syndrome</td>
<td>Small-cell carcinoma of lung</td>
<td>ACTH or ACTH-like substance</td>
</tr>
<tr>
<td></td>
<td>Pancreatic carcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neural tumors</td>
<td></td>
</tr>
<tr>
<td>Syndrome of inappropriate antidiuretic hormone secretion</td>
<td>Small-cell carcinoma of lung; intracranial neoplasms</td>
<td>Antidiuretic hormone or atrial natriuretic hormones</td>
</tr>
<tr>
<td>Hypercalcemia</td>
<td>Squamous cell carcinoma of lung</td>
<td>Parathyroid hormone-related protein, TGF-α, TNF, IL-1</td>
</tr>
<tr>
<td></td>
<td>Breast carcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Renal carcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adult T-cell leukemia/lymphoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ovarian carcinoma</td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>Fibrosarcoma</td>
<td>Insulin or insulin-like substance</td>
</tr>
<tr>
<td></td>
<td>Other mesenchymal sarcomas</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
<td></td>
</tr>
<tr>
<td>Carcinoid syndrome</td>
<td>Bronchial adenoma (carcinoid)</td>
<td>Serotonin, bradykinin</td>
</tr>
<tr>
<td></td>
<td>Pancreatic carcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gastric carcinoma</td>
<td></td>
</tr>
<tr>
<td>Polycythemia</td>
<td>Renal carcinoma</td>
<td>Erythropoietin</td>
</tr>
<tr>
<td></td>
<td>Cerebellar hemangioma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
<td></td>
</tr>
<tr>
<td>Nerve and Muscle Syndrome</td>
<td>Bronchogenic carcinoma</td>
<td>Immunologic</td>
</tr>
<tr>
<td>Myasthenia</td>
<td>Breast carcinoma</td>
<td></td>
</tr>
<tr>
<td>Disorders of the central and peripheral nervous systems</td>
<td>Bronchogenic carcinoma</td>
<td>Immunologic</td>
</tr>
<tr>
<td>Dermatologic Disorders</td>
<td>Gastric carcinoma</td>
<td>Immunologic; secretion of epidermal growth factor</td>
</tr>
<tr>
<td>Acanthosis nigricans</td>
<td>Lung carcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uterine carcinoma</td>
<td></td>
</tr>
<tr>
<td>Dermatomyositis</td>
<td>Bronchogenic, breast carcinoma</td>
<td>Immunologic</td>
</tr>
<tr>
<td>Osseous, Articular, and Soft-Tissue Changes</td>
<td>Bronchogenic carcinoma</td>
<td>Unknown</td>
</tr>
<tr>
<td>Hypertrophic osteoarthropathy and clubbing of the fingers</td>
<td>Bronchogenic carcinoma</td>
<td>Unknown</td>
</tr>
<tr>
<td>Vascular and Hematologic Changes</td>
<td>Pancreatic carcinoma</td>
<td>Tumor products (mucins that activate clotting)</td>
</tr>
<tr>
<td>Venous thrombosis (Trousseau phenomenon)</td>
<td>Bronchogenic carcinoma</td>
<td>Other cancers</td>
</tr>
<tr>
<td>Nonbacterial thrombotic endocarditis</td>
<td>Advanced cancers</td>
<td>Hypercoagulability</td>
</tr>
<tr>
<td>Anemia</td>
<td>Thymic neoplasms</td>
<td>Unknown</td>
</tr>
<tr>
<td>Others</td>
<td>Nephrotic syndrome</td>
<td>Tumor antigens, immune complexes</td>
</tr>
<tr>
<td></td>
<td>Various cancers</td>
<td></td>
</tr>
</tbody>
</table>

ACTH, adrenocorticotropic hormone; TGF, transforming growth factor; TNF, tumor necrosis factor; IL, interleukin.

SUMMARY

Clinical Aspects of Tumors
- Cachexia, defined by progressive loss of body fat and lean body mass, accompanied by profound weakness, anorexia, and anemia, is caused by release of cytokines by the tumor or host.
Laboratory Diagnosis of Cancer

Morphologic Methods

In most instances, the laboratory diagnosis of cancer is not difficult. The two ends of the benign–malignant spectrum pose no problems; however, in the middle lies a "no man's land" where the wise tread cautiously. Clinicians tend to underestimate the contributions they make to the diagnosis of a neoplasm. Clinical data are invaluable for optimal pathologic diagnosis. Radiation-induced changes in the skin or mucosa can be similar to those of cancer. Sections taken from a healing fracture can mimic an osteosarcoma. The laboratory evaluation of a lesion can be only as good as the specimen submitted for examination. The specimen must be adequate, representative, and properly preserved.

Several sampling approaches are available, including excision or biopsy, fine-needle aspiration, and cytologic smears. When excision of a lesion is not possible, selection of an appropriate site for biopsy of a large mass requires awareness that the margins may not be representative and the center may be largely necrotic. Analogously with disseminated lymphoma (i.e., involving many nodes), nodes in the inguinal region that drain large areas of the body often undergo reactive changes that may mask neoplastic involvement. Requesting frozen-section diagnosis is sometimes desirable, as, for example, in determining the nature of a mass lesion or in evaluating the regional lymph nodes in a patient with cancer for metastasis. This method, in which a sample is quick-frozen and sectioned, permits histologic evaluation within minutes. In experienced, competent hands, frozen-section diagnosis is accurate, but there are particular instances in which the better histologic detail provided by the more time-consuming routine methods is needed. In such instances, it is better to wait a few days, despite the drawbacks, than to perform inadequate or unnecessary surgery.

Fine-needle aspiration of tumors is another approach that is widely used. It involves aspiration of cells from a mass, followed by cytologic examination of the smear. This procedure is used most commonly with readily palpable lesions affecting the breast, thyroid, lymph nodes, and salivary glands. Modern imaging techniques permit extension of the method to deeper structures, such as the liver, pancreas, and pelvic lymph nodes. It obviates surgery and its attendant risks. Although it entails some difficulties, such as small sample size and sampling errors, in experienced hands it can be extremely reliable, rapid, and useful.

Cytologic (Papanicolaou) smears provide another method for the detection of cancer. Historically, this approach has been used widely for the discovery of carcinoma of the cervix, often at an in situ stage, but now it is used with many other forms of suspected malignancy, such as endometrial carcinoma, bronchogenic carcinoma, bladder and prostate tumors, and gastric carcinomas; for the identification of tumor cells in abdominal, pleural, joint, and cerebrospinal fluids; and, less commonly, with other forms of neoplasia. Neoplastic cells are less cohesive than others and so are shed into fluids or secretions (Fig. 6–34). The shed cells are evaluated for features of anaplasia indicative of their origin from a tumor. The gratifying control of cervical cancer is the best testament to the value of the cytologic method.

Figure 6–34

A, Normal Papanicolaou smear from the uterine cervix. Large, flat cells with small nuclei. B, Abnormal smear containing a sheet of malignant cells with large hyperchromatic nuclei. There is nuclear pleomorphism, and one cell is in mitosis. There are few interspersed neutrophils with compact lobated nuclei and much smaller size. (Courtesy of Dr. Richard M. DeMay, Department of Pathology, University of Chicago, Chicago, Illinois.)
Immunocytochemistry offers a powerful adjunct to routine histology. Detection of cytokeratin by specific monoclonal antibodies labeled with peroxidase points to a diagnosis of undifferentiated carcinoma rather than large-cell lymphoma. Similarly, detection of prostate-specific antigen (PSA) in metastatic deposits by immunohistochemistry allows definitive diagnosis of a primary tumor in the prostate. Immunocytochemical detection of estrogen receptors allows prognostication and directs therapeutic intervention in breast cancers.

Flow cytometry is used routinely in the classification of leukemias and lymphomas. In this method, fluorescent antibodies against cell surface molecules and differentiation antigens are used to obtain the phenotype of malignant cells.

Tumor Markers

Biochemical assays for tumor-associated enzymes, hormones, and other tumor markers in the blood cannot be utilized for definitive diagnosis of cancer; however, they contribute to finding cancers and in some instances are useful in determining the effectiveness of therapy or the appearance of a recurrence. The application of these assays is considered with many of the specific forms of neoplasm discussed in other chapters, so only a few examples suffice here. PSA, used to screen for prostatic adenocarcinoma, may be one of the most used, and most successful, tumor markers in clinical practice. Prostatic carcinoma can be suspected when elevated levels of PSA are found in the blood. However, PSA screening also highlights problems encountered by virtually every tumor marker. Although PSA levels are often elevated in cancer, PSA levels also may be elevated in benign prostatic hyperplasia (Chapter 18). Furthermore, there is no PSA level that ensures that a patient does not have prostate cancer. Thus, the PSA test suffers from both low sensitivity and low specificity. Other tumor markers occasionally used in clinical practice include carcinoembryonic antigen (CEA), which is elaborated by carcinomas of the colon, pancreas, stomach, and breast, and α-fetoprotein, which is produced by hepatocellular carcinomas, yolk sac remnants in the gonads, and occasionally teratocarcinomas and embryonal cell carcinomas. Unfortunately, like PSA, both of these markers can be produced by a variety of non-neoplastic conditions as well. Thus, CEA and α-fetoprotein assays lack both specificity and sensitivity required for the early detection of cancers. They are still particularly useful in the detection of recurrences after excision. With successful resection of the tumor, these markers disappear from the serum; their reappearance almost always signifies the beginning of the end. CEA is further discussed in Chapter 15 and α-fetoprotein in Chapter 16.

Molecular Diagnosis

An increasing number of molecular techniques are being used for the diagnosis of tumors and for predicting their behavior.

1. Diagnosis of malignancy. Because each T and B cell has unique rearrangement of its antigen receptor genes, polymerase chain reaction (PCR)–based detection of T-cell receptor or immunoglobulin genes allows distinction between monoclonal (neoplastic) and polyclonal (reactive) proliferations. Many hematopoietic neoplasms, and a few solid tumors, are defined by particular translocations, and thus the diagnosis can be made by detection of such translocations. For example, fluorescence in situ hybridization (FISH) or PCR (Chapter 7) can be used to detect translocations characteristic of Ewing sarcoma and several leukemias and lymphomas. PCR-based detection of BCR-ABL transcripts provides the molecular diagnosis of chronic myeloid leukemia.

2. Prognosis and behavior. Certain genetic alterations are associated with a poor prognosis, and thus the presence of these alterations determines the patient’s subsequent therapy. FISH and PCR methods can be used to detect amplification of oncogenes such as HER-2/NEU and N-MYC, which provide prognostic and therapeutic information for breast cancers and neuroblastomas.

3. Detection of minimal residual disease. Another emerging use of molecular techniques is detection of minimal residual disease after treatment. For example, detection of BCR-ABL transcripts by PCR gives a measure of residual disease, in patients treated for chronic myeloid leukemia.

4. Diagnosis of hereditary predisposition to cancer. Germ-line mutation of several tumor suppressor genes, such as BRCA1, increases a patient’s risk of developing certain types of cancer. Thus, detection of these mutated alleles may allow the patient and physician to devise an aggressive screening protocol, as well as to consider prophylactic surgery. In addition, such detection allows genetic counseling of relatives at risk.

Molecular Profiling of Tumors

One of the most exciting advances in the molecular analysis of tumors has been made possible by DNA-microarray analysis. This technique allows simultaneous measurement of the expression levels of several thousand genes. The principle of this so-called gene chip technology is illustrated in Figure 6–35 and described briefly here.

As can be seen, the process begins by extraction of mRNA from any two sources (e.g., normal and malignant, normal and preneoplastic, or two tumors of the same histologic type). cDNA copies of the mRNA are synthesized in vitro with fluorescently labeled nucleotides. The fluorescence-labeled cDNA strands are hybridized to sequence-specific DNA probes linked to a solid support, such as a silicon chip. A 1-cm² chip can contain thousands of probes arranged in an array of columns and rows. After hybridization, high-resolution laser scanning detects fluorescent signals from each of the spots. The fluorescence intensity of each spot is proportional to the level of expression of the original mRNA used to synthesize the cDNA hybridized to that spot. For each sample, therefore, the expression level of thousands of genes is obtained, and by using bioinformatic tools,
the relative levels of gene expression in different samples can be compared. In essence, a molecular profile is generated for each tissue analyzed.

Such analysis has revealed that phenotypically identical large B-cell lymphomas (Chapter 12) from different patients are heterogeneous with respect to their gene expression. Nevertheless, clusters of gene expression patterns can be detected that allow segregation of phenotypically similar tumors into distinct subcategories with dramatically different survival rates. This type of molecular profiling indicates that the currently available morphologic and molecular tools are insufficient for stratification of tumors into prognostically different subgroups. Similar analyses have been performed on breast cancers and melanomas. Although the data currently available have to be validated by prospective analysis of a larger cohort of patients, the proof of principle has been obtained. It is likely that, in the near future, molecular profiling will become an adjunct in the diagnosis, classification, and management of cancer. This type of analysis may also reveal novel gene targets for development of new drugs. Thus, therapy may be tailored to the specific genes dysregulated in a given tumor. Who knows, advertisements for “designer genes” may appear side by side with ads for “designer jeans”!

### SUMMARY

**Laboratory Diagnosis of Cancer**

- Several sampling approaches exist for the diagnosis of tumors, including excision, biopsy, fine-needle aspiration, and cytologic smears.
- Immunohistochemistry and flow cytometry help in the diagnosis and classification of tumors, because distinct protein expression patterns define different entities.
- Proteins released by tumors into the serum, such as PSA, can be used to screen populations for cancer and to monitor recurrence following treatment.
- Molecular analyses are used to determine diagnosis, prognosis, the detection of minimal residual disease, and the diagnosis of hereditary predisposition to cancer.
- Molecular profiling of tumors by cDNA arrays can determine expression of large segments of the genome at once and can be useful in molecular stratification of otherwise identical tumors for the purpose of treatment and prognostication.

### BIBLIOGRAPHY

Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 100:57, 2000. [An excellent, brief account of the fundamental properties of cancer and their molecular basis. The organization of genetic changes in cancer is based on this article.]


