CHAPTER 6

Neoplasia and Tumor Biology[1]

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Key Readings Index

Neoplasia is an important concern for veterinary practitioners, diagnosticians, and researchers. Tumor diagnosis and treatment for individual animals is becoming an increasingly prominent part of small animal practice. In farm animals, neoplasms caused by infectious or environmental agents can have a major impact on herd or flock health and result in economic losses due to carcass or organ condemnation. Furthermore, animal models of neoplasia provide important insights into the cause and treatment of cancer in human beings.

General Nomenclature

Neoplasia

Neoplasia is a process of "new growth" in which normal cells undergo irreversible genetic changes, which render them unresponsive to ordinary controls on growth exerted from within the "transformed" cell or by surrounding "normal" cells. With continued proliferation the cells expand beyond their normal anatomic boundaries, creating a macroscopically (grossly) or microscopically detectable *neoplasm*. Other common terms for neoplasms, such as *tumor* ("swelling") or *cancer* ("crab"), describe the clinical appearance or infiltrative behavior of these abnormal growths. In fact, *oncology*, the study of neoplasia, is derived from the Greek word *oncos* ("tumor"). Although the terms "neoplasm" and "tumor" may refer to either benign or malignant growths, the term "cancer" always denotes a malignant growth. It is important to note that a gross lesion described clinically as a "tumor" or "mass" may be a neoplasm or a nonneoplastic lesion like a granuloma.

Benign ("harmless") tumors do not invade surrounding tissue or spread to new anatomic locations within the body; thus these tumors are usually curable and are rarely responsible for death of the animal. *Malignant* ("harmful") tumors, if left untreated, invade locally, spread by *metastasis* ("change of place"), and ultimately kill the animal by interfering with critical body functions. Although nervous system tumors are often localized and very rarely metastasize, they may cause clinical signs and death by interrupting important neurologic pathways via compression of axons or critical clusters of neuron cell bodies (see Chapter 14).

Preneoplastic Changes

With the recognition that tumor development is a stepwise process, potentially preneoplastic changes, including hyperplasia, hypertrophy, metaplasia, and dysplasia, have assumed new diagnostic and clinical significance (Fig. 6-1). These preneoplastic changes often signal an increased risk or likelihood for progression to neoplasia in the affected tissue. *Hyperplasia* is an increase in the number of cells in a tissue through mitotic division of cells, in other words, through cellular proliferation. It must be distinguished from *hypertrophy*, which is an increase in individual cell size through the addition of cytoplasm (cytosol) and associated organelles. *Metaplasia*, the transformation of one differentiated cell type into another, is seen most commonly in epithelial tissues. For example, in several species of animals, vitamin A deficiency is characterized by transformation of columnar or cuboidal respiratory and digestive epithelium into squamous epithelium (squamous metaplasia). *Dysplasia* is an abnormal pattern of tissue growth and usually refers to disorderly arrangement of cells within the tissue.

In general, preneoplastic changes are reversible. They may arise in response to physiologic demands, injury, or irritation but often resolve with the removal of the inciting factor. For example, epidermal hyperplasia is a normal part of wound repair, and skeletal muscle

[1]For a glossary of abbreviations and terms used in this chapter see E-Glossary 6-1.

E-Glossary 6-1 Glossary of Terms

Adaptive immune system–Cell-mediated and humoral immune responses directed against specific antigens
Adenocarcinoma–A malignant tumor of glandular epithelial origin with a tubular or acinar microscopic growth pattern
Adenoma–A benign tumor of epithelial origin either arising from a gland or characterized by a tubular growth pattern
Amplification–A mutational process resulting in the presence of multiple copies of a DNA sequence
Amyloid–An amorphous eosinophilic substance composed of abnormal proteins arranged in β-pleated fibrils that is sometimes found in tumor stroma
Anaplasia–Loss of cellular differentiation indicative of neoplasia; also termed *atypia*
Anaplastic neoplasm–A tumor in which cells do not resemble any normal cell type and for which the issue of origin cannot be determined; also termed *undifferentiated neoplasm*
Aneuploidy–An abnormal number of chromosomes
Angiogenesis–Formation of new blood vessels
Angiogenic switch–Change that occurs when tumor cells acquire the ability to induce and sustain new tumor vasculature
Antibody-dependent cell-mediated cytotoxicity (ADCC)–A mechanism of immune attack carried out by macrophages and natural killer (NK) cells armed with tumor-specific antibodies bound by their constant regions
Apoptosis–Programmed cell death
Atypia–Loss of cellular differentiation indicative of neoplasia; also termed *anaplasia*
Autophagy–Autodigestion of cell organelles within phagosomes
Benign tumor–A tumor that does not invade surrounding tissue or metastasize
B lymphocyte–A lymphocyte that produces antibodies
Cachexia–Cancer-related muscle wasting and fat loss with associated debility
Cancer–A malignant neoplasm; also termed *neoplasm* or *tumor*
Cancer syndrome–Spectrum of tumors associated with a specific genetic mutation carried by related individuals
Capsule–Connective tissue structure surrounding a tumor
Carcinogen–An agent known to cause cancer
Carcinogenesis–Process by which tumors develop as the result of successive genetic and epigenetic changes; also termed *neoplastic transformation*; sometimes used synonymously with *stepwise tumor development* or *multistage carcinogenesis*
Carcinoma–A malignant neoplasm of epithelial origin
Carcinoma in situ–A preinvasive epithelial malignancy that has not yet penetrated the epithelial basement membrane; also termed *intraepithelial neoplasia*
Carcinomatosis–A condition in which a carcinoma widely disseminates within a body cavity
Cellular senescence–Permanent cellular growth arrest in the G_1 phase of the cell cycle
Choristoma–A normal tissue found in an abnormal location, such as a dermoid cyst
Clonal–Derived from a single transformed cell
Clonally transmissible cancers–Tumors that can spread to other individuals via direct physical contact with the original host
Clonal selection–The process by which tumor cells with a selective advantage come to predominate in a tumor; also termed *tumor evolution*
Complete carcinogen–A carcinogen capable of both initiating and promoting tumorigenesis
Conditional gene expression–Experimentally controlled gene expression at a specific time or location
Cytolysis–Immune cell-mediated induction of cell death in a target cell
Cytotoxic T lymphocyte (CTL)–A T lymphocyte that recognizes and kills tumor cells
Deletion–A mutational change consisting of loss of a genomic DNA segment
Desmoplastic response–Extensive nonneoplastic fibrous connective tissue growth in response to tumor cell factors; also termed *scirrhous* response
Differentiation antigen–An antigen expressed by normal cells only at specific times during cellular differentiation but which may be reexpressed by tumor cells
Direct-acting carcinogen–A carcinogen that is effective in the form in which it enters the body and does not require metabolic activation
Disseminated intravascular coagulation (DIC)–A paraneoplastic condition characterized by widespread intravascular coagulation and consumption of clotting factors
DNA methylation–Addition of a methyl group to carbon 5 of cytosine in DNA
Dormancy–A state of arrested proliferation of metastatic tumor cells
Driver mutation–A mutation in an oncogene or tumor suppressor gene causally linked to carcinogenesis
Dysplasia–Abnormal or disorganized tissue growth; often a preneoplastic change
Ectopic hormone production–The formation of hormonally active substances by nonendocrine tumor tissue
Epigenetic–A term describing a change in gene expression not due to an altered DNA sequence but to reversible processes like DNA methylation or histone modification; may be heritable
Epithelial-mesenchymal transition (EMT)–Acquisition of mesenchymal cell characteristics by epithelial cells; characterized by loss of intercellular adhesion, enhanced cell motility, and increased cell invasiveness
Euchromatin–DNA wound around histones in a loosely packed configuration that allows gene transcription
Extracellular matrix (ECM)–An aggregate of proteins and glycoproteins embedded in a matrix of proteoglycans that constitutes a large proportion of tumor stroma
Extravasation–The process by which intravascular tumor cells exit the vasculature and enter the extracellular space
Frameshift mutation–A mutation that results in alteration of the reading frame during protein synthesis
Germline mutation–A genomic mutation that is present in all cells of the body and can be transmitted to offspring; germline mutations in oncogenes or tumor suppressor genes may be responsible for *cancer syndromes*
Global hypomethylation–Lowered levels of genome methylation, which is often seen in cancer cells
Grade–A measure of how similar or dissimilar a neoplastic cell population is to its normal counterpart; may be used to determine treatment plan and prognosis
Hamartoma-–Mature but disorganized tissue found in its normal anatomic location
Haploinsufficiency–Inactivation of only one allele of a gene within a cell
Heritable–Passed from a cell or animal to its progeny
Heterochromatin–DNA wound around histones in an closed configuration that prevents gene transcription
Hit-and-run–A poorly understood mechanism by which some viruses initiate cancer but do not persist in the host cell
Humoral hypercalcemia of malignancy (HHM)–A paraneoplastic condition due to secretion of parathyroid hormone–related peptide by tumor cells

Continued

E-Glossary 6-1 Glossary of Terms–cont'd

Hyperchromatic–Describes nuclei that stain darkly due to increased DNA content
Hypermethylation–Increased methylation of genes that can paradoxically occur concurrently with global hypomethylation in tumor cell genomes
Hyperplasia–Increase in cell numbers through cell proliferation; may be a preneoplastic change
Hypertrophic osteoarthropathy–A paraneoplastic condition characterized by extensive new bone growth that is often associated with masses in the thoracic cavity
Hypertrophy–Increase in individual cell size through addition of cytoplasm and organelles
Hyperviscosity syndrome–A paraneoplastic condition characterized by marked hyperproteinemia
Immunologic synapse–A temporary structure formed at the interface between a natural killer cell (NK cell) or cytotoxic lymphocyte (CTL) and a tumor target cell
Immunosurveillance–Immune cell-mediated recognition and destruction of tumor cells with altered antigenicity
Imprinting–An epigenetic change resulting in expression of only the maternal or paternal allele of a gene
Indirect-acting carcinogen–A procarcinogen that requires metabolic activation to form a carcinogen
Initiating agent–A chemical or physical carcinogen that induces genetic alterations that drive carcinogenesis; also termed an *initiator*
Initiation–Introduction of an irreversible genetic change in a normal cell that gives the initiated cell a growth advantage; first step in carcinogenesis
Initiator–A chemical or physical carcinogen that induces genetic alterations that drive carcinogenesis; also termed an *initiating agent*
Innate immune system–Nonspecific immune responses that serve as the first line of defense against cells or organisms recognized as foreign
Insertion–A mutational change consisting of the addition of a DNA segment to the genome
Insertional mutagenesis–The mechanism by which some retroviruses lacking viral oncogenes drive carcinogenesis by enhancing the transcription of cellular oncogenes
Intravasation–The process by which tumor cells enter blood or lymphatic vessels
Karyotype–The number and arrangement of condensed chromosomes in a cell
Knockout mouse–A mouse in which a functional gene has been experimentally inactivated
Latent period–The interval of tumor growth before a tumor becomes clinically detectable
Leukemia–Cancer arising from blood cells or hematopoietic precursors
Lymphangiogenesis–Formation of new lymphatic vessels
Macrophage–A phagocytic cell that constitutes part of the innate immune system
Malignant progression–Development of increasingly aggressive behavior in a malignant tumor due to a combination of genetic and epigenetic changes
Malignant transformation–The process by which a benign tumor evolves into a malignant tumor
Malignant tumor–A neoplasm that is locally invasive or metastatic
Maturation arrest–Blockade of normal differentiation at an immature stage; characteristic of some tumors
Membrane attack complex (MAC)–A complement-derived complex that mediates antibody-mediated killing of tumor cells
Metaplasia–Change from one differentiated cell type to another; often a preneoplastic change
Metastasis–Tumor spread to sites in the body distant from the original tumor location
MicroRNA–Small noncoding RNA that posttranscriptionally regulates the expression of other genes
Mismatch repair–A DNA repair mechanism that locates and repairs single nucleotide mismatches between complementary DNA strands
Mitotic index–The average number of neoplastic cells with mitotic figures within a 400× power microscopic field
Mixed tumor–A neoplasm that contains multiple cell types
Modifier genes–Genes that do not drive cancer development but that influence cancer susceptibility or outcome; also called *quantitative trait loci*
Monosomy–A condition in which only a single copy of a chromosome is present in a cell
Multicentric–Arising at multiple sites throughout the body
Multistage carcinogenesis–The process by which tumors develop as the result of successive genetic and epigenetic changes; also termed *stepwise tumor development*; the less specific terms *carcinogenesis* or *neoplastic transformation* are sometimes used synonymously
Mutagen–Agent that gives rise to genetic mutations
Mutation–Heritable alteration in DNA sequence
Mutation fixation–Reproduction of an altered base sequence in subsequent rounds of genetic replication
Myelofibrosis–A paraneoplastic condition characterized by overgrowth of nonneoplastic fibroblasts in the bone marrow
Neoplasia–The process by which normal cells undergo irreversible genetic changes that render them unresponsive to ordinary growth controls
Neoplasm–Grossly or microscopically detectable mass composed of benign or malignant neoplastic cells; also termed *tumor* or *cancer*; the term *cancer* is applied only to malignant neoplasms
Neoplastic transformation–The process by which tumors develop as the result of successive genetic and epigenetic changes; also termed *carcinogenesis*; sometimes used synonymously with *stepwise tumor development* or *multistage carcinogenesis*
Noncoding RNA–An RNA transcript that does not encode a protein
Nucleotide excision repair–A DNA repair process involving removal of a DNA lesion and surrounding nucleotides followed by resynthesis of the excised DNA segment
Oncofetal antigen–Embryonic antigens not normally expressed in adult tissue but that may be reexpressed in tumors
Oncogene–A gene that, when activated, allows cellular proliferation unresponsive to normal growth inhibitory signals, resulting in tumor formation
Oncogenic virus–A virus that causes cancer
Oncology–The study of neoplasia
Papilloma–A benign tumor of epithelial origin that typically extends above tissue surface in an exophytic growth pattern; sometimes used interchangeably with *polyp*
Paraneoplastic syndrome–Systemic clinical signs caused by release of tumor cell products
Parenchyma–The neoplastic cell population within a tumor
Pleomorphism–Variation in cell or nuclear size and shape that is often seen in neoplastic cells
Polyp–Benign tumor of epithelial origin; sometimes used interchangeably with *papilloma*
Progression–Transition from a benign to a malignant neoplasm due to ongoing genetic and epigenetic changes; third step in carcinogenesis
Promoters–Nonmutagenic stimuli that drive overgrowth of initiated cells during tumor development

E-Glossary 6-1 Glossary of Terms–cont'd

Promotion–The process by which cells with irreversible genetic damage (initiated cells) outgrow surrounding cells; second step in carcinogenesis
Proto-oncogene–A normal cellular gene that regulates cell growth and differentiation but that can be converted into an oncogene by overexpression, mutation, or viral transduction
Quantitative trait loci–Genes that do not drive cancer development but that influence cancer susceptibility or outcome; also called *modifier genes*
Regulatory T cell (T reg)–T lymphocyte that induces tolerance
Sarcoma–Malignant tumor of mesenchymal origin
Scirrhous response–Extensive nonneoplastic fibrous connective tissue growth in response to tumor cell factors; also termed *desmoplastic response*
Somatic mutation–A mutation confined to an individual cell and its progeny; somatic mutations are found in sporadic tumors
Stage–Indication of extent of tumor growth and spread throughout the body; used to determine treatment plan and prognosis
Stepwise tumor development–The process by which tumors develop as the result of successive genetic and epigenetic changes; also termed *multistage carcinogenesis*; the less specific terms *carcinogenesis* or *neoplastic transformation* are sometimes used synonymously
Stroma–The nonneoplastic supporting tissues within a tumor, including connective tissue and blood vessels
Tissue-specific antigen–Antigen shared by tumors and the normal tissues from which they originate
Transgene–An exogenous gene experimentally introduced into the mouse genome
Translocation–A mutation that occurs when pieces of two separate chromosomes break off and reattach inappropriately
Trisomy–A condition in which three copies of a chromosome are present in a cell
Tumor–A relatively nonspecific term describing a tissue swelling; may refer to benign, malignant, or even nonneoplastic masses; also termed *neoplasm* or *cancer*
Tumor antigen–A protein, glycoprotein, glycolipid, or carbohydrate expressed on the tumor cell surface
Tumor-associated antigen–An antigen present on both tumor and normal cells
Tumor evolution–The process by which tumor cells with a selective advantage come to predominate in a tumor; also termed *clonal selection*
Tumor giant cell–Large tumor cell with a very large nucleus
Tumor-specific antigen–A tumor antigen expressed only on tumor cells, not on nonneoplastic cells
Tumor-specific shared antigen–An antigen encoded by genes that have very limited expression in adult tissue but that are expressed by many types of tumor tissue
Tumor suppressor gene–A gene that ordinarily prevents uncontrolled cellular proliferation; inactivation of tumor suppressor genes contributes to tumor development
"Two-hit" hypothesis–The theory that both alleles of a tumor suppressor gene must be inactivated to trigger carcinogenesis
Undifferentiated neoplasm–A tumor in which cells do not resemble any normal cell type and for which the issue of origin cannot be determined; also termed *anaplastic neoplasm*

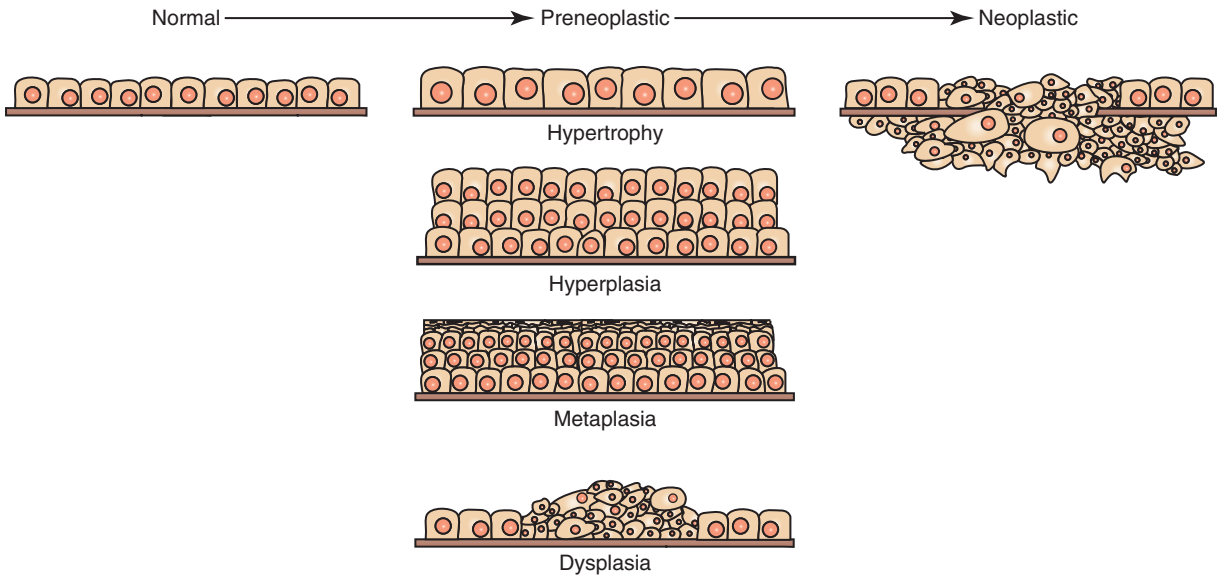


Figure 6-1 Preneoplastic Changes Preceding Tumor Emergence. Preneoplastic changes in tissues include alterations in cell size (hypertrophy), cell number (hyperplasia), and organization (metaplasia, dysplasia). In this example, preneoplastic changes are illustrated in simple cuboidal epithelium, although such changes may also be seen in other epithelial and mesenchymal tissue types. The metaplastic change shown is squamous metaplasia, that is, the conversion of simple cuboidal epithelium into stratified squamous epithelium. (Redrawn with permission from Dr. D.F. Kusewitt, Health Sciences Center, University of New Mexico.)

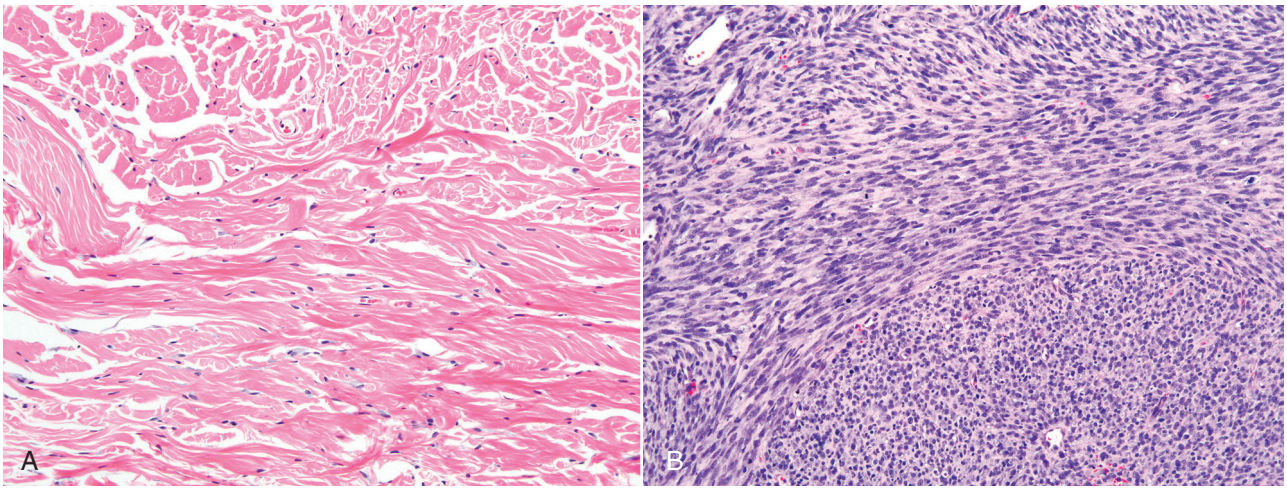


Figure 6-2 Comparison of Benign and Malignant Tumors of Fibroblast Origin. A, Fibroma, subcutis, dog. The benign fibroma is composed primarily of mature collagenous connective tissue with relatively few neoplastic fibroblasts that are indistinguishable from normal fibroblasts. H&E stain. **B,** Fibrosarcoma, subcutis, dog. The fibrosarcoma is composed of interlacing bundles of large fibroblasts with plump elongate nuclei and moderate amounts of eosinophilic cytoplasm; mature collagen is sparse to absent. H&E stain. (Courtesy College of Veterinary Medicine, University of Tennessee.)

hypertrophy is an adaptive response to increased workload. The terms "hyperplasia" and "hypertrophy" are not appropriate in descriptions of true neoplasms, but the terms "dysplasia" and "metaplasia" may describe changes that persist during the transition from preneoplasia to neoplasia. *Anaplasia* is the term used to describe loss of cellular differentiation and reversion to more primitive cellular morphologic features; anaplasia often indicates irreversible progression to neoplasia.

Tumor Types

Microscopically, most tumors consist of a single cell type, either mesenchymal or epithelial, and the name of the neoplasm reflects the cell type from which the tumor is thought to arise.

Mesenchymal Tumors

Mesenchymal tumors arise from cells of embryonic mesodermal origin. These tumors are generally composed of spindle cells arranged in streams and bundles. Benign tumors originating from mesenchymal cells are usually named by adding the suffix *-oma* to the name of the cell of origin. Thus a fibroma is a benign tumor of fibroblast origin (Fig. 6-2, *A*). A malignant tumor of mesenchymal origin is a *sarcoma* ("fleshy growth"). A prefix or modifier indicates the tissue of origin. For example, a fibrosarcoma is a tumor composed of malignant fibroblasts (see Fig. 6-2, *B*). The cells of the hematopoietic system are also mesenchymal; thus tumors arising from these cells are sarcomas. For instance, a malignant tumor of lymphocytes is called lymphosarcoma; by convention, lymphosarcoma is often

shortened to lymphoma, but this term should not be mistaken for the name of a benign mesenchymal growth. These solid sarcomas of hematopoietic cell origin are composed of sheets of round cells (Fig. 6-3). Malignancies arising from circulating blood cells or their precursors are termed *leukemias* ("white blood") when characterized by large numbers of abnormal hematopoietic cells in the peripheral blood.

Epithelial Tumors

All three embryonic cell layers, endoderm, mesoderm, and ectoderm, can give rise to epithelial tissues and tumors derived from these tissues.

The terms *adenoma*, *papilloma*, and *polyp* refer to benign epithelial tumors. "Adenoma" denotes either a tumor arising from glandular epithelium like mammary epithelium or a tumor derived from nonglandular epithelial tissue that exhibits a tubular pattern microscopically, such as a renal tubular adenoma. The term "papilloma" refers to a benign, usually exophytic ("growing outward"), growth arising from a cutaneous or mucocutaneous surface, whereas a "polyp" is a grossly visible, benign epithelial tumor projecting from a mucosal surface (Fig. 6-4, *A*); however, the terms "polyp" and "papilloma" are sometimes used interchangeably.

All malignant tumors of epithelial origin are termed *carcinomas* ("cancers"). Tumors termed "carcinomas" may contain nests, cords, or islands of neoplastic epithelial cells, whereas the more specific term *adenocarcinoma* refers to carcinomas with a distinct glandular growth pattern, as indicated by the presence of tubules or acini (see Fig. 6-4, *B*). By definition, carcinomas are invasive and have the

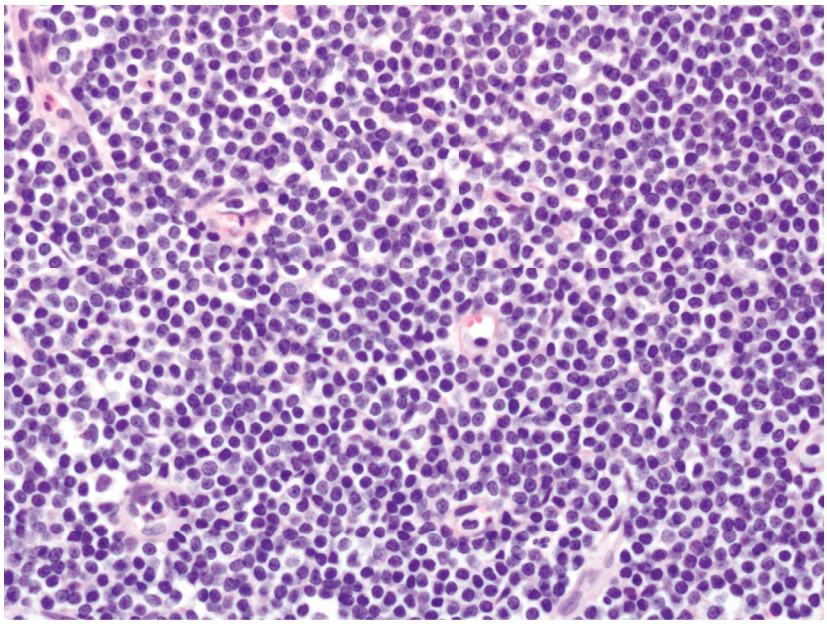


Figure 6-3 Lymphoma (Lymphosarcoma), Lymph Node, Dog. The tumor is composed of a solid sheet of neoplastic round cells (lymphocytes). The neoplastic cells are monomorphic, meaning that there is little variation in cell or nuclear size or shape. H&E stain. (Courtesy College of Veterinary Medicine, University of Tennessee.)

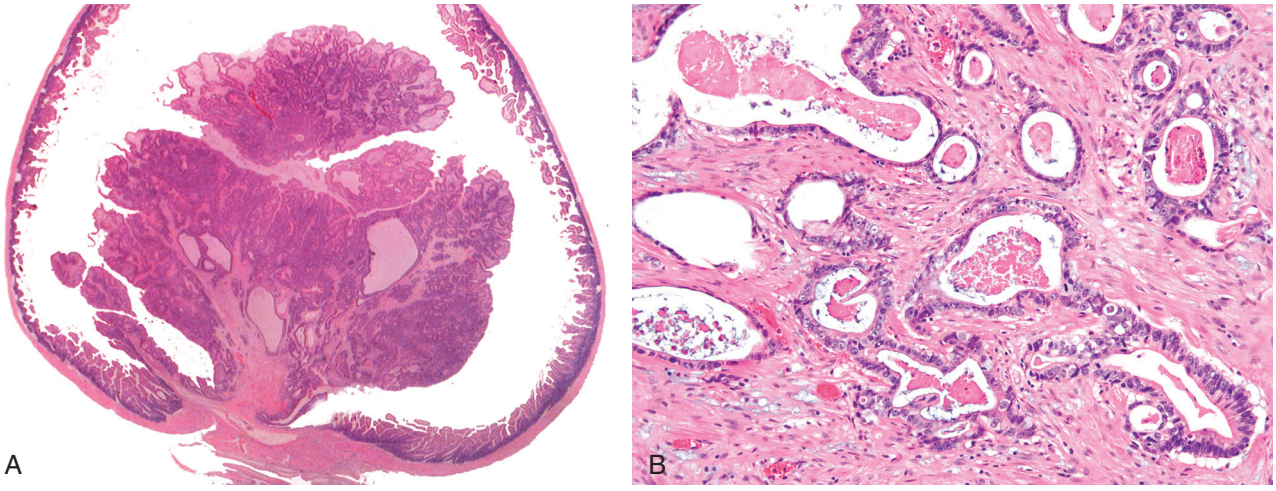


Figure 6-4 Comparison of Benign and Malignant Epithelial Tumors. A, Polyp, small intestine, mouse. The neoplastic growth arises from the mucosa and extends into the lumen of the intestine. There is no invasion of the intestinal wall. H&E stain. **B,** Adenocarcinoma (carcinomatosis), mesentery, cat. Irregular acini of neoplastic epithelial cells, presumably of biliary or pancreatic origin, have invaded mesenteric connective tissue. H&E stain. (**A** courtesy College of Veterinary Medicine, The Ohio State University. **B** courtesy College of Veterinary Medicine, University of Tennessee.)

potential to metastasize. The term *carcinoma* in situ, however, refers to a preinvasive form of carcinoma that remains within the epithelial structure from which it arises and that does not penetrate the basement membrane or invade underlying stroma.

As with mesenchymal tumors, the general terms "adenoma" and "carcinoma" may be further modified to indicate the organ of origin, as in "hepatocellular adenoma" or "hepatocellular carcinoma". In addition, these terms are frequently modified by prefixes or adjectives describing their microscopic appearance. For instance, the adjective "squamous" is applied to an epithelial neoplasm that demonstrates squamous differentiation similar to that seen in normal stratified squamous epithelia. The neoplastic epithelial cells of "mucinous" adenocarcinomas produce abundant mucin. Carcinomas that stimulate significant *desmoplasia*, the formation of abundant collagen in surrounding connective tissue, may be termed *scirrhous*.

Undifferentiated Tumors

The primitive or markedly heterogeneous microscopic appearance of some malignant tumors gives no clue to their cell of origin; thus they are termed *undifferentiated* or *anaplastic neoplasms*.

Mixed Tumors

A tumor containing multiple cell types is called a *mixed tumor*. Mixed tumors are believed to arise from a single pluripotent or totipotent stem cell capable of differentiating into a variety of more mature cell types. The benign mixed mammary gland tumor of dogs is a good example of a mixed tumor, because it typically contains a variable mixture of neoplastic epithelial or glandular elements, including luminal epithelium and myoepithelium, and mesenchymal elements, including fibrous connective tissue, fat, cartilage, and bone (Fig. 6-5). Teratomas and teratocarcinomas, which arise from totipotential germ cells, contain tissues normally derived from all three embryonic cell layers and thus may be composed of a bizarre mixture of adult and embryonic tissue types.

Tumor-Like Lesions

Several lesions may appear neoplastic grossly but are actually nonneoplastic growths when examined microscopically. *Hamartomas* are disorganized but mature mesenchymal or epithelial tissues found in their normal anatomic location (see Fig. 7-47). Many of the hamartomas identified in animals consist of abnormal proliferations of blood vessels. Hamartomas may be the result of aberrant differentiation in development rather than true neoplasia, and their behavior is completely benign. *Choristomas* are composed of normal mature tissue located at an ectopic site. An example is the dermoid, a mass consisting of mature skin and adnexa, which may be found in a variety of unusual sites, including the cornea (see Fig. 21-34 and E-Fig. 21-39).

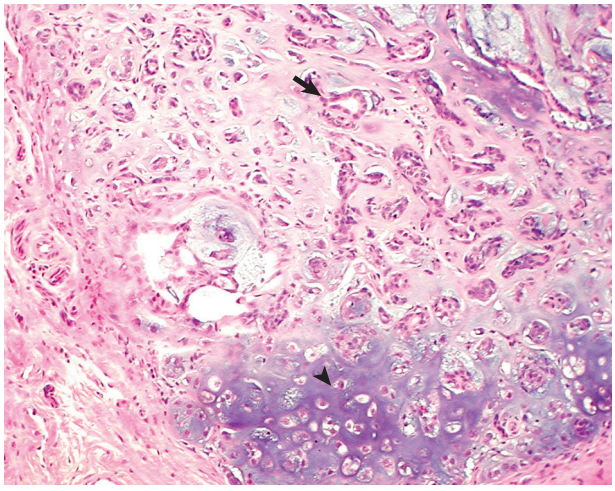


Figure 6-5 Mixed Mammary Tumor, Mammary Gland, Dog. Mixed mammary tumors of dogs contain both epithelial structures *(arrow)* and mesenchymal elements, such as cartilage and bone *(arrowhead)*. H&E stain. (Courtesy College of Veterinary Medicine, The Ohio State University.)

Veterinary Nomenclature

In Table 6-1 the names of common benign neoplasms in animals and their malignant counterparts are shown. The names given are those commonly employed in veterinary medicine. The terms used by veterinary pathologists to describe tumors in animals may differ from the terms used by medical pathologists to describe tumors in human beings. This inconsistency is partly because conventional usage plays an important role in tumor nomenclature; thus tumor nomenclature may be dictated by historic precedent rather than by logic. Moreover, attempts to standardize diagnostic terms for tumors in veterinary medicine have lagged far behind such efforts in human medicine. Thus more than one name for a given type of neoplasm may be reported in medical literature. For instance, a significant difference between veterinary and human nomenclature is that a benign tumor arising from melanocytes is termed a "benign melanoma" or "melanocytoma" by veterinary pathologists and a "nevus" by medical pathologists. Medical pathologists reserve the term "melanoma" for a malignant tumor of melanocyte origin, whereas veterinary pathologists term such tumors "malignant melanomas." Consideration of established precedents in tumor nomenclature and use of precise terminology are therefore critical for accurate communication among pathologists, practitioners, and researchers across medical disciplines.

Tumor Characteristics (Essential Concept 6-1)

Benign Versus Malignant Tumors

Benign tumors are generally expansile and may compress adjacent tissue, whereas malignant tumors are usually invasive. In malignant tumors, alterations in cell adhesion, motility, and protease production allow tumor cells to leave the tumor mass and penetrate surrounding tissue. Moreover, for malignant cells to invade and ultimately metastasize, they must become completely independent of local growth regulatory controls and acquire an independent blood supply. Acquisition of these features allows a tumor to spread well beyond its site of origin.

ESSENTIAL CONCEPT 6-1 Tumor Characteristics

Tumors may arise from virtually any normal tissue in the body. Benign tumors are expansile masses and may compress but do not invade surrounding tissue and do not spread to other sites in the body. In contrast, malignant tumors are locally invasive and have the potential to metastasize to distant sites. Tumor characteristics include the following:

1. Loss of differentiation as indicated by morphologic variability in tumor cells, abnormal tissue architecture, and loss of specialized cell function
2. Unlimited proliferative potential due to continuous cell division and resistance to cell death

Clinically, tumor grade (degree of differentiation) and stage (extent of spread) are used to establish prognosis and determine treatment options.

Table 6-1 Tumor Nomenclature

Origin	Tissue of Origin	Cell of Origin	Benign	Malignant
MESENCHYMAL				
Connective tissue and related tissue	Fibrous connective tissue	Fibroblast	Fibroma	Fibrosarcoma
	Fat	Adipocyte	Lipoma	Liposarcoma
	Cartilage	Chondrocyte	Chondroma	Chondrosarcoma
	Bone	Osteoblast	Osteoma	Osteosarcoma
Endothelium and related tissue	Blood vessel	Vascular endothelium	Hemangioma	Hemangiosarcoma
	Lymphatic vessel	Lymphatic endothelium	Lymphangioma	Lymphangiosarcoma
	Synovium	Synovial lining cell	Synovioma	Synovial sarcoma
	Mesothelium	Mesothelial cell	*	Mesothelioma
	Meninges	Meningeal connective tissue cell	Meningioma	Malignant meningioma
	Ovary	Modified mesothelium†	Adenoma	Adenocarcinoma
Hematopoietic and lymphoid tissue	Lymphoid tissue	Lymphocytes	*	Lymphoma
	Bone marrow	Leukocytes and erythrocytes	*	Leukemia
	Connective tissue	Mast cell	Mast cell tumor	Mast cell tumor
		Histiocytes	Histiocytoma	Histiocytic sarcoma (malignant histiocytosis)
Muscle	Smooth muscle	Smooth muscle cell	Leiomyoma	Leiomyosarcoma
	Skeletal muscle	Skeletal muscle cell	Rhabdomyoma	Rhabdomyosarcoma
EPITHELIAL				
Lining or covering epithelia	Skin	Squamous epithelial cell	Papilloma	Squamous cell carcinoma
		Adnexal cells	Adenoma	Adenocarcinoma Carcinoma
		Melanocyte	Benign melanoma (melanocytoma)	Malignant melanoma
	Upper alimentary tract (oral cavity, esophagus)	Squamous epithelial cell	Papilloma	Carcinoma
	Lower alimentary tract (intestine)	Columnar epithelium	Adenoma	Adenocarcinoma Carcinoma
	Upper respiratory tract (nasal cavity, trachea)	Columnar respiratory epithelium	Adenoma	Adenocarcinoma Carcinoma
	Lower respiratory tract (lung)	Columnar epithelium of bronchi and bronchioles	Adenoma	Adenocarcinoma Carcinoma
		Alveolar lining epithelium		
	Urinary tract	Transitional epithelium	Papilloma	Transitional cell carcinoma
	Uterus	Columnar epithelium	Uterine polyp	Endometrial carcinoma Endometrial adenocarcinoma
	Lining of glands or ducts	E.g., prostate, thyroid, bile ducts of liver	Adenoma	Adenocarcinoma Carcinoma
Solid epithelial organs	Glands	Pancreas, salivary gland, and others	Adenoma	Adenocarcinoma
	Liver	Hepatocyte	Hepatoma	Hepatocellular carcinoma
	Kidney	Renal tubular cell	Renal tubular adenoma	Renal cell carcinoma
	Testicle	Sertoli cell	Sertoli cell tumor	Malignant Sertoli cell tumor
		Interstitial cell	Interstitial/Leydig cell tumor	*
		Germ cell	Seminoma	Malignant Seminoma
			Teratoma	Teratocarcinoma
	Ovary	Stromal cell	Granulosa cell tumor	*
			Luteoma	*
			Thecoma	*
		Germ cell	Dysgerminoma	Dysgerminoma
			Teratoma	Teratocarcinoma
NERVOUS TISSUE				
Glial cells	Central nervous system	Astrocyte	*	Astrocytoma Glioblastoma
		Oligodendrocyte	*	Oligodendroglioma
		Microglial cell	*	Microgliomatosis
	Peripheral nervous system	Schwann cell	Benign peripheral nerve sheath tumor (schwannoma)	Malignant peripheral nerve sheath tumor (malignant schwannoma)

Table 6-1 Tumor Nomenclature–cont'd

Origin	Tissue of Origin	Cell of Origin	Benign	Malignant
Neural cells	Central nervous system	Neuron	*	Primitive neuroectodermal tumor
	Peripheral nervous system	Neuron	Ganglioneuroma	*
MIXED TUMORS				
Various	Mammary gland	Epithelium and myoepithelium	Adenoma Benign mixed mammary tumor (dog)	Adenocarcinoma Carcinoma Malignant mixed mammary tumor (dog)
	Testicle	Germ cell	Teratoma	Teratocarcinoma
	Ovary	Germ cell	Teratoma	Teratocarcinoma

*Not generally recognized.
†In contrast to the nomenclature for other mesenchymal tumors, tumors arising from modified ovarian mesothelium (i.e., surface epithelium, rete ovarii, or subsurface epithelial structures) are designated as adenomas/carcinomas based on the epithelioid cell morphology rather than spindle-shaped or round cell morphology of the tumor cells.

Table 6-2 Comparisons between Benign and Malignant Tumors

Characteristic	Benign	Malignant
Differentiation	Well-differentiated morphologic features and function	Poorly differentiated morphologic features and function
	Structure similar to tissue of origin	Tissue of origin sometimes unclear
	Little or no anaplasia	Variable degrees of anaplasia
Growth rate	Slow, progressive expansion	Rapid growth
	Rare mitotic figures	Frequent mitotic figures
	Normal mitotic figures	Abnormal mitotic figures
	Little necrosis	Necrosis if poor blood supply
Local invasion	No invasion	Local invasion
	Cohesive and expansile growth	Infiltrative growth
	Capsule often present	Capsule often absent or incomplete
Metastasis	No metastasis	Metastasis sometimes present

Although benign tumors are ultimately distinguished from their malignant counterparts based on invasiveness, a variety of morphologic and behavioral features are generally considered to predict the potential for malignant behavior (Table 6-2). Both benign and malignant tumors are composed of proliferating cells, but malignant tumors have essentially unlimited replicative potential. Malignant tumors are relatively independent of exogenous growth stimulatory molecules and are insensitive to growth inhibitory signals from their environment. Furthermore, malignant cells are better able than benign cells to evade apoptotic cell death (see Chapter 1). Compared with benign tumors, malignant tumors stimulate marked angiogenesis (the formation of new blood vessels), which ensures adequate tumor nutrition and promotes vascular invasion and metastasis. However, because of the rapid growth rate of many malignant tumors, areas of necrosis are often found within these tumors.

Because some benign tumors evolve into malignant neoplasms and some malignant tumors develop increasingly aggressive behavior over time in a process termed *malignant progression*, tumors may be graded to reflect where they lie on the continuum from benign to highly malignant or staged to indicate the extent of tumor spread. Together, the grade and stage of the tumor, discussed later in this chapter, indicate the risk the tumor poses to the animal and help determine a therapeutic strategy. It should be noted, however, that many benign tumors, such as sebaceous gland adenomas in dogs, have little or no malignant potential and rarely evolve into malignant tumors.

Differentiation

Morphology

Each normal, fully differentiated, mature tissue type has a characteristic gross and microscopic appearance that varies little from individual to individual of an animal species. To a variable extent, neoplastic tissues lose these mature differentiated features of cellular morphology and organization. In general, malignant tumors appear less differentiated than benign tumors. Many of the morphologic changes seen in neoplastic cells reflect frequent cell division, chromosomal abnormalities, and the active metabolic state that characterizes these cells.

Neoplastic cells often show considerable morphologic variability when compared with the normal tissue from which they are derived. Tumor cells, especially malignant tumor cells, may exhibit *anaplasia* or cellular *atypia*. Anaplastic cells are poorly differentiated cells with a wide variation in cell size (*anisocytosis*) and shape (*pleomorphism*). In some tumors, bizarre *tumor giant cells* with very large nuclei (*karyomegaly*) are observed (Fig. 6-6). There may also be extreme variability in nuclear size (*anisokaryosis*), shape, and pattern of chromatin distribution, and cells may contain multiple nuclei (Fig. 6-7). Anaplastic nuclei are often darkly staining (*hyperchromatic*) because of increased DNA content and are disproportionately large relative to cell size, resulting in an increased nuclear to cytoplasmic ratio. Prominent or multiple nucleoli may be present. The *mitotic figures* seen in dividing cells may be numerous, and atypical mitotic figures may be present.

Many tumor cells have noticeably basophilic cytoplasm as a result of the presence of large numbers of ribosomes required for rapid cell growth and frequent cell division. Neoplastic cells often exhibit loss of characteristic cytoplasmic features such as cilia or pigment. However, even in poorly differentiated tumors, special stains or immunohistochemical stains may be able to identify a characteristic morphologic feature retained in at least a subpopulation of tumor cells. For example, although poorly differentiated melanomas may lose their pigmentation, there is often positive

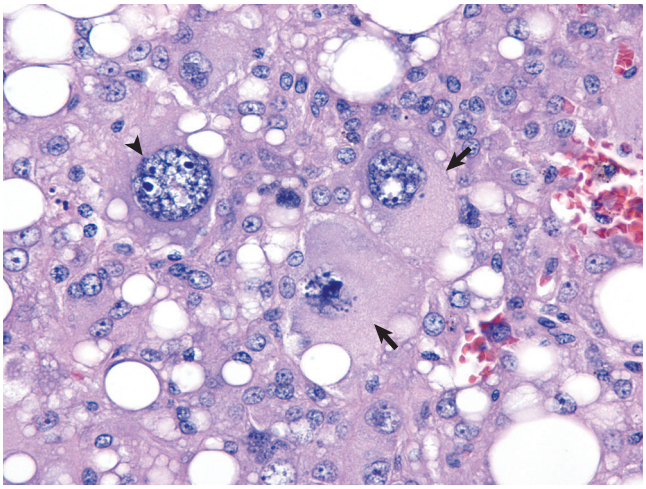


Figure 6-6 Anaplastic Liposarcoma, Subcutis, Dog. Anaplastic tumors of epithelial or mesenchymal cell origin often contain bizarre tumor giant cells such as the cells indicated by the arrows. Note also the large nuclei with abundant coarsely aggregated chromatin and multiple nucleoli *(arrowhead)*. The term "anaplastic" is used because the neoplastic cells bear very little resemblance to the adipocytes from which the tumor developed. H&E stain. (Courtesy College of Veterinary Medicine, University of Illinois.)

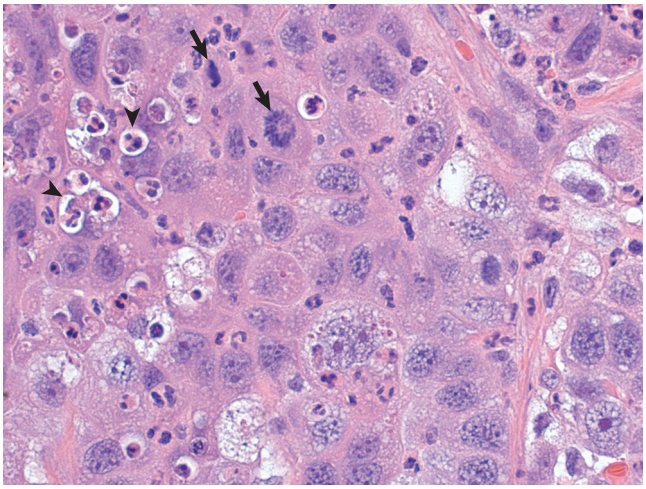


Figure 6-7 Anaplastic Bronchoalveolar Carcinoma, Dog. This tumor exhibits marked nuclear pleomorphism, as evidenced by the variation in nuclear and cellular size and shape. Note the prominent mitotic figures *(arrows)* and phagocytosis of neutrophils by the tumor cells *(arrowheads)*. H&E stain. (Courtesy Dr. J. F. Zachary, College of Veterinary Medicine, University of Illinois.)

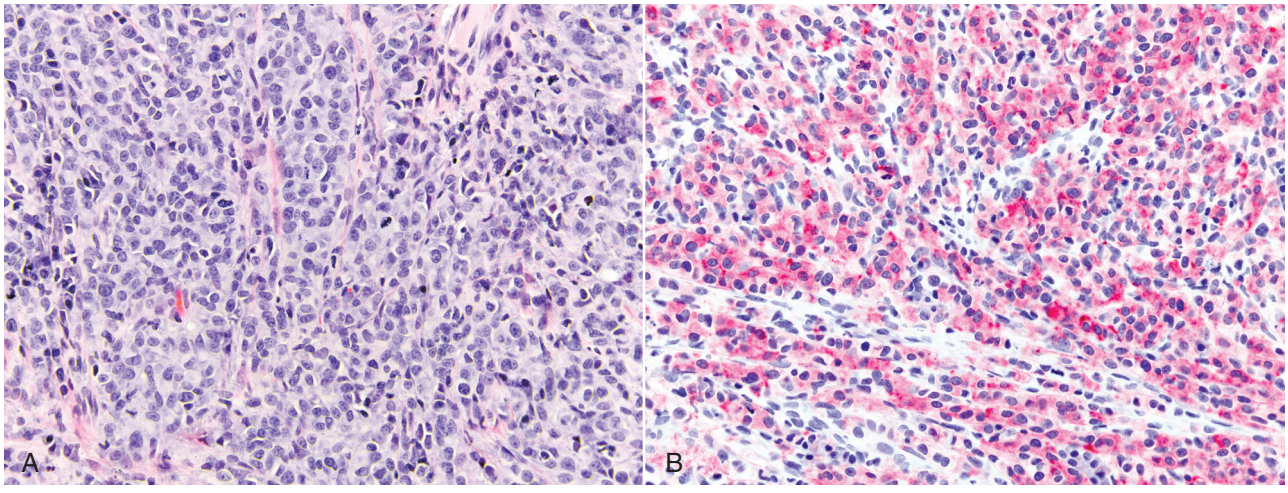


Figure 6-8 Amelanotic Melanoma, Oral Cavity, Dog. A, The tumor is composed of relatively uniform round to polygonal cells that lack obvious cytoplasmic pigmentation. H&E stain. **B,** Subsequent immunohistochemical staining for Melan A (red staining), a melanocyte marker, shows the tumor to be a melanoma. IHC for Melan A. (Courtesy College of Veterinary Medicine, University of Tennessee.)

immunohistochemical staining for the melanoma markers Melan-A (MART1) or dopachrome tautomerase (TYRP2) in amelanotic melanomas (Fig. 6-8).

In tumors, normal tissue organization is frequently lost. Increasing loss of normal architecture in tumors correlates with increasing independence of tumor cells from their surrounding tissues. As an example, lymphomas arising in lymph nodes often consist of solid sheets of neoplastic cells that partially or completely efface the normal follicular lymph node architecture (Fig. 6-9). In tissues that normally undergo continual renewal, such as the skin and oral mucosa, the normal maturation sequence may be altered. Thus in squamous cell carcinomas the orderly morphologic progression from basal cell layer to fully keratinized stratum corneum may not occur (Fig. 6-10).

Function

Loss of specialized function frequently accompanies loss of differentiated morphologic features in tumors. Neoplastic cells arising in the small intestinal epithelium may lack microvilli and thus lose their absorptive capabilities. However, in some tumors, aspects of normal function may be retained. For example, thyroid adenomas may continue to produce thyroid hormones, and plasma cell tumors may secrete immunoglobulins. However, in the majority of cases, these functions are no longer regulated appropriately because the neoplastic cells have lost responsiveness to and dependence on normal regulatory pathways. Thus thyroid adenomas may produce clinical hyperthyroidism, and plasma cell tumors may cause hypergammaglobulinemia.

Differentiation Therapy

Although tumor cells are generally less differentiated than normal cells, some tumor cells can be forced to differentiate into more mature, near-normal cells. Vitamin A derivatives are routinely employed to treat acute promyelocytic leukemia in human patients, vitamin D compounds are showing some promise in differentiation therapy of human epithelial tumors, and compounds that

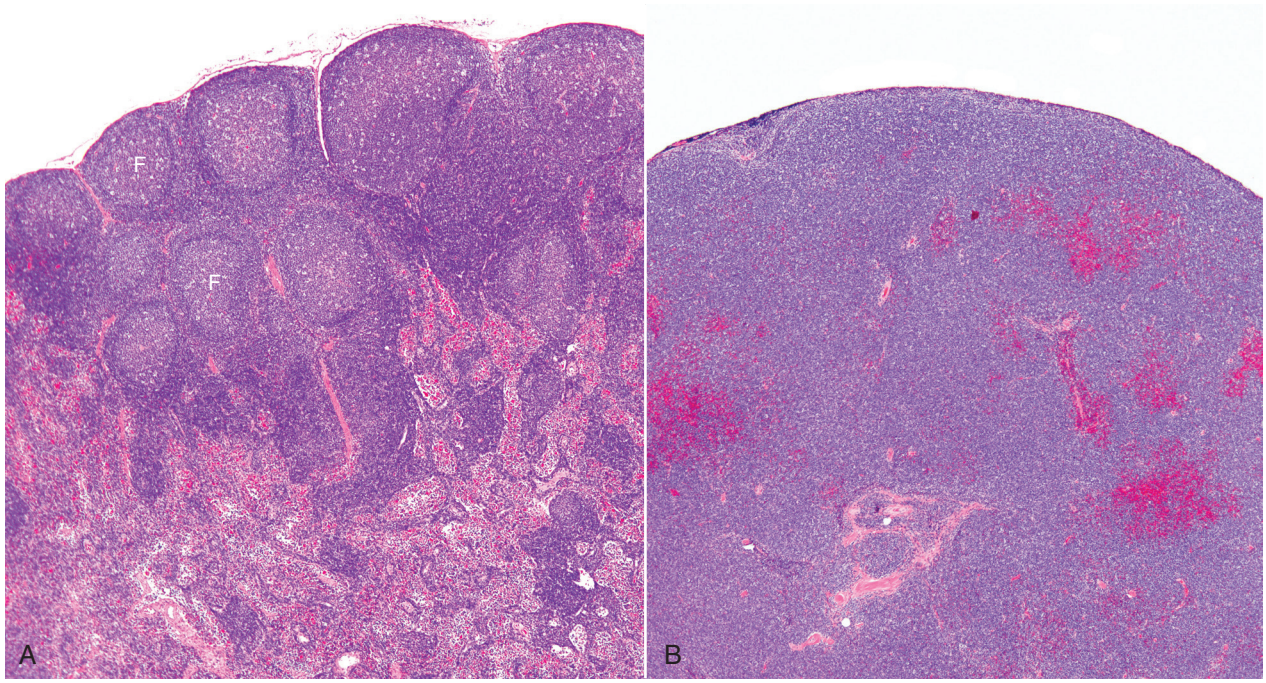


Figure 6-9 Comparison of Lymphoid Hyperplasia and Lymphoma. A, Lymphoid hyperplasia, lymph node, cat. Although there is an expansion of lymphoid elements in this lymph node, lymph node architecture is maintained, and several lymphoid follicles *(F)* are evident. Only two of several follicles are labeled. **B**, Lymphoma (lymphosarcoma), lymph node, dog. Solid sheets of neoplastic lymphocytes completely efface normal lymph node architecture. H&E stain. (Courtesy College of Veterinary Medicine, University of Tennessee.)

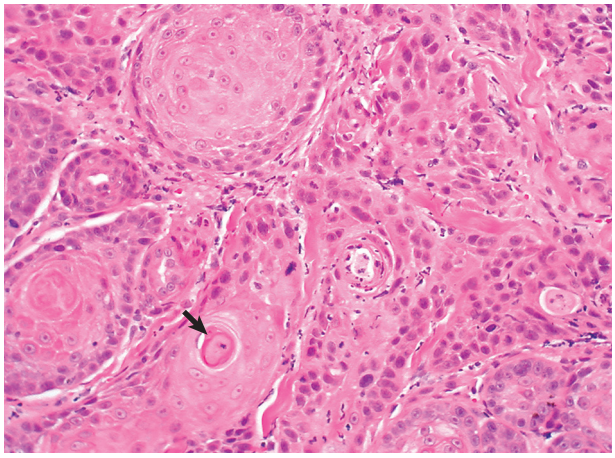


Figure 6-10 Squamous Cell Carcinoma, Tongue, Cat. The orderly pattern of epidermal maturation seen in normal oral mucosa is absent from this squamous cell carcinoma. An occasional "keratin pearl" *(arrow)* identifies the keratinized stratified squamous epithelial origin of this tumor. H&E stain. (Courtesy College of Veterinary Medicine, The Ohio State University.)

epigenetically alter tumor cells by modifying the histones in chromatin may also enhance differentiation of tumor cells. The assumption underlying differentiation therapies is that more differentiated tumor cells will have a less stem cell–like phenotype and will thus have reduced proliferative potential.

Proliferation

Tumor Growth

Essentially unlimited proliferative potential is a hallmark of neoplasia, especially of malignant neoplasms. Unlike normal cells, many tumor cells are immortal. In general, neoplastic cells escape normal limits on cell division, become independent of external growth stimulatory and inhibitory factors, and lose their susceptibility to apoptotic signals. These characteristics result in an imbalance between cell production and cell loss and a net increase in tumor size. However, it should be noted that the growth of a tumor is not completely exponential. A proportion of tumor cells is continually lost from the replicative pool because of irreversible cell cycle arrest, differentiation, and death.

Cell Division

Normal cell proliferation is largely controlled by soluble or contact-dependent signals from the microenvironment that either stimulate or inhibit cell division. An excess of stimulators or a deficiency of inhibitors leads to net growth. As discussed in Chapter 1, the cell cycle consists of G_1 (presynthetic), S (DNA synthetic), G_2 (premitotic), and M (mitotic) phases. Quiescent cells are in a physiologic state called G_0. In adult tissue, many cells reside in G_0 and are unable to enter the cell cycle at all or do so only when stimulated by extrinsic factors. In response to DNA damage, even actively dividing normal cells undergo cell cycle arrest, usually at one of several cell cycle checkpoints. Cell cycle arrest is initiated by the multifunctional tumor suppressor gene product p53 and gives the cell time to repair DNA damage, as discussed in more detail later in the chapter. Many neoplastic cells no longer respond to extrinsic or intrinsic signals directing them into G_0 and no longer express functional p53. Thus the cells move continuously through the cell cycle. Moreover, because the tumor cells do not undergo cell cycle arrest after DNA damage, they progressively accumulate potentially mutagenic DNA damage (Fig. 6-11).

The *mitotic index* is usually defined as the average number of tumor cells per 400× power microscopic field that contain condensed chromosomes and lack nuclear membranes (Fig. 6-12). Such cells are interpreted as being actively dividing, and the mitotic index of a tumor is considered to indicate its malignant potential. However, the mitotic index can be misleading. The fraction of tumor cells

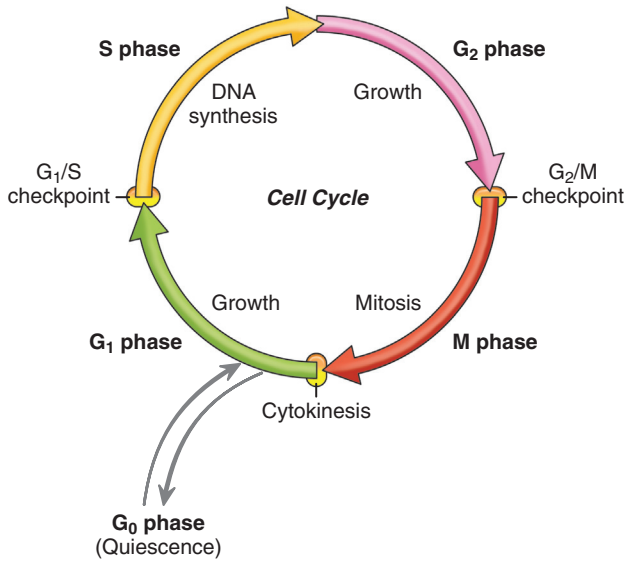


Figure 6-11 Cell Cycle Landmarks. This figure shows the cell cycle phases (G_1, S, G_2, and M). Prophase, metaphase, anaphase, and telophase constitute the M phase, whereas interphase encompasses G_1, S, and G_2. Actively dividing cells cycle continuously. Under the appropriate conditions, cells may exit the cell cycle to enter G_0, a quiescent state, or cells in G_0 may reenter the cell cycle. The G_1/S and G_2/M checkpoints are sites at which cell cycle arrest in response to DNA damage may occur.

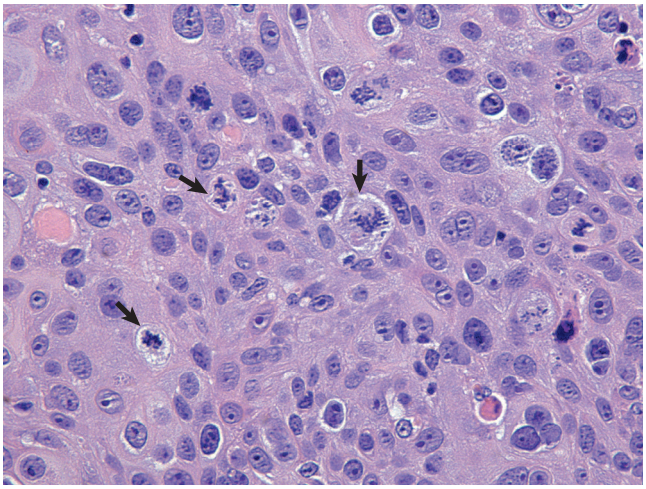


Figure 6-12 Anaplastic Neoplasm, Site Unknown, Dog. The arrows identify a few of the mitotic figures present. This tumor has a high mitotic index. H&E stain. (Courtesy College of Veterinary Medicine, University of Illinois.)

observed to be in mitosis depends not only on the number of cells undergoing mitosis but also on the length of time required to complete the process. In tumor cells the time required for completion of the cell cycle is generally as long as or even longer than for normal cells. Mitotic figures may persist in cancer cells that are unable to complete cell division; abnormal mitotic figures may also be observed.

For homeostasis to be maintained, normal cells must engage in a continual dialogue with their environment. There is a constant exchange of information among cells via soluble mediators, including growth stimulatory factors, growth inhibitory factors, and hormones (see Chapters 1 and 12). These soluble mediators tightly control the growth of nonneoplastic cells. Neoplastic cells, on the other hand, often lose both their dependence on extrinsic growth stimulatory substances and their susceptibility to growth inhibitory signals from their environment. The end result is that tumor cells are no longer responsive to the needs of the organism as a whole and develop the capacity to drive their own replication.

Cell Death

Senescence. In response to DNA damage, oxidative stress, and telomere shortening, proliferating cells may undergo a permanent arrest in the G_1 phase of the cell cycle termed *cellular senescence*. This growth arrest limits the life span of neoplastic cells and prevents unlimited tumor cell proliferation. Senescence is mediated by activation of the p53 or retinoblastoma pathways of cell cycle arrest. Senescent cells often express senescence-associated β-galactosidase.

Because DNA replication machinery is unable to duplicate the extreme ends of DNA templates, the telomeres that form the ends of chromosomes are shortened at each cell division. Embryonic cells express telomerase, a riboprotein enzyme that allows telomeres to be replicated and even expanded; however, most adult cells do not express this protein, and their telomeres shrink with each round of cell division. Very short telomeres are incompatible with continued cell division and trigger cellular senescence in normal cells. However, many neoplastic cells regain the ability to produce telomerase and thus to replicate their telomeres. Reexpression of telomerase appears to play an important role in the escape of tumor cells from senescence and their consequent immortality.

Apoptosis. *Apoptosis* is a form of "programmed cell death" that serves both as a normal physiologic process and as a response to injurious stimuli (see Chapter 1). In proliferative tissue, such as gut epithelium, terminally differentiated cells undergo apoptosis and are thus removed from the cell population. Apoptosis may occur in response to withdrawal of survival or growth factors from the cell environment or to binding of death factors, such as Fas ligand and tumor necrosis factor-α (TNF-α), to cell surface receptors. Cell hypoxia and lack of essential nutrients may end in apoptosis. DNA damage may also induce apoptosis; in this case, apoptosis is triggered by p53. Apoptosis may be stimulated by the activity of cytotoxic immune cells, including T lymphocytes and natural killer (NK) cells. Signals for apoptosis activate a variety of signaling pathways, many of which ultimately result in the release of cytochrome *c* from mitochondria. The final effectors of apoptosis are the caspases, intracellular proteases that selectively destroy cellular organelles and degrade genomic DNA into nucleosome-sized fragments (see E-Fig. 1-22). The morphologic hallmarks of apoptosis include margination of chromatin, condensation and fragmentation of the nucleus, and condensation of the cell with preservation of organelles. Ultimately, the cell breaks into membrane-bound apoptotic bodies that are engulfed by surrounding cells without stimulating an inflammatory response (Fig. 6-13).

Although virtually all normal cells in the body can undergo apoptosis in response to appropriate physiologic signals, many cancer cells acquire resistance to apoptosis. Because apoptosis is a major route of tumor cell loss, such resistance enhances the overall growth rate of the tumor. Many tumor cells circumvent apoptosis by functional inactivation of the *p53* gene, thus removing a key proapoptotic molecule. Additionally, tumor cells may constitutively activate survival signaling pathways, rendering the cells independent of exogenous survival factors. Finally, tumor cells may develop mechanisms for inactivating death factor signaling pathways, thus evading apoptosis in response to homeostatic signals from the cellular environment.

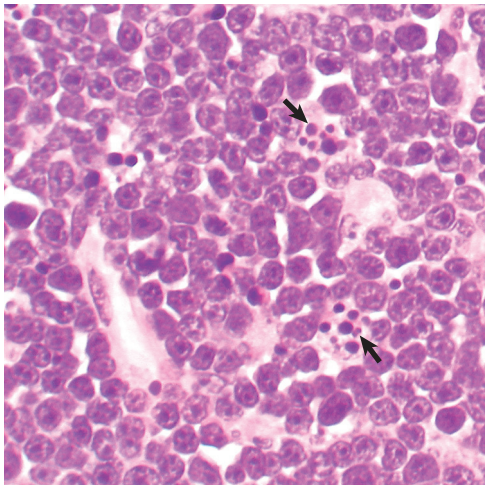


Figure 6-13 Lymphoma (Lymphosarcoma), Lymph Node, Horse. Histologically, apoptosis is characterized by condensation and fragmentation of nuclei *(arrows)*, cell shrinkage, and engulfment of apoptotic bodies by surrounding cells. Note the absence of associated inflammation. H&E stain. (Courtesy Dr. R. Tan, College of Veterinary Medicine, University of Illinois.)

Autophagy. *Autophagy* refers to degradation of a cell's own organelles within autophagosomes (see Fig. 1-24). Autophagy can be a mechanism for cell survival in the face of nutrient deprivation, because it salvages important cellular components for reuse; however, extensive autophagy can also lead to a form of programmed cell death. Autophagy plays a poorly understood and somewhat paradoxical role in tumor growth. In many tumors, autophagy is suppressed, thus presumably preventing autophagic tumor cell death. The mammalian target of rapamycin (mTOR) kinase is the major cellular inhibitor of autophagy, and mTOR inhibitors have shown limited promise as cancer therapeutics. However, in other tumors, increased autophagy may also enhance tumor cell survival under the conditions of reduced nutrient availability that arise during therapy.

Neoplastic Transformation (Essential Concept 6-2)

Latency

As illustrated in Figure 6-14, the *latent period* for a tumor is the time before a tumor becomes clinically detectable. The smallest clinically detectable mass is usually approximately 1 cm in diameter and contains approximately 10^9 cells. To form a tumor that size, a single transformed cell must undergo approximately 30 rounds of cell division, assuming all the progeny remain viable and capable of

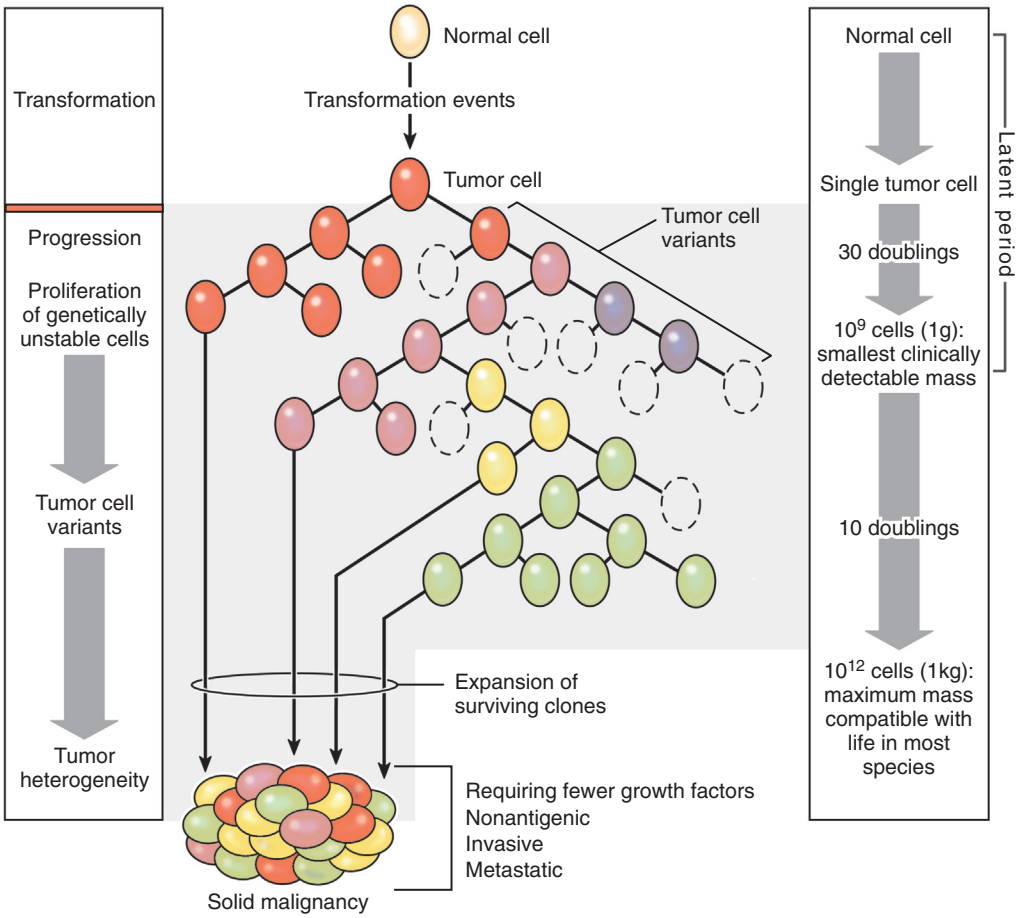


Figure 6-14 Biology of Solid Tumor Growth. The center panel illustrates clonal tumor evolution and generation of tumor cell heterogeneity. Subclones arise from descendents of the original transformed cell. As it grows, the tumor becomes enriched for those variant subclones that proliferate faster or are better able to evade host defenses; such subclones tend to be more aggressive, thus more invasive and likely to metastasize. The left panel shows the corresponding stages of tumor progression. The right panel shows, roughly, the tumor-cell doublings that precede the formation of a clinically detectable tumor. The maximum tumor size compatible with life depends to some extent on the species affected.

ESSENTIAL CONCEPT 6-2 Neoplastic Transformation

Neoplasia occurs by the step-wise transformation of normal cells into tumor cells able to escape ordinary mechanisms of growth control. Steps in neoplastic transformation include the following:

1. Initiation: An irreversible alteration of genetic material
2. Promotion: The selective outgrowth of initiated cells to form a benign tumor
3. Progression: The gradual development of features of malignancy due to a combination of genetic and epigenetic changes

replication. Thus, by the time most tumors become clinically evident, they have probably been developing in the host for many years. However, once tumors reach a clinically detectable size, their growth may appear to be very rapid, because only 10 subsequent doubling cycles are required to convert a 1-g tumor into a 1-kg tumor. Actually, volume doubling times for tumors vary considerably, depending on the rate at which tumor cells divide, the fraction of tumor cells that are replicatively competent, and the rate at which tumor cells die. In general, benign neoplasms grow more slowly than malignant tumors, although there is considerable variation among tumors. Moreover, tumors may grow erratically, depending on several additional factors, including blood supply, extrinsic growth-regulating factors such as hormones, the efficacy of the host immune response, and the emergence of subpopulations of particularly aggressive tumor cells.

Stepwise Tumor Development

Neoplasms develop as the result of multiple genetic and epigenetic changes that occur over a relatively long time course. It is the cumulative effect of these alterations that ultimately creates a tumor. Tumor development thus takes place in a gradual fashion and is described by the term *stepwise tumor development*. Another term applied to this process is *multistage carcinogenesis*. In many chapters in this book the less specific terms *neoplastic transformation* and *carcinogenesis* are used to describe the process of stepwise tumor development discussed here. The stepwise evolution of tumors has been studied most thoroughly in carcinomas. There are several types of carcinoma that develop in an orderly and predictable fashion. For instance, squamous cell carcinoma arises from the epithelium of the eyelid in many species of animals, including cattle, horses, cats, and dogs. In all species these tumors develop through the same sequence of steps: epidermal hyperplasia, carcinoma in situ, and invasive carcinoma. Extensive studies of experimentally induced squamous cell carcinomas in the skin of mice have revealed a similar morphologic pattern of tumor evolution (Fig. 6-15) and have led to a detailed model of stepwise carcinoma development (Fig. 6-16).

In a few well-studied tumor types, such as chemically induced skin tumors in mice and colonic carcinomas in human beings, the stepwise molecular changes that underlie morphologic changes in the tumors have also been determined, as discussed later in the chapter. Many of these genetic changes are associated with cell proliferation, DNA repair, angiogenesis, and invasiveness.

Initiation

The first step of carcinogenesis is *initiation*, the introduction of an irreversible genetic change into normal cells by the action of a mutagenic *initiating agent* or *initiator*. Initiators are chemical or physical carcinogens that damage DNA. Mutation induction requires not only the introduction of a DNA lesion, but also mispairing of the

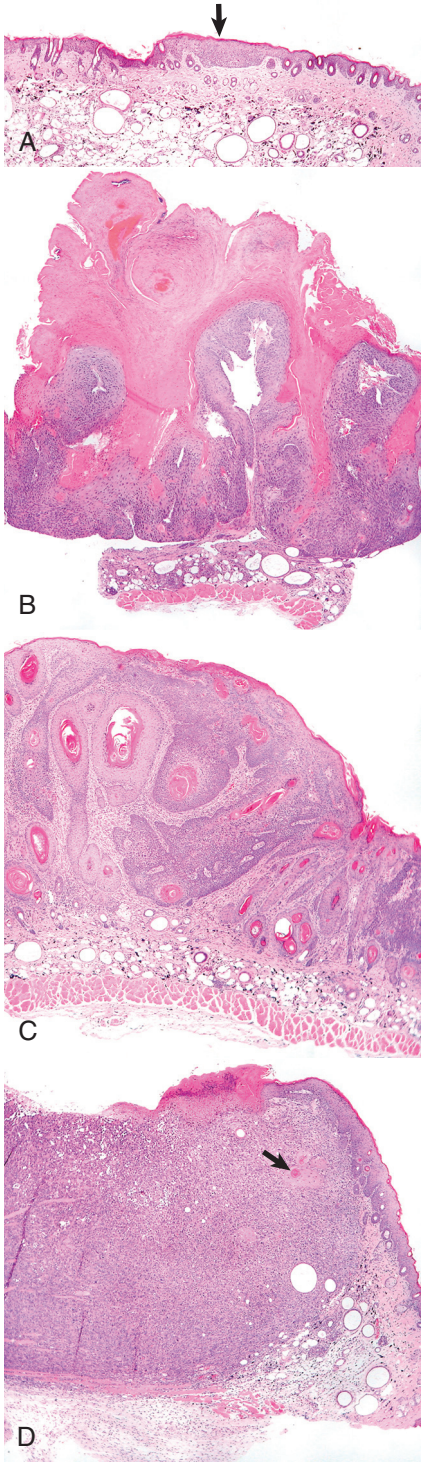


Figure 6-15 Squamous Cell Carcinoma Development, Skin, Hairless Mouse Chronically Exposed to Ultraviolet Radiation. A, A focus of epidermal hyperplasia *(arrow)* is the earliest lesion seen. **B,** This lesion develops into a papilloma, a benign exophytic papillary growth that is highly keratinized and does not penetrate the underlying dermis. **C,** As the papilloma undergoes conversion into a malignant squamous cell carcinoma, it begins to invade the dermis and to lose the regular pattern of epithelial differentiation. **D,** A fully developed squamous cell carcinoma has lost most differentiated characteristics and extends deep into the dermis and panniculus carnosus (muscle). Only a few keratin "pearls" *(arrow)* identify the origin of this tumor from the skin epidermis. All figures were taken at the same magnification. H&E stain. (Courtesy Dr. T.M. Oberyszyn, The Ohio State University.)

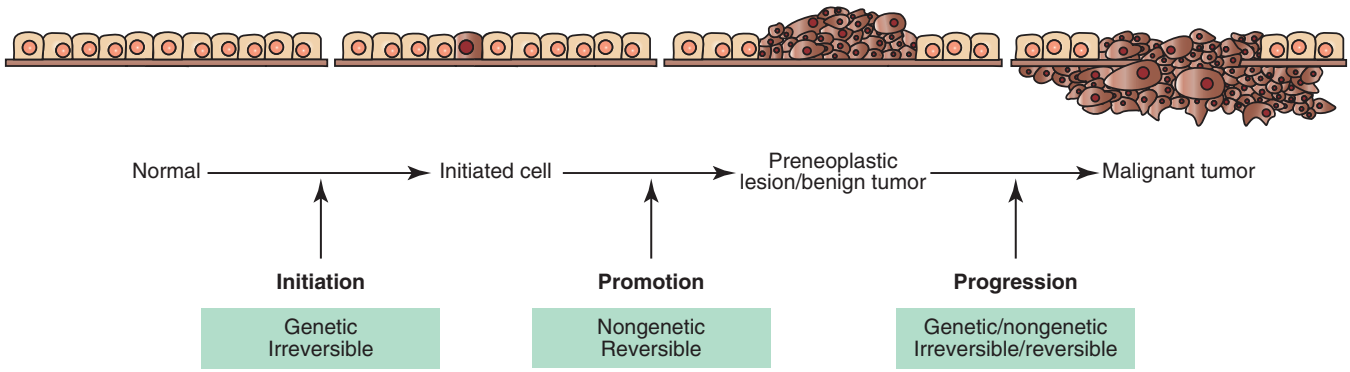


Figure 6-16 Stepwise Tumor Development. Initiated cells have irreversible genetic damage. In the presence of a promoter, these initiated cells expand to form a preneoplastic lesion or benign tumor. With further genetic and epigenetic alterations, a malignant tumor emerges from a subclone of cells within the benign precursor lesion. (Redrawn with permission from Dr. D.F. Kusewitt, Health Sciences Center, University of New Mexico.)

DNA lesion during subsequent DNA replication to produce an altered complementary DNA strand. Thus at least a single round of DNA replication is necessary for the genetic change to become permanent. Initiated cells may appear morphologically normal and may remain quiescent for years. However, these cells harbor mutations that could provide them with a growth advantage under special conditions. For example, the initiated cells may respond more vigorously to mitogenic signals or be more resistant to apoptosis-inducing stimuli than neighboring cells.

Promotion

The second stage of tumor development is *promotion*, the outgrowth of initiated cells in response to selective stimuli. Most of these selective stimuli, termed *promoting agents* or *promoters*, drive proliferation. In general, promoters are not mutagenic; instead, they create a proliferative environment in which initiated cells have a growth advantage. Because promoters are nonmutagenic, their effects are usually reversible. However, the proliferative response to promoters creates a large population of initiated cells at risk for further mutations. What emerges at the end of the promotion phase of tumor development is a benign tumor.

Progression

In *progression*, the final stage of tumor development, a benign tumor evolves into an increasingly malignant tumor in a process termed *malignant transformation*. Malignant tumors may ultimately become metastatic. Malignant transformation represents an irreversible change in the nature of the developing tumor. Progression is a complex and poorly understood process involving both genetic and epigenetic changes in tumor cells, as well as alterations in the tumor environment, that select for increasingly malignant clones of tumor cells. Genetic instability in tumor cells and increasing tumor cell heterogeneity are hallmarks of progression.

Tumor Heterogeneity and Clonal Selection

Most tumors are believed to be of *clonal* origin, that is, they are derived from a single transformed cell. Tumor cell heterogeneity is generated during the course of tumor growth by the progressive accumulation of heritable changes in tumor cells (see Fig. 6-14). With each new genetic alteration, the progeny of a single tumor cell with this new mutation will constitute a subclone of tumor cells. The generation of subclones is fostered by the marked genomic instability of tumor cells compared with normal cells. Successful subclones are those that have a high proliferative rate, are able to evade the animal's immune response, can stimulate the development of an independent blood supply, and become independent of exogenous growth factors. These characteristics give successful subclones a selective advantage over other subclones of cells within the tumor. A tumor subclone with a selective advantage will eventually predominate and, if acquiring certain additional traits, can metastasize from the tumor of origin. This overall process is referred to as *clonal selection* or *tumor evolution*.

Stem Cells and Cancer

Most tumors are composed of cells that lack fully differentiated morphologic, functional, and behavioral characteristics. Furthermore, many neoplastic cells acquire features similar to the embryonic cells that gave rise to the mature tissue in which the tumor originated. This similarity between embryonic cells and neoplastic cells may be accounted for in two different ways. First, normal mature cells may dedifferentiate as they evolve into tumor cells, leading to the reemergence of more primitive characteristics. Second, tumors may arise directly from the small population of *stem cells* found in all adult tissues; such stem cells are required for normal tissue renewal and often have unlimited replicative potential. The appearance and behavior of the tumor cells that develop from a neoplastic stem cell are determined by the stage of differentiation at which the malignant phenotype is manifested; the neoplastic stem cell is said to have undergone *maturation arrest* at that stage of its development. The diversity of cell types that can arise from a single progenitor stem cell is limited by the differentiation potential of that cell.

Totipotent stem cells, such as embryonic stem cells, can give rise to all tissues of the body, whereas multipotent or pluripotent stem cells can give rise to a smaller variety of tissue types. The plasticity of some adult stem cells is relatively restricted. Leukemias provide excellent examples of neoplasms arising from stem cells. Leukemia almost always arises from a single hematopoietic stem cell that has undergone neoplastic transformation. The progeny of this stem cell all exhibit the same genetic change, although the cell type and degree of differentiation of the progeny may vary. Thus in myelomonocytic leukemia a neoplastic multipotential stem cell may give rise to leukemic cells from both the granulocytic and monocytic series (Fig. 6-17). The concept of a stem cell origin for cancer explains not only the embryonic characteristics of neoplastic cells but also the success of certain treatment strategies that use differentiating agents such as retinoids, vitamin A derivatives that are used to induce maturation of some human leukemia cells.

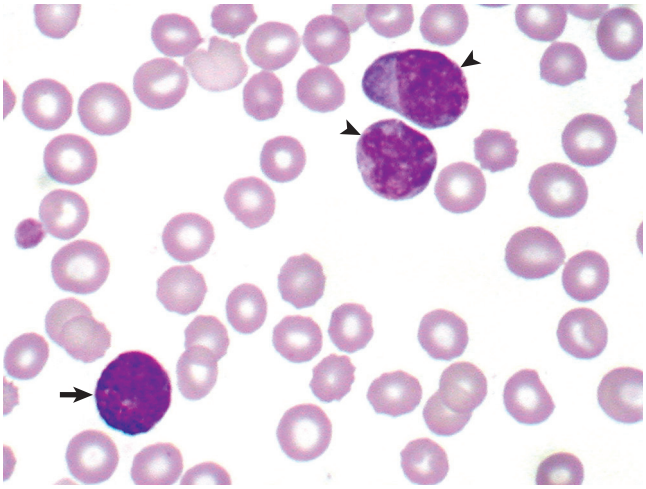


Figure 6-17 Myelomonocytic Leukemia, Peripheral Blood, Dog. In this unusual case, leukemic cells of both monocytic *(arrowheads)* and granulocytic (basophil) *(arrow)* origin were present in peripheral blood. The animal had a marked leukocytosis (103,000 white blood cells/μL) and thrombocytopenia. Wright's stain. (Courtesy Dr. M.J. Burkhard, College of Veterinary Medicine, The Ohio State University.)

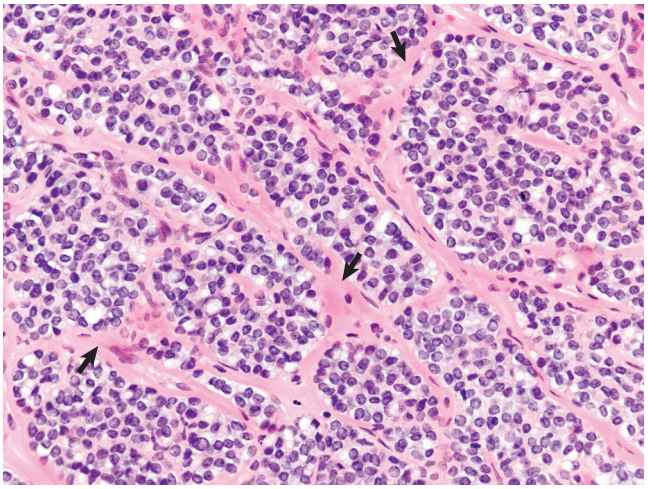


Figure 6-18 Trichoblastoma, Skin, Dog. Neoplastic basal epithelial cells are divided into incomplete lobules by the tumor stroma *(arrows)* composed of collagen and extracellular matrix components in which blood vessels, fibroblasts, and inflammatory and immune cells are embedded. H&E stain. (Courtesy College of Veterinary Medicine, University of Tennessee.)

Tumor Microenvironment (Essential Concept 6-3)

Tumor Stroma

Composition of the Stroma

A tumor consists of the tumor cells proper, termed the *parenchyma*, and a nonneoplastic supporting structure called the *stroma* (Fig. 6-18). The stroma is composed largely of extracellular connective tissue and consists of proteins and glycoproteins, such as collagen, embedded in a complex matrix of proteoglycans. This aggregate is called the *extracellular matrix* (ECM) throughout this book and is discussed in greater detail in Chapter 1. The stroma also contains the blood vessels that supply nutrients to the tumor, fibroblasts that synthesize collagen and other ECM components, and a variety of inflammatory and immune cells. The amount of stroma associated with tumors varies considerably. The extracellular material in the stroma of epithelial tumors is produced primarily by surrounding nonneoplastic mesenchymal cells, whereas many mesenchymal tumors produce their own stroma. For example, many osteosarcomas produce bone, a specialized form of connective tissue stroma. Stromal tissue may form a connective tissue *capsule* around tumors (Fig. 6-19), which may help to limit neoplastic spread. In general, encapsulated tumors have a better prognosis than unencapsulated tumors.

Rarely, the tumor stroma contains an amorphous eosinophilic substance termed *amyloid*. Amyloid consists of one of a variety of abnormal proteins arranged in β-pleated fibrils. The proteins that form amyloid are usually secreted by the tumor cells themselves. For example, λ-light chain protein secreted by neoplastic plasma cells forms the amyloid sometimes seen in the extramedullary plasmacytomas of various species. See Chapters 1 and 5 for more information on amyloid.

Tumor-Stromal Interactions

Tumor cells interact with their stroma in a complex fashion, exchanging a wide variety of signaling molecules, including growth factors, cytokines, hormones, and inflammatory mediators (Fig. 6-20). These exchanges modulate the growth rate, differentiation state, and behavior of both stromal cells and tumor cells. As an example, platelet-derived growth factor (PDGF) released by tumor cells stimulates tumor-associated fibroblasts to increase the production of collagen. In some cases this process leads to an extensive fibrous reaction, termed a "scirrhous" or "desmoplastic" response, in the stroma (Fig. 6-21). Some tumors produce transforming growth factor-α (TGF-α), which stimulates tumor-associated fibroblasts to differentiate into myofibroblasts, which have contractile capabilities. Tumor-associated fibroblasts may acquire special characteristics that distinguish them from normal fibroblasts. In some tumors, heritable genetic and epigenetic changes in tumor-associated fibroblasts

ESSENTIAL CONCEPT 6-3 Tumor Interactions with Other Tissues

Interactions between tumors and nonneoplastic tissues and organs of the body include the following:

1. Tumor-stromal interactions: A tumor consists of the tumor cells proper and a nonneoplastic supporting stroma composed of extracellular matrix, blood vessels, fibroblasts, inflammatory cells, and immune cells. Tumor cells and their stroma exert considerable mutual control. A particularly important effect of tumors on their stroma is the ability to stimulate angiogenesis, the formation of new blood vessels that support continued tumor growth.
2. Tumor immunity: The immune system may recognize tumor antigens as foreign and destroy tumor cells by a variety of mechanisms. Antitumor effector cells include those of the innate immune system (natural killer cells, macrophages) and the adaptive immune system (cytotoxic T lymphocytes, B lymphocytes). Tumor cells may employ a number of strategies to evade immunosurveillance.
3. Paraneoplastic effects: Once established, primary or metastatic tumors cause clinical disease through direct means, such as compression or effacement of normal tissues, or through paraneoplastic effects, such as the secretion of hormones by the tumor. Paraneoplastic effects important in veterinary medicine include cachexia, hypercalcemia, and anemia.

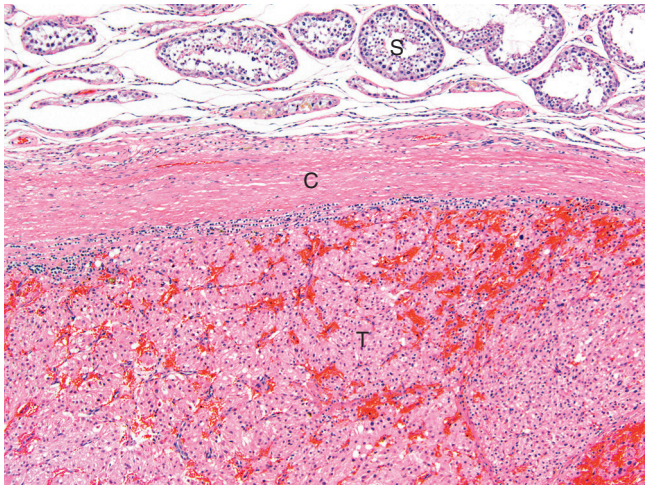


Figure 6-19 Interstitial Cell Tumor, Testis, Dog. This interstitial (Leydig) tumor (*T*) lies within the testis and is surrounded by a thick connective tissue capsule (*C*). Fibrous capsules are more common surrounding benign tumors than surrounding malignant tumors. *S*, Seminiferous tubule. H&E stain. (Courtesy College of Veterinary Medicine, The University of Tennessee.)

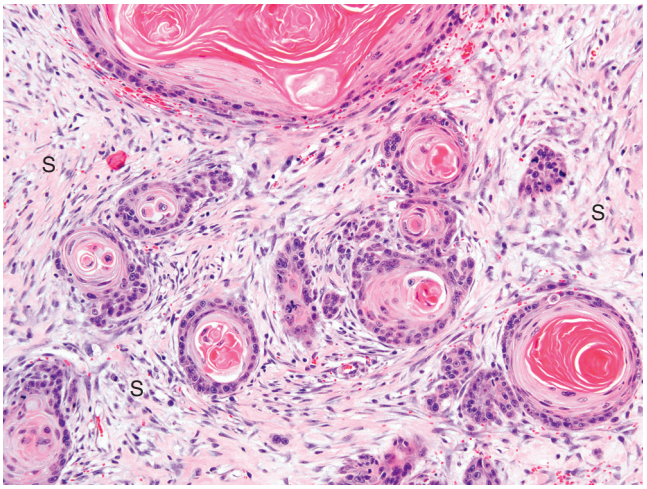


Figure 6-21 Squamous Cell Carcinoma. Carcinomas and adenocarcinomas that stimulate the formation of abundant collagen in surrounding connective tissue (desmoplasia) may be termed "scirrhous." Tumor-associated fibroblasts may secrete a fetal type of extracellular matrix and coevolve with adjacent tumor cells. In this photomicrograph, nests of squamous carcinoma cells with central keratin pearls are separated by abundant stroma containing large numbers of immature fibroblasts and collagen (*S*). H&E stain. (Courtesy College of Veterinary Medicine, The University of Tennessee.)

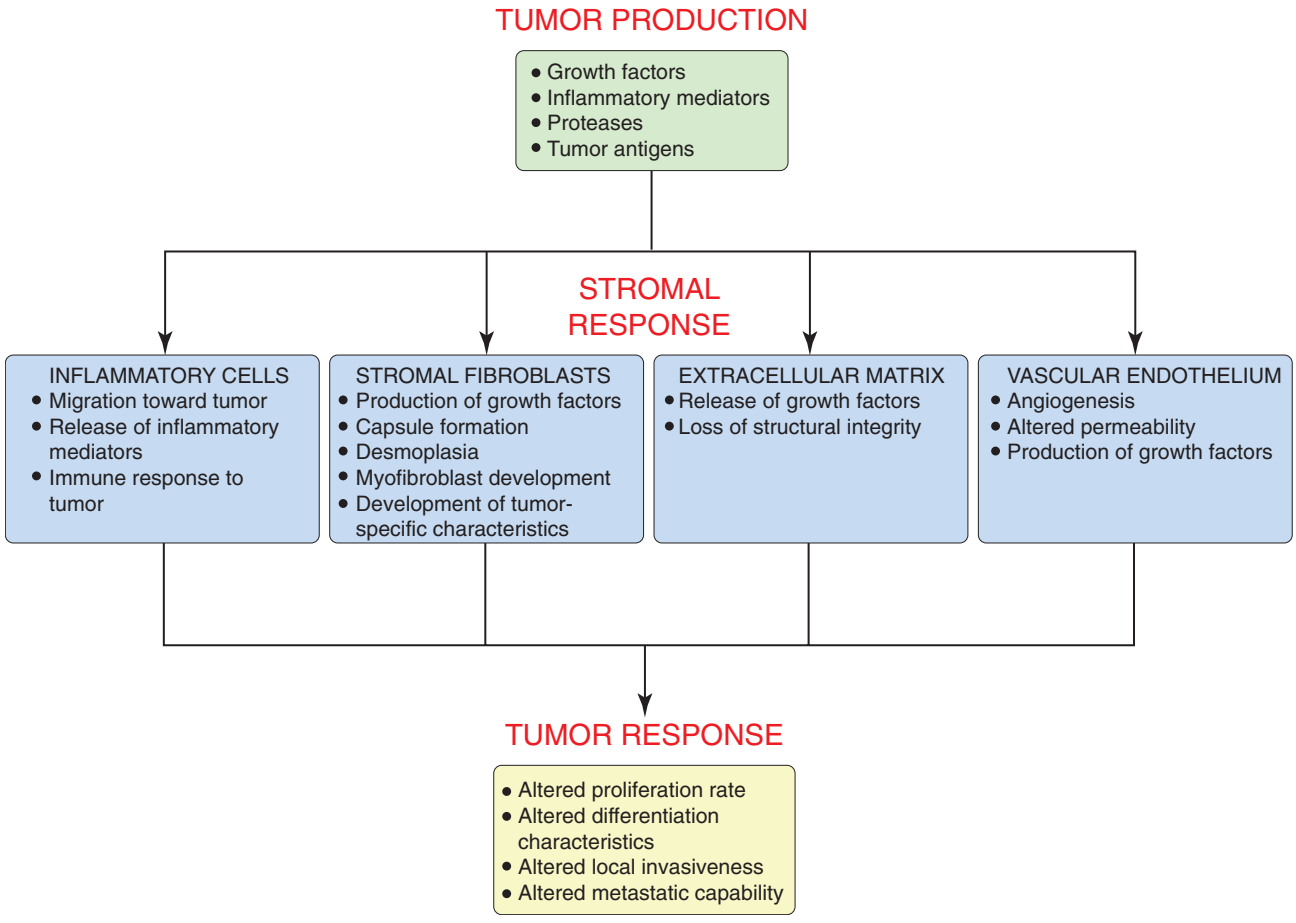


Figure 6-20 Tumor-Stromal Interactions. Tumor cells and the stroma in which they are embedded interact in a variety of ways that serve to modify the growth and behavior of both elements. Tumor stroma may both enhance and limit tumor development and spread. (Redrawn with permission from Dr. D.F. Kusewitt, Health Sciences Center, University of New Mexico.)

allow them to synchronize their growth with that of adjacent tumor cells. Tumor cells may induce surrounding stromal cells to produce cytokines that promote tumor cell proliferation and motility or attract inflammatory cells. Furthermore, growth factors are sequestered in the ECM of the stroma, where they bind to proteoglycans. Proteases secreted by tumor cells, stromal fibroblasts, or inflammatory cells can release these growth factors from the ECM, thus fostering tumor cell proliferation and migration.

Angiogenesis

Continued growth of solid tumors depends absolutely on an adequate blood supply to provide oxygen and nutrients to tumor cells. Without the development of new blood vessels, a process termed *angiogenesis* (see Chapter 2), tumors are limited to a maximum diameter of 1 to 2 mm. At some point during tumor development, an *angiogenic switch* occurs that allows tumor cells to induce and sustain new tumor vasculature. Angiogenesis is a complex process involving recruitment of endothelial cells from preexisting blood vessels, endothelial cell proliferation, directed migration of endothelial cells through the ECM, and maturation and differentiation of the capillary sprout. Angiogenesis is controlled by the balance between a plethora of angiogenesis-stimulating and angiogenesis-inhibiting factors. Tumors initiate angiogenesis by producing angiogenic factors, such as vascular endothelial growth factor (VEGF), or by downregulating production of antiangiogenic factors, such as thrombospondin. In addition, angiogenic and antiangiogenic factors bound to ECM components within the stroma can be released and activated by tumor protease activity. VEGF and fibroblast growth factors (FGFs) are among the most potent angiogenic factors produced by tumors. The tumor blood vessels that develop in response to angiogenic signals are usually more dilated, more tortuous, and more permeable (leaky) than normal blood vessels (Fig. 6-22).

In addition to supplying nutrients, tumor vasculature plays other roles in tumor development. Vessel leakiness allows perivascular deposition of a fibrin network that promotes formation of collagenous tumor stroma. The endothelial cells of tumor blood vessels produce growth factors, such as PDGF and interleukin-1 (IL-1), that stimulate tumor cell proliferation. Moreover, without access to the circulatory system, tumors cannot metastasize. Because solid tumor growth depends absolutely on an adequate blood supply, therapeutic strategies to inhibit angiogenesis have been developed; however, the clinical results using antiangiogenic agents have thus far been disappointing.

The development of lymphatic vasculature in tumors, termed *lymphangiogenesis*, shares many features with tumor angiogenesis. Tumor-associated lymphatic vessels sprout from preexisting lymphatic vessels in response to tumor-secreted factors such as VEGF. Tumor-associated lymphatic vessels are essential for metastasis of solid tumors to regional lymph nodes. In tumors of human beings, there is a strong correlation between the levels of VEGF expression and lymphatic metastasis; and in genetically engineered mice that do not express VEGF, lymphatic metastases do not occur.

Inflammation

Many tumors are heavily infiltrated with neutrophils, eosinophils, mast cells, lymphocytes, histiocytes, or a combination of these cells.

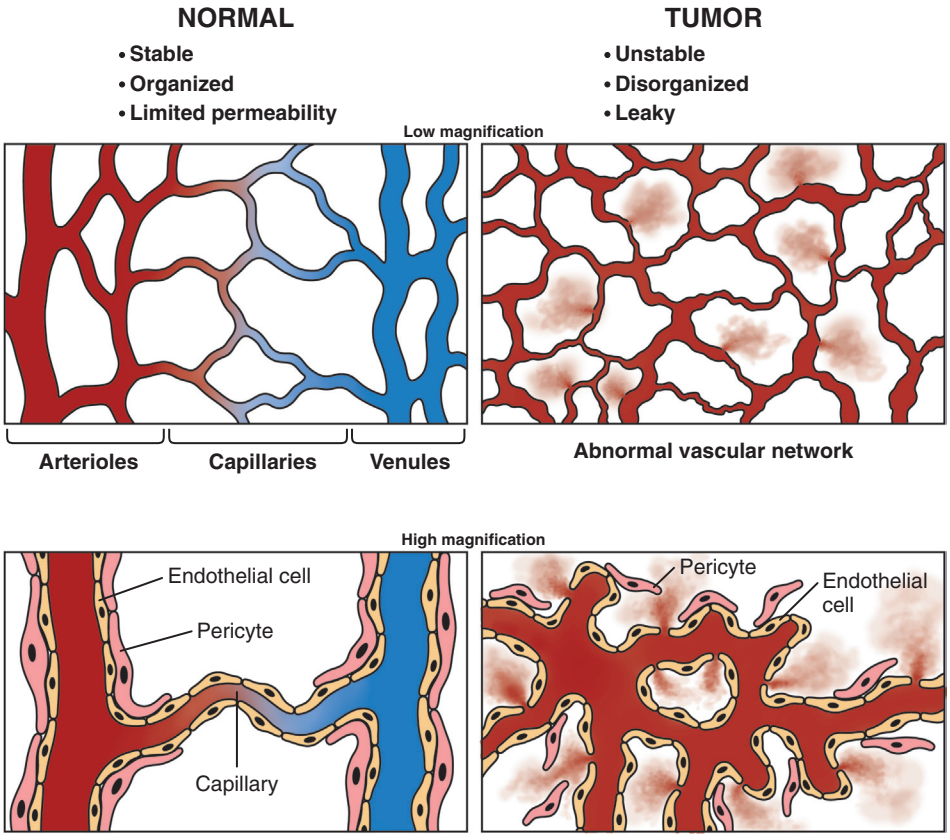


Figure 6-22 Tumor Angiogenesis. Compared with normal vessels *(left panels)*, tumor vessels are tortuous and irregularly shaped *(right panels)*. Arterioles, capillaries, and venules are clearly distinguishable in normal vasculature; in tumors, vessels are disorganized, and specific vessel types cannot be identified. In contrast to the stable vascular network of normal tissue, the networks formed by tumor vessels are unstable and leaky. Thus both the structure and function of tumor vasculature are abnormal.

Inflammatory cells are attracted to tumors by chemokines and cytokines released by tumor cells. Infiltrating inflammatory cells can then recruit additional leukocytes to the tumor. Inflammatory cells serve as a source of prostaglandins, leukotrienes, and reactive oxygen species. In general, inflammation does not appear to protect against tumors. In fact, many chronic inflammatory conditions increase the risk for cancer in affected organs. As an example, the development of vaccine-associated sarcomas in cats is clearly linked to the presence of inflammation at sites of inoculation. Moreover, in human beings, epidemiologic studies suggest that nonsteroidal antiinflammatory drugs (NSAIDs) reduce the incidence of some cancers.

Tumor Immunity (see Essential Concept 6-3)

Immunosurveillance

The vertebrate immune system evolved for the primary purpose of recognizing and destroying infectious organisms and the host cells they infect. However, the immune system also attacks tissue transplanted from genetically dissimilar animals of the same or different species. The same mechanisms used to identify and kill microbially infected cells or foreign cells can be directed against some self-antigens on tumor cells. This process is termed *immunosurveillance*. It is believed that effective immunosurveillance suppresses tumor development and that a failure of immunosurveillance allows tumors to emerge. The immunosurveillance hypothesis is supported by the dramatically increased tumor susceptibility of immunosuppressed human transplant recipients. The inability of these individuals to mount effective antitumor responses apparently allows the emergence of many tumors usually eliminated by immunosurveillance. The presence of lymphocyte and macrophage infiltrates within and around tumors of many types in many species also suggests that tumors can elicit an immune response. Finally, there is abundant experimental evidence that mice can mount an effective immune response against some chemically induced tumors.

Tumor Antigens

Tumor antigens are proteins, glycoproteins, glycolipids, or carbohydrates expressed on the surface of tumor cells (Fig. 6-23). They include both *tumor-specific antigens* restricted to tumor cells and *tumor-associated antigens* present on both tumor cells and normal cells. Tumor antigens can be exploited for both diagnostic and therapeutic purposes. Tumor antigens released into the bloodstream allow noninvasive detection of tumors and monitoring of tumor response to treatment. In combination with sophisticated imaging techniques, antibodies against tumor-restricted antigens can be used to identify the location of tumors and detect metastases. Some tumor antigens can serve as the targets of effective immunosurveillance. However, many tumor antigens are not appropriate therapeutic targets. The antigens may not be restricted solely to tumor cells or they may not elicit a strong cytotoxic response from immune cells.

Some tumor-specific antigens are newly expressed molecules, such as antigens derived from oncogenic viruses, or altered cellular products encoded by mutated genes. In these instances, productive viral infection or gene mutation is restricted to tumor cells and their progeny. Embryonic antigens or *oncofetal antigens*, normally not expressed in adult tissue but reexpressed in tumor tissue, may also behave as tumor antigens. For example, the developmental antigens, carcinoembryonic antigen (CEA) and α-fetoprotein, are

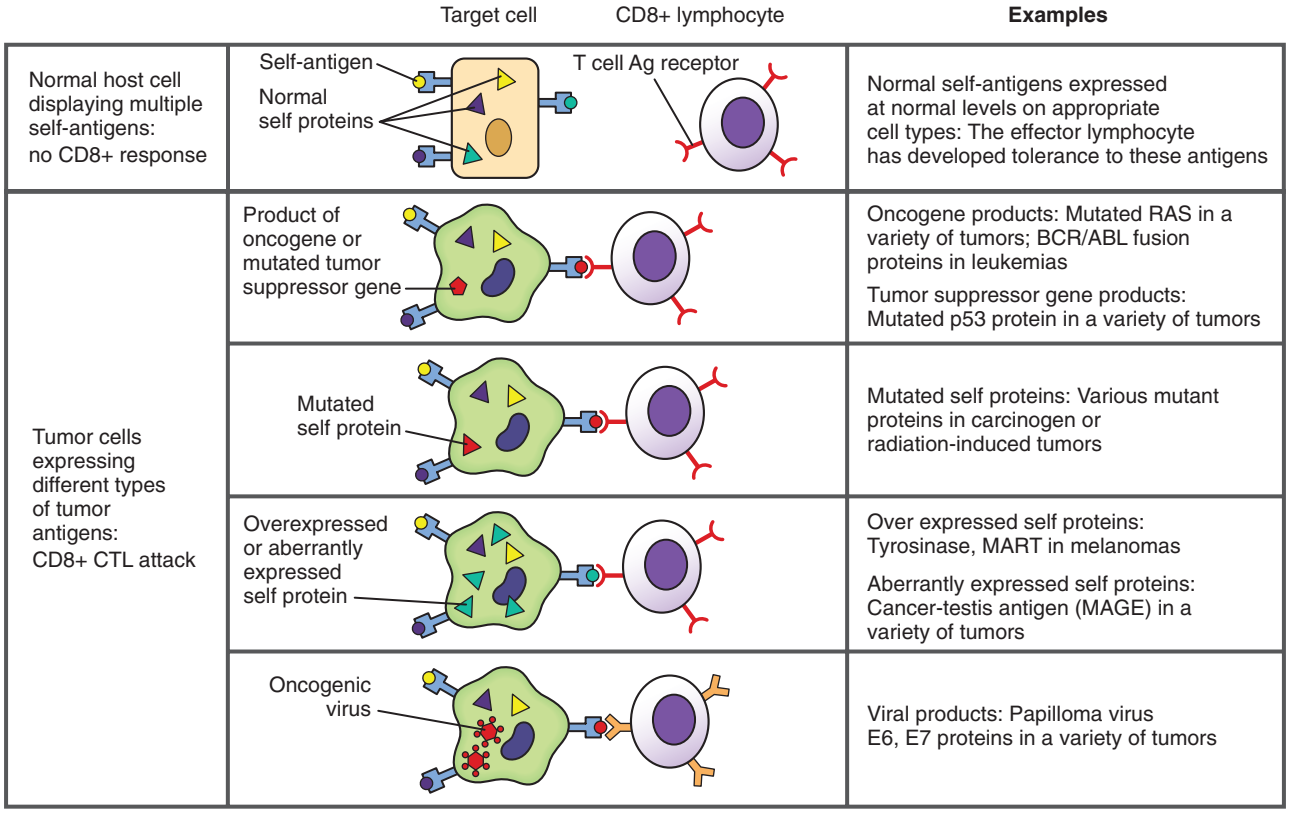


Figure 6-23 Tumor Antigens Recognized by CD8⁺ T Lymphocytes. All tumor cell target antigens are presented to cytotoxic CD8⁺ T lymphocytes by major histocompatibility complex class I molecules bound to the surface of the tumor cells. T lymphocyte receptors on the surface of the CD8⁺ lymphocytes recognize tumor antigens in this context but fail to recognize normal self-antigens to which the immune system has been tolerized.

reexpressed in some tumors in a variety of species and may be released into the circulation. Serologic testing for these antigens is widely used to test for recurrence of liver and intestinal tumors in human beings. *Tumor-specific shared antigens* are encoded by genes that have very limited expression in adult tissue but that are expressed by many types of tumor tissue. A striking example of useful tumor-specific shared antigens is the MAGE family of proteins, found in human beings and other animal species. These antigens are not present on the surface of normal adult cells; however, they are expressed by a wide variety of tumor types and are promising candidates for antitumor immunotherapy. *Tissue-specific antigens* are shared by tumors and the normal tissues from which they arise. In some cases these antigens are expressed only at specific stages of differentiation in the normal tissue and are thus termed *differentiation antigens*. When tissue-specific or differentiation antigens are expressed at considerably higher levels on tumor cells than normal cells, they may function much like tumor-specific antigens.

Antitumor Effector Mechanisms

The body may mount a variety of immune responses against tumor antigens, as illustrated in Fig. 6-24. For a more detailed discussion of immune responses, see Chapter 5. The type of immune response and its effectiveness against tumor cells are largely determined by the inherent immune responsiveness of the animal and the characteristics of the tumor antigen under attack. The least specific immune response to tumor cells is carried out by the *innate immune system*, which is responsible for immediate inflammatory responses. The innate immune response is believed to be the first line of defense against cancer cells. Antitumor effectors of the innate immune system, including NK cells and macrophages, do not require antigen-specific priming by dendritic cells. Innate immune responses do not create lasting antitumor immunity.

More specific immune responses are undertaken by the *adaptive immune system*, consisting of both cell-mediated and humoral components. The cell-mediated immune response is believed to mount the most effective antitumor defenses. Any adaptive antitumor immune response requires that tumor antigens be presented to appropriate immune effector cells in a recognizable context. Dendritic cells capture antigens that are secreted by viable tumor cells or released from dying tumor cells. The dendritic cells ingest these antigens, fragment them to a suitable size, link them to class I or class II major histocompatibility complex (MHC) antigens, and present them on the cell surface in association with appropriate costimulatory molecules. A dendritic cell can then interact with many different lymphocytes to prime their response to the specific tumor antigen presented by the dendritic cell. Antigen-activated $CD8^+$ and $CD4^+$ T lymphocytes develop into tumor-specific cytotoxic and T helper (T_H) lymphocytes, respectively, whereas B lymphocytes develop into immunoglobulin-secreting plasma cells. $CD8^+$ lymphocytes recognize tumor antigens in the context of MHC class I antigens, whereas $CD4^+$ cells recognize these antigens only in association with MHC class II molecules.

Natural Killer Cells

Natural killer (NK) cells are lymphocytes that lack many of the usual markers of T or B lymphocytes. NK cells display a variety of receptors, both inhibitory and activating, that recognize MHC molecules and stress-induced ligands on tumor cells. NK cells can kill a wide variety of neoplastic and virally infected cells. Cells that express MHC class I molecules are preferentially spared by NK cells, whereas cells lacking MHC molecules are specifically targeted. When an NK cell recognizes and attaches to its target cell, a well-organized structure, termed an *immunologic synapse*, is rapidly formed at the site of cell-to-cell contact and persists for more than an hour. At this interface the NK cell releases lytic granules containing perforin, a pore-forming protein, and granzymes, which are serine proteases. Perforin mediates the entry of granzymes into the target cell. Once inside the target cell, granzymes initiate both caspase-dependent and caspase-independent apoptosis. This mechanism of cell killing, termed *cytolysis*, is shared with T lymphocytes.

Macrophages

Macrophages are migratory phagocytic cells capable of killing tumor cells by releasing reactive oxygen intermediates, lysosomal enzymes, nitric oxide, and tumor necrosis factor. Their antitumor activity is stimulated by interferon-γ (IFN-γ), which is produced by both T lymphocytes and NK cells. Macrophage-mediated tumor cell killing is independent of MHC antigens, tumor-specific antigens, and the type of transformed cell being targeted, but direct contact between the macrophage and tumor cell is required.

Although macrophages have long been considered to be tumoricidal, recent evidence suggests that some macrophages actually promote tumorigenesis. It has been demonstrated experimentally that tumor-associated macrophages can promote angiogenesis and enhance tumor cell invasion and metastasis. In addition, macrophages may be immunosuppressive, blocking the antitumor activity of NK cells and lymphocytes. Early studies suggest that depleting tumor-associated macrophages may be useful as part of cancer therapy.

T Lymphocytes

Cytotoxic T lymphocytes (CTLs) are the primary effector cells of the adaptive antitumor immune response. Most CTLs are $CD8^+$ T lymphocytes that have been primed by dendritic cells to recognize and engage tumor antigens on the surface of tumor cells. Tumor cells are then killed by cytolysis. $CD4^+$ T_H lymphocytes enhance the function of $CD8^+$ CTLs and antigen-producing B lymphocytes by secreting cytokines, such as IL-2 and IFN-γ, which stimulate $CD8^+$ T lymphocyte proliferation and differentiation.

There is, however, one T cell population, composed of *regulatory T cells* (T reg), which actually protects tumors against attack by other immune cells. T reg cells accumulate in tumors, where they induce tolerance to tumor tissue. Tolerance is established via a complex set of interactions with other lymphocyte types, macrophages, and dendritic cells. These interactions are mediated by both soluble factors and cell-cell contact. In human beings, administration of antibodies that block the immunosuppressive effects of suppressor T cells allows a robust antitumor CTL response against melanoma.

B Lymphocytes

Many tumor antigens can incite both cell-mediated and humoral immune responses. Antibody-producing *B lymphocytes* mediate the humoral immune response to tumors. Antibodies that recognize tumor antigens kill tumor cells by binding to the cells and activating a local complement cascade (see Chapters 3 and 5). Activation of the complement cascade generates a *membrane attack complex* (MAC) that induces loss of tumor cell membrane integrity and rapid cell death with the morphologic hallmarks of necrosis. In addition, antitumor antibodies may be bound by their constant regions to NK cells or macrophages, leaving the variable regions of the immunoglobulins available for specific recognition of tumor antigens. This arrangement allows the effector immune cells to recognize, attach to, and kill tumor cells by the mechanism of *antibody-dependent cell-mediated cytotoxicity* (ADCC).

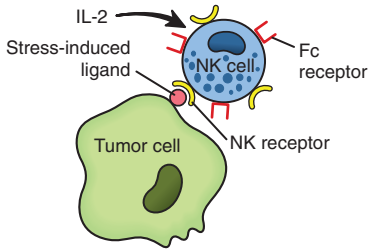
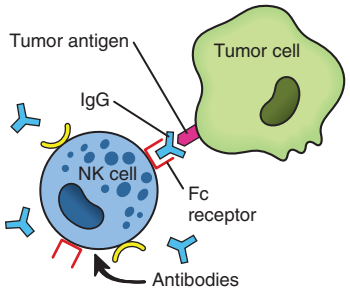
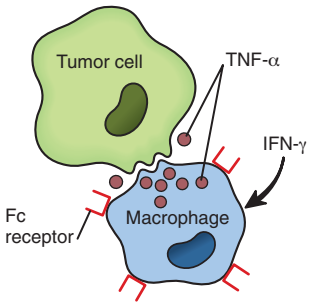
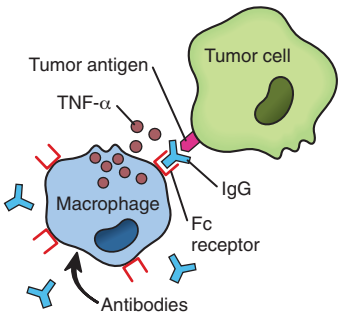
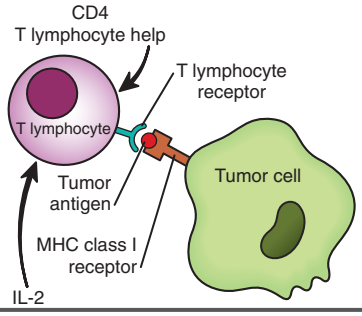
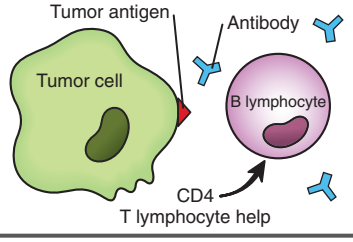
Effector Cell		Activation/ Assistance	Target	Mode of Attack
NK cell		IL-2	Stress-induced ligand	Immunologic synapse (perforin/granzymes)
NK cell (ADCC)		Antibodies produced by B lymphocytes	Tumor antigen	Immunologic synapse (perforin/granzymes)
Macrophage		IFN-γ activation	Tumor cell membrane	TNF-α, reactive oxygen species, nitric oxide, lysosomal enzymes
Macrophage (ADCC)		Antibodies produced by B lymphocytes	Tumor antigen	TNF-α, reactive oxygen species, nitric oxide, lysosomal enzymes
T lymphocyte (CTL)		Help by CD4 T lymphocyte, IL-2 activation	Tumor antigen presented in MHC class I context	Immunologic synapse (perforin/granzymes)
B lymphocyte		Help by CD4 T lymphocyte	Tumor antigen	Membrane-attack complex (activated complement)

Figure 6-24 Cells Involved in Immunosurveillance Against Tumors. Antitumor responses involve a variety of immune cells, including natural killer (NK) cells, macrophages, and T and B lymphocytes. NK cells and macrophages can attack tumor cells directly or via the mechanism of antibody-dependent cell-mediated cytotoxicity (ADCC). In ADCC, macrophages and NK cells bind tumor-specific antibodies by their constant regions, allowing the variable regions of the antibodies to interact with specific tumor antigens. Most cytotoxic T lymphocytes (CTL) are $CD8^+$ lymphocytes.

Evasion of the Immune Response

Many tumors are able to evade immunosurveillance, using one or more of mechanisms illustrated in Fig. 6-25 and discussed in the following sections.

Altered Major Histocompatibility Complex Expression

CTLs recognize tumor antigens only on tumor cells that display the antigens in the context of MHC class I molecules. Thus tumor cells that lose or downregulate expression of class I MHC antigens may evade detection and have a distinct selective advantage. However, tumors that fail to express class I antigens are also more susceptible to NK cell killing. Tumors may also downregulate expression of class II MHC antigens. Class II antigens are required for activation of T_H lymphocytes that stimulate CTL differentiation, and loss of these antigens prevents the generation of an optimal antitumor CTL response.

Antigen Masking

Tumors may become invisible to the immune system by losing or masking their tumor antigens. The outgrowth of clonal tumor variants that do not express tumor antigens is favored during tumor evolution. Tumor antigens on the cell surface may be hidden from the immune system if they are complexed with glycocalyx molecules, fibrin, or even antibodies. Thus some humoral responses to tumor antigens may actually promote tumor survival by protecting tumor antigens from recognition by CTLs.

Tolerance

Although the immune system responds vigorously to nonself-antigens, it is tolerant to self-antigens. Thus tumor antigens shared with normal tissue usually are not able to evoke an immune response because the body has already been "tolerized" to the antigen. If nonself-antigens are presented in the absence of costimulatory molecules required for effective T lymphocyte activation, that is, in a "tolerogenic" context, tolerance may also result. Moreover, it has recently been shown that T reg cells in tumors can actively promote tolerance to tumor tissue.

Immunosuppression

Tumor cells or their secretory products may be immunosuppressive. Many tumors produce TGF-α, which inhibits the proliferation and function of lymphocytes and macrophages. Tumors may also produce Fas ligand. Fas ligand expressed by tumor cells binds to Fas receptors on nearby T lymphocytes and triggers their apoptosis. By this

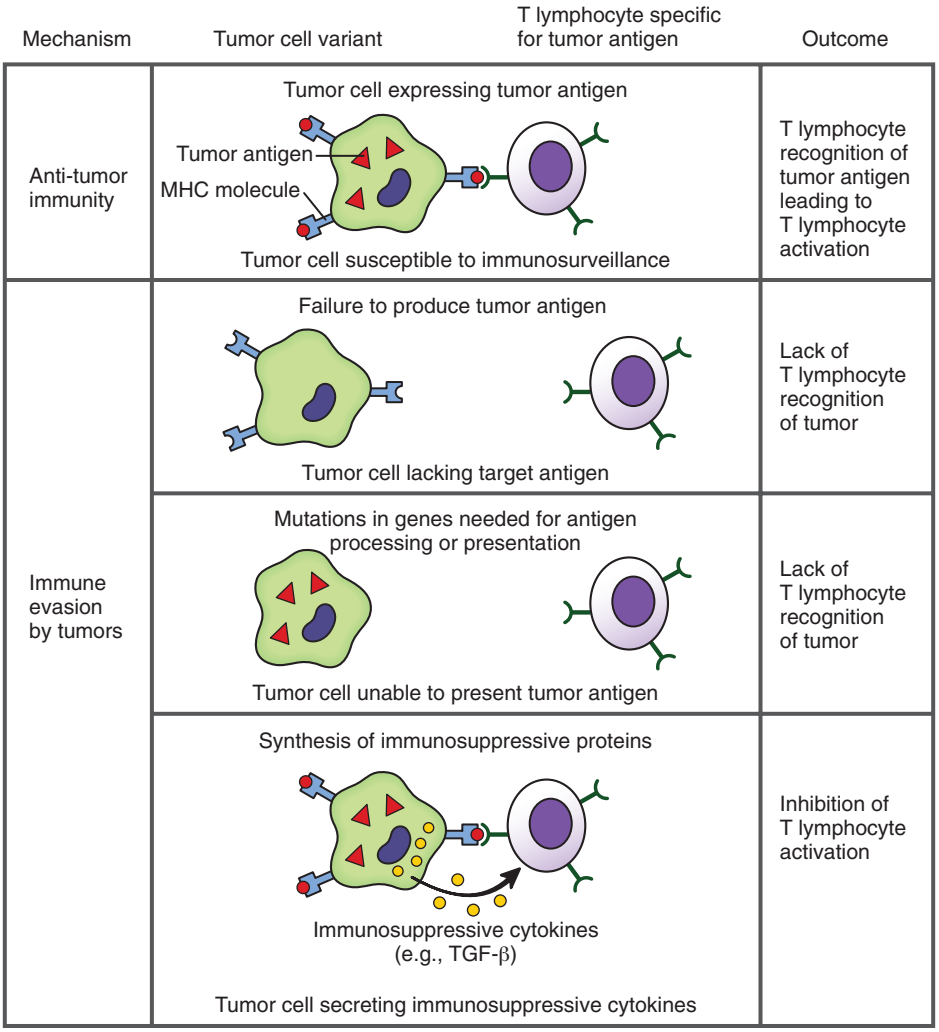


Figure 6-25 Mechanisms by Which Tumors Evade the Immune System. Tumors employ a variety of mechanisms to evade attack by cytolytic T lymphocytes. Tumor cells may cease expressing tumor antigens, fail to appropriately process these antigens, or lack expression of the major histocompatability antigen necessary to present tumor cell antigens. Tumor cells may also secrete cytokines such as transforming growth factor-β (TGF-β) that inhibit cytolytic T lymphocyte activation.

mechanism, T lymphocyte clones that recognize a tumor may be specifically deleted, thus protecting the tumor from attack. Finally, tumor cells may release tumor antigens into the circulation that form immune complexes with antibodies, and these immune complexes may be immunosuppressive. In addition, tumor-associated macrophages and T reg cells have immunosuppressive functions that protect the tumor from immune attack.

Tumor Immunotherapy

The fact that an antitumor immune response attacks only the tumor cells and not normal tissue makes it an attractive candidate as a therapeutic modality. Moreover, effective immunotherapy would reduce or eliminate the need to use highly cytotoxic chemotherapeutic agents that indiscriminately target both normal and neoplastic dividing cells and thus cause significant morbidity and mortality in cancer patients. In general, immunotherapeutic strategies are aimed at (1) providing the patient with mature effector cells or antibodies that recognize and destroy tumors (passive immunotherapy) or (2) stimulating the immune response of the animal against the tumor (active immunotherapy).

Administration of monoclonal antibodies raised against tumor antigens generates rapid but short-lived passive tumor immunity. However, the coupling of toxins to monoclonal antibodies may allow targeted delivery of therapeutic agents to tumor cells. Monoclonal antibodies raised in other species have limited usefulness, because the tumor host may develop an immune response to these antibodies that abrogates their effectiveness. Antitumor lymphocytes are generated by removing lymphocytes from the human patient's blood or tumor and expanding them in vitro by incubation with IL-2; these autologous immune cells are then readministered to the patient.

Many approaches to stimulate the active immunity of human patients against their tumors have been attempted, including vaccination with tumor cells or tumor antigens to generate antitumor CTLs, administration of cytokines to increase effector cell number and function, and nonspecific stimulation of the immune system by treatment with proinflammatory substances, such as bacterial products. These approaches have proved particularly effective against malignant melanomas. A melanoma vaccine for dogs stimulates an immune response against human tyrosinase; antibodies against the human protein are formed that then attack canine melanocytes.

As mentioned earlier, blocking the activity of tumor-associated macrophages and T reg cells is a new and promising approach to tumor immunotherapy.

Tumor Dissemination (Essential Concept 6-4)

The Significance of Metastasis

Primary tumors are not usually the proximate cause of death for the animal or human cancer patient. Instead death is usually due to tumor metastasis to distant organs and interference with critical bodily functions. For example, widespread metastases to the lung from an osteosarcoma can result in death or euthanasia due to respiratory distress caused by interference with oxygen exchange. Indeed, it has been estimated that, in human beings, tumor metastasis is responsible for approximately 90% of cancer mortality from solid tumors. Fortunately, metastasis is a rather inefficient process. Very few cells within the primary tumor are capable of entering blood or lymphatic vessels, only a few of the circulating cells are able to exit vessels, and only a few of those that exit the vessels are able to survive at new sites within the body. In some cases, metastatic cancer cells enter a state of *dormancy* during which metastatic cells grow only slowly or not at all. However, dormant cancer cells can resume their growth at a later date, leading to cancer recurrence, sometimes after prolonged periods of remission. The mechanisms by which dormancy is established and the means by which dormant cancer cells are reactivated remain poorly understood.

ESSENTIAL CONCEPT 6-4 Tumor Dissemination

A tumor first grows locally, but acquisition of additional genetic and epigenetic changes allows the tumor to metastasize. Metastasis is responsible for most cancer mortality. Metastasis may occur through lymphatic vessels, via blood vessels, or by direct dissemination throughout a body cavity. To metastasize, tumor cells must escape the primary tumor mass through loss of intercellular attachments and acquisition of migratory and invasive capabilities. To avoid detection and elimination, tumor cells must also escape immunosurveillance. Once present at a distant site, tumor cells must be able to exit vessels and establish themselves in the new tissue.

Mechanisms of Tumor Invasion and Metastasis

A tumor's metastatic potential reflects the cumulative effect of a wide variety of genetic and epigenetic changes involving tumor cell adhesion, motility, and protease production.

Adhesion

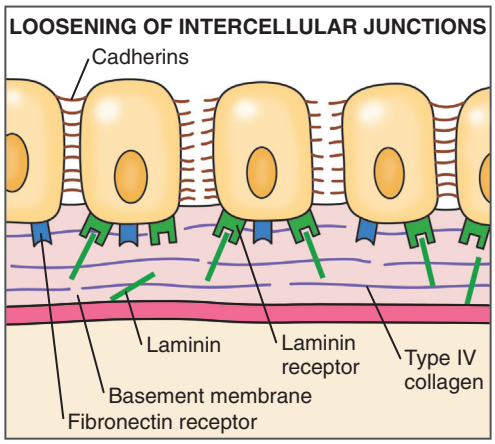
As a first event in invasion and metastasis, tumor cells must detach from the main tumor mass, penetrate the basement membrane, and enter the ECM. For cells to separate from each other, intercellular adhesion structures, including desmosomes and adherens junctions, must be dismantled. In many tumor cells of epithelial origin, this process is due to loss of cadherins or catenins, molecules that are essential structural elements of intercellular junctions. At the same time that tumor cells detach from each other, they must also establish contacts with ECM elements within the tumor stroma. Integrins and other specific receptors on tumor cell membranes recognize and bind to a variety of ECM components such as fibronectin, laminin, collagen, and vitronectin. During invasion and metastasis, carcinoma cells often express increased numbers of these receptors. Tumor cells are also able to modulate the types and distribution of ECM receptors that they express, allowing them to adapt to the ECM of different microenvironments.

Migration

At many points during invasion and metastasis, tumor cells migrate actively. This migration is mediated by alterations in the cytoskeleton and the cellular adhesion structures to which the cytoskeletal components are anchored. Tumor cell migration is stimulated by autocrine growth factors, such as hepatocyte growth factor (HGF), also called "scatter factor," and by cleavage products of ECM components, including fragments of collagen.

Stromal Invasion

Epithelial cells normally rest on a specialized extracellular structure called the basement membrane, to which they are firmly attached by hemidesmosomes (see Figs. 1-5 and 1-6). In benign epithelial tumors, the basement membrane remains intact. In contrast, the neoplastic epithelial cells of malignant tumors actively degrade basement membrane and ECM components by increasing the net protease activity in their vicinity (Fig. 6-26). This allows them to penetrate the basement membrane and invade surrounding tissue. Net protease activity is determined by a variety of interacting factors, including the rate of protease synthesis and activation and



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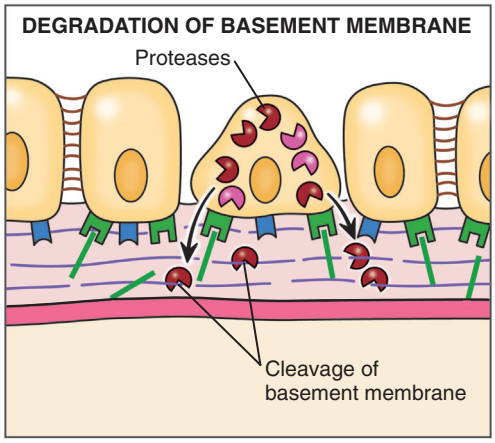

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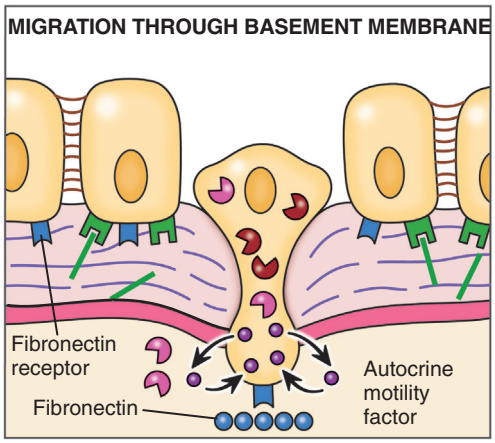


C

Figure 6-26 Tumor Cell Invasion of Epithelial Basement Membrane. **A,** Tumor cells detach from one another because of dissolution of intercellular junctions. **B,** Proteolytic enzymes, such as type IV collagenase and urokinase, secreted by tumor cells degrade the basement membrane. **C,** With degradation of the basement membrane, tumor cells are able to migrate into underlying tissue. Migration is enhanced by altered expression of receptors for extracellular matrix components on tumor cells and secretion of autocrine motility factors.

the rate at which protease inhibitors are produced. Proteases and antiproteases may be produced and activated by the tumor cells themselves, or tumor cells may induce nonneoplastic stromal cells to produce these enzymes. Proteases implicated in promoting tumor metastasis include matrix metalloproteinases, such as type IV collagenase, and urokinase, a serine protease.

Epithelial-Mesenchymal Transition

As they progress, some carcinomas undergo a change termed *epithelial-mesenchymal transition* (EMT). EMT is characterized by loss of intercellular adhesion structures, enhanced expression of proteases, acquisition of migratory capabilities, reduced expression of epithelial cytokeratins, and de novo expression of vimentin, a mesenchymal cell marker. During EMT, sessile neoplastic cells are transformed into motile fibroblast-like cells. Cells that have undergone EMT are typically spindle shaped and express little or no E-cadherin, a component of adherens junctions. EMT allows neoplastic epithelial cells to dissociate and migrate, thus fostering local invasion and distant metastasis. Most drivers of EMT are transcription factors, proteins that determine the timing and levels of DNA transcription into RNA. These transcription factors coordinate the expression of genes involved in cellular adhesion, migration, and protease production.

Intravasation

Cancer cells invade blood or lymphatic vessels by penetrating endothelial basement membranes and passing between or through endothelial cells into the vessel lumen (Fig. 6-27). This process is termed *intravasation*. Tumor cells are attracted to vessels by chemotactic factors produced by multiple cell types and migrate through the ECM with the aid of tumor-derived proteases. Tumor cell migration and vessel penetration are facilitated by tumor-associated macrophages that accompany the invading tumor cells.

Tumor Emboli

Once inside a lymphatic or blood vessel, tumor cells tend to clump together to form small emboli held together by shared adhesion molecules. While in the vessels, tumor cells may be recognized and attacked by host lymphocytes or may be surrounded by platelets. Interestingly, platelets may actually protect the tumor embolus from immune-mediated destruction, thereby increasing the potential for metastasis.

Extravasation

Intravascular tumor cells leave vessels by the process of *extravasation*. The site at which tumor cells exit the blood vascular or lymphatic system is largely determined by the ability of tumor cells to interact with adhesion molecules on endothelial cells. Once attached to vascular endothelium, tumor cells pass between or through endothelial cells and penetrate the basement membrane to enter the ECM, thus establishing a metastatic site. Metastatic sites must provide a suitable microenvironment for tumor cell growth, or metastatic tumor cells will not become established. Some tumors preferentially metastasize to specific sites; for example, prostate carcinomas in both human beings and dogs frequently spread to bone (Fig. 6-28).

Pathways of Tumor Metastasis

Lymphatic Spread

Most carcinomas and some sarcomas metastasize via the lymphatic system. The pattern of lymph node involvement is usually dictated by preexisting routes of normal lymphatic drainage. The lymph nodes closest to the tumor are usually affected first and develop the

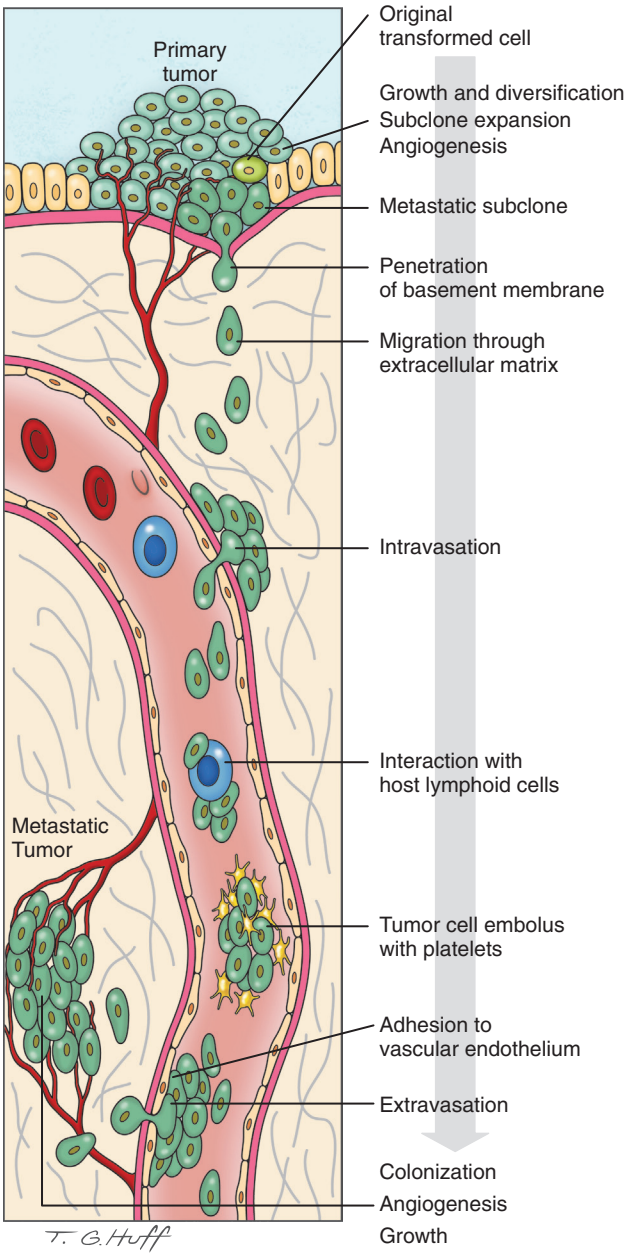


Figure 6-27 The Metastatic Cascade. Although this figure illustrates the sequential steps in the hematogenous spread of an epithelial tumor, similar steps occur during lymphatic spread. Metastasis is a complex process involving multiple steps and interactions of tumor cells with many different normal cell and tissue types. Failure at any point in the metastatic process prevents tumor spread; thus metastasis is usually an inefficient process.

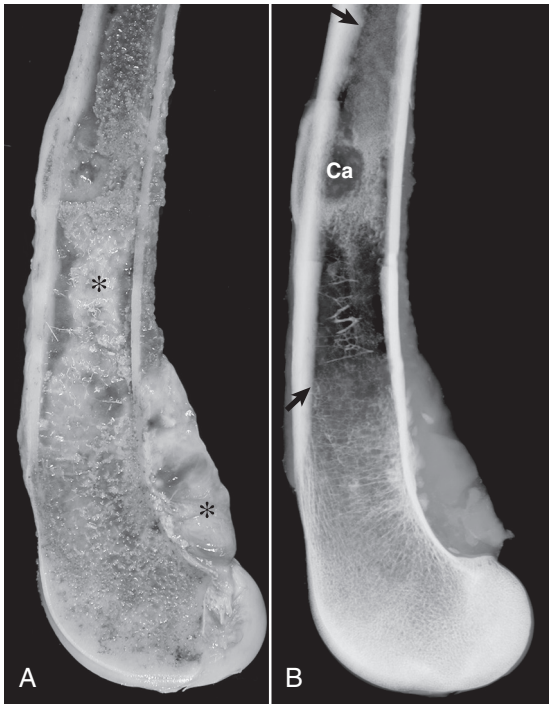


Figure 6-28 Prostate Carcinoma, Metastasis, Femur, Dog. A, The gross photograph of a sectioned femur reveals metastatic prostate carcinoma (*). B, The radiograph illustrates an osteolytic bone metastasis (*Ca*). In the region between the arrows, extensive proliferation of new bone has occurred in response to the tumor. (Modified from Rosol TJ, Tannehill-Gregg SH, LeRoy BE, et al: Animal models of bone metastasis. In Keller ET, Chung LWK, editors: *Cancer treatment and research*, Boston, 2004, Kluwer Academic Publishers.)

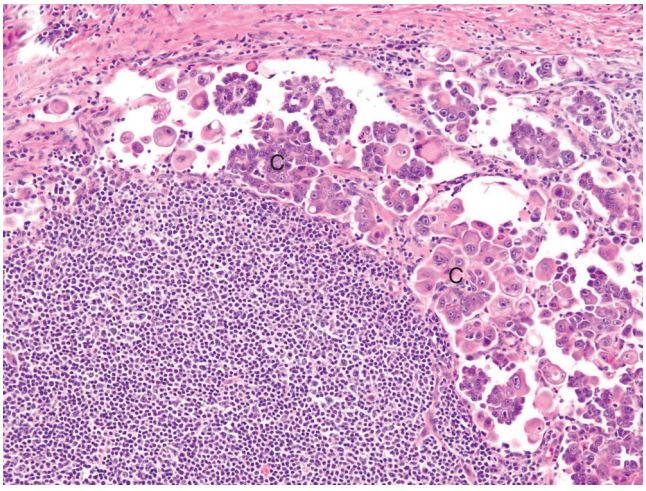


Figure 6-29 Mammary Carcinoma, Metastatic, Regional Lymph Node, Dog. Mammary carcinoma cells (C) are present in the subcapsular sinus of a lymph node that drains the affected mammary gland. Tumor cells spreading via lymphatic vessels typically lodge first at this location in lymph nodes draining the tumor site. H&E stain. (Courtesy College of Veterinary Medicine, The University of Tennessee.)

largest metastatic tumor masses (Fig. 6-29). For example, adenocarcinomas of the intestine usually metastasize first to the mesenteric lymph nodes and later to other lymph nodes within and outside the abdominal cavity. For many years it was assumed that cancers spread in a stepwise manner from the primary site to regional lymph nodes, then to distant sites such as the lung, and that regional lymph nodes acted as a mechanical barrier to the spread of cancer. Based on this assumption, it was believed that removal of all affected regional lymph nodes would prevent further spread of the tumor. However, regional lymph nodes may be bypassed as a result of natural, tumor-related, or treatment-induced anomalies in lymphatic drainage, resulting in distant metastases before the development of regional metastases. More recent studies suggest that lymphatic spread does not occur in an orderly fashion and that metastasis to regional lymph nodes indicates that systemic spread has likely already occurred.

Hematogenous Spread

Because lymphatic vessels connect with the vascular system, the distinction between lymphatic and hematogenous spread is

somewhat artificial. However, sarcomas do tend to use the hematogenous route of spread more frequently than carcinomas. Tumors generally invade veins rather than arteries because venous walls are much thinner and easier to penetrate than arterial walls. Tumor cells that enter veins ultimately reach the vena cava, pass through the heart, and lodge in capillary beds, particularly in the lungs (Fig. 6-30). Tumors that invade portal vessels tend to lodge in the liver. Some tumors have a notable predilection for invading veins; for example, pheochromocytomas, particularly those arising from the right adrenal gland, frequently invade the adjacent caudal vena cava (see Figs. 12-40 and 12-41).

Transcoelomic Spread

When cancers arise on the surface of an abdominal or thoracic structure, they encounter few anatomic barriers to spread. Thus mesotheliomas may be confined to the peritoneal, pericardial, or pleural cavities, but the tumor cells within these cavities readily spread to cover visceral and parietal surfaces. In both human beings and dogs, ovarian and pancreatic adenocarcinomas preferentially spread transcoelomically, resulting in multiple tumor masses throughout the abdomen, a condition termed *carcinomatosis* (Fig. 6-31). Even in the absence of invasion into the underlying organs, tumors such as mesotheliomas and ovarian and pancreatic adenocarcinomas are extremely difficult to treat and are generally fatal.

Metastasis Suppression

Expression of some gene products in tumor cells appears to suppress metastasis. For example, sustained expression of E-cadherin, a transmembrane protein that forms part of adherens junctions, maintains adherence between tumor cells and prevents them from dissociating to invade surrounding tissues and lymphatic vessels. The E-cadherin gene is thus a candidate metastasis suppressor gene. Determining the expression levels of metastasis suppressor genes in tumors may provide valuable prognostic information. Moreover, drug-induced reactivation of metastasis suppressor genes is a potentially valuable therapeutic strategy.

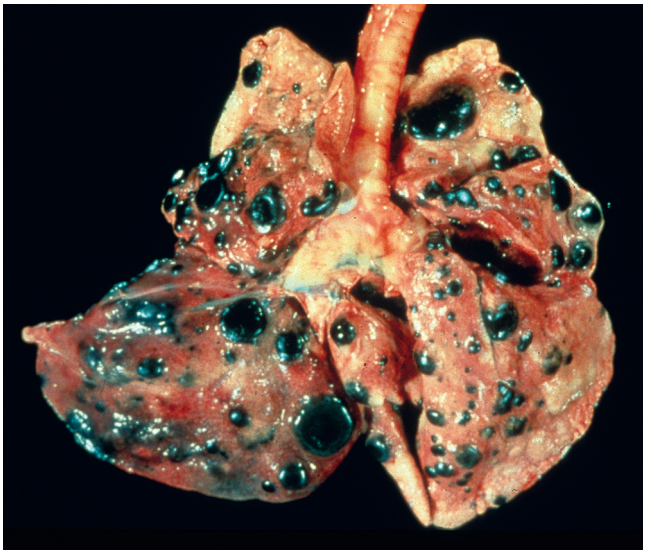


Figure 6-30 Melanoma, Metastatic, Lung, Dog. The multifocal (embolic) distribution of tumor nodules throughout the lung is characteristic of hematogenous metastasis. (Courtesy College of Veterinary Medicine, The University of Tennessee.)

Transmissible Tumors

A few tumors, termed *clonally transmissible cancers*, have been shown to spread beyond the original host via physical transplantation following direct physical contact between animals of the same species. Examples include the transmissible venereal tumor (TVT) of dogs and devil facial tumor disease (DFTD) of Tasmanian devils. In these syndromes, tumors isolated from multiple affected animals have essentially identical cytologic and genetic characteristics, which differ from those of the hosts. This finding indicates that all of the tumors arose from a single tumor that was subsequently disseminated to multiple animal hosts. Because TVT transmission occurs during mating, the tumors are found on the genitalia or face. DFTD is transmitted during territorial fighting; thus it tends to occur on the head and neck.

Systemic Clinical Effects on the Animal (see Essential Concept 6-3)

Direct Effects

Tumors directly compromise the function of the organs in which they arise by replacing (effacing) normal tissue and by disrupting normal anatomic relationships of affected organs. In both the tissue of origin and in metastatic sites, expanding tumor tissue may compress surrounding normal tissue or the blood vessels that supply this tissue, resulting in pressure atrophy or necrosis. This situation is particularly a problem in the calvarium, where an expanding tumor will quickly compress and damage the brain, because the overlying bone cannot expand to accommodate the growth of the tumor. Therefore even benign tumors arising in the brain that are not surgically accessible may prove fatal. Seizure activity is a common manifestation of brain tumors. Tumor invasion into the wall of a hollow

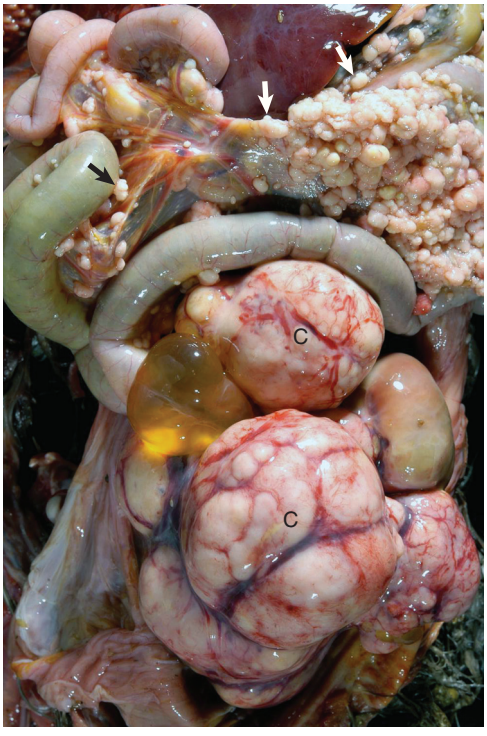


Figure 6-31 Ovarian Carcinoma, Coelomic Cavity, Chicken. This figure illustrates transcoelomic spread of an ovarian carcinoma. The primary tumor (*C*) has given rise to multiple tumor nodules (*arrows*) throughout the coelomic cavity. This condition is termed "carcinomatosis." (Courtesy College of Veterinary Medicine, The University of Tennessee.)

organ, such as the stomach, may create an obstruction or lead to organ rupture. Tumors may also erode blood vessel walls, causing acute hemorrhage, or extend into blood vessels, creating tumor emboli that may produce infarcts or metastases at distant sites.

Paraneoplastic Effects

In addition to the direct effects discussed earlier, tumors may cause a variety of systemic clinical signs termed *paraneoplastic syndromes*. Paraneoplastic disorders are indirect and usually remote effects caused by tumor cell products rather than by the "mass effect" of the primary tumor or its metastases. Approximately 75% of human cancer patients develop paraneoplastic syndromes, but the incidence in veterinary cancer patients is unknown. These syndromes are best described for the dog, although some affecting the cat and the horse have also been reported (E-Table 6-1). Recognition of paraneoplastic syndromes is important because (1) these syndromes may facilitate early tumor diagnosis if they arise in the initial stages of tumor development, (2) treatment of metabolic abnormalities associated with paraneoplastic syndromes may be required to ensure effective cancer management, and (3) the severity of paraneoplastic abnormalities may reflect the tumor burden; thus monitoring such abnormalities may be useful in determining tumor response to therapy and identifying tumor recurrence or spread.

Cachexia

Many animals with cancer show notable weight loss and debility, a condition referred to as *cachexia*. In cancer cachexia, both muscle and fat are lost, whereas in simple starvation fat is lost preferentially. The compensatory decrease in basal metabolic rate seen with starvation is not observed in cancer cachexia. Extra calories do not prevent or reverse the catabolic state of cancer cachexia. The etiology of cancer cachexia is complex. Cancer cachexia is due, in part, to cytokines and hormones, particularly TNF-α (also known as cachectin), IL-1, IL-6, and prostaglandins, which cause anorexia and debilitation. Other contributing factors include impaired digestion, nutritional demands of tumor tissue, nutrient loss in cancer-related effusions or exudates, and a variety of metabolic and endocrine derangements.

Endocrinopathies

Endocrine Tumors. A functioning endocrine tumor produces the hormonal products of the tissue of origin. For example, thyroid follicular tumors produce thyroid hormone. In endocrine glands with more than one cell type, such as the pancreatic islet, the anterior pituitary, the thyroid, and the adrenal, generally only a single cell type becomes neoplastic. Thus a pancreatic islet cell adenoma typically produces only a single hormone, such as insulin, glucagon, gastrin, or somatostatin, and not a combination of hormones. A functional tumor overproduces a hormone as the consequence of increased numbers of hormone-secreting tumor cells, increased production of hormone by individual neoplastic cells, or both.

Several clinically significant endocrinopathies occur commonly in veterinary medicine, and their effects and clinical presentations depend on the hormones being produced. Thyroid follicular adenomas in cats cause a syndrome of hyperthyroidism characterized by an increased metabolic rate. Functioning tumors of the pancreatic islet β-cells result in hyperinsulinemia with subsequent hypoglycemia. Because of the absolute dependence of the nervous system on glucose for energy, clinical signs of hypoglycemia are mostly neurologic and may include lethargy, incoordination, muscle weakness, and seizures. Profound hypoglycemia of unknown origin may also occur with other tumor types.

Nonendocrine Tumors. A variety of nonendocrine neoplasms may also produce hormonally active substances not normally found in the tissue of tumor origin. This relationship is termed *ectopic hormone production*. The hormone produced may be identical to the normal hormone, may be a modified form of the normal hormone, or may be the product of a gene that encodes a protein related to but not identical with the true hormone. In veterinary medicine the most common example of ectopic hormone production is secretion of parathyroid hormone–related peptide (PTHrP) by tumor cells, resulting in humoral hypercalcemia of malignancy. In dogs, *humoral hypercalcemia of malignancy* is seen most frequently with adenocarcinoma of the anal sac (≈90% of cases), lymphoma (≈20% of cases), and multiple myeloma (≈15% of cases). Hypercalcemia of malignancy in cats appears to be relatively rare. Like parathyroid hormone, PTHrP increases serum calcium level by increasing calcium release from bones, enhancing reabsorption of calcium in the kidneys, and stimulating absorption of calcium in the intestine. Clinical signs of hypercalcemia include muscle weakness, cardiac arrhythmia, anorexia, vomiting, and renal failure. Hypercalcemia and associated clinical signs may also occur as a result of excess production of parathyroid hormone by a parathyroid neoplasm. Hypercalcemia may also be due to tumor metastasis to bone and resultant bone resorption; however, this is not a true paraneoplastic disorder because it is a direct effect of the tumor.

Skeletal Syndromes

Hypertrophic osteopathy is a condition associated with extensive periosteal new bone growth, particularly on the extremities (Fig. 6-32). It is strongly associated with both neoplastic and nonneoplastic space-occupying thoracic lesions. This condition, which is seen in cats, dogs, and horses, presents as symmetric lameness. The cause of hypertrophic osteopathy is not known, although abnormalities of growth hormone production are suspected.

Another skeletal manifestation of neoplasia is *myelofibrosis*. Myelofibrosis results from overgrowth of nonneoplastic fibroblasts in

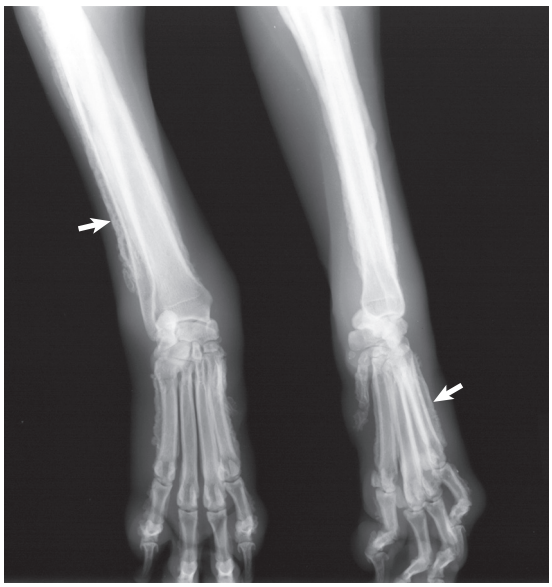


Figure 6-32 Hypertrophic Osteopathy, Forelimbs, Dog with a Pulmonary Tumor. On this radiograph the arrows indicate newly deposited bone that is less dense than normal cortical bone. Note that multiple bones on both limbs are affected and that new bone deposits are located primarily in the diaphyseal region of the long bones. (Courtesy Dr. J. Mattoon, College of Veterinary Medicine, The Ohio State University.)

E-Table 6-1 Paraneoplastic Syndromes in Animals

System	Syndrome
Systemic	Anorexia/cachexia
	Fever
Endocrine	Hypercalcemia
	Hypoglycemia
	Hyperestrogenism
	Hypergastrinemia
	Thyrotoxicosis
	Hyperhistaminosis
	Hypercatecholaminemia
	Cushing's disease
Skeletal	Hypertrophic osteoarthropathy
	Myelofibrosis
Vascular/ hematopoietic	Leukocytosis
	Leukopenia
	Thrombocytosis
	Thrombocytopenia
	Erythrocytosis
	Anemia
	Eosinophilia
	Disseminated intravascular coagulation
	Hyperviscosity syndrome
Neurologic	Peripheral neuropathy
	Myasthenia gravis
Cutaneous	Alopecia
	Nodular dermatofibrosis

Modified from McCullen JM, Page R, Misdorp W: An overview of cancer pathogenesis, diagnosis, and management. In Meuten DJ, editor: *Tumors in domestic animals,* ed. 5, Hoboken, NJ, 2010, John Wiley and Sons.

the bone marrow, which impairs normal hematopoiesis and results in cytopenias. It may be associated with a local myeloproliferative disease like lymphoma or with distant tumors. The cause of myelofibrosis is also unknown.

Vascular and Hematologic Syndromes

Nonhematopoietic cancer in animals may result in a variety of vascular and hematologic syndromes, including eosinophilia and neutrophilia. The cause of these conditions is unclear, but they are likely due to alterations in circulating cytokine concentrations. Anemia is commonly seen in veterinary cancer patients. There are numerous potential causes for anemia in these animals, including anemia of chronic disease, bone marrow invasion, myelofibrosis, blood loss, and hemolysis. Polycythemia associated with ectopic production of erythropoietin has been reported. Thrombocytopenia is seen in approximately one-third of all dogs with cancer. Thrombocytopenia may be due to rapid consumption of platelets. For example, *disseminated intravascular coagulation* (DIC) leading to thrombocytopenia and concurrent anemia is frequently seen in dogs with hemangiosarcoma. In addition, platelets may display surface antigens similar to tumor antigens; antibodies directed against tumor antigens then cross-react with platelet antigens, resulting in an immune-mediated thrombocytopenia (see Chapter 5). Excessive immunoglobulin production by tumors, particularly monoclonal gammopathies caused by multiple myeloma, can result in massive hyperproteinemia and *hyperviscosity syndrome*, manifested as altered neurologic function, congestive heart failure, or bleeding disorders.

Miscellaneous Syndromes

Information on this topic, including E-Fig. 6-1, is available at www.expertconsult.com.

Heritable Alterations in Cancer (Essential Concept 6-5)

Cancer occurs as the result of the progressive accumulation of genetic and epigenetic abnormalities in cells. These abnormalities lead to changes in cell growth, cell death, apoptosis, alterations in cellular differentiation, defective DNA repair, and dysfunction of other critical pathways, endowing the cancer cell with its neoplastic characteristics. Alterations in DNA sequence, termed *mutations*, result from inaccurate DNA repair and are passed along to all progeny of the cancer cell (see Chapter 1). Some epigenetic alterations may also persist over multiple cell divisions. Thus, a cancer phenotype is *heritable*. Specific genes that play important roles in cancer development are discussed in a later section of this chapter.

ESSENTIAL CONCEPT 6-5 Heritable Alterations in Cancer

Cancer occurs as the result of the progressive accumulation of heritable genetic and epigenetic abnormalities in cells. The initial change in genetic material may be due to inherited germline mutations or to somatic mutations acquired as the result of DNA damage by a chemical carcinogen, radiation, or an oncogenic virus. Genetic changes that activate oncogenes like the *ras* genes or inactivate tumor suppressor genes like the *p53* gene are considered to be the mutations that drive cancer development. However, preexisting modifier genes, for example, genes that encode DNA repair enzymes, may affect cancer susceptibility and development, although less dramatically.

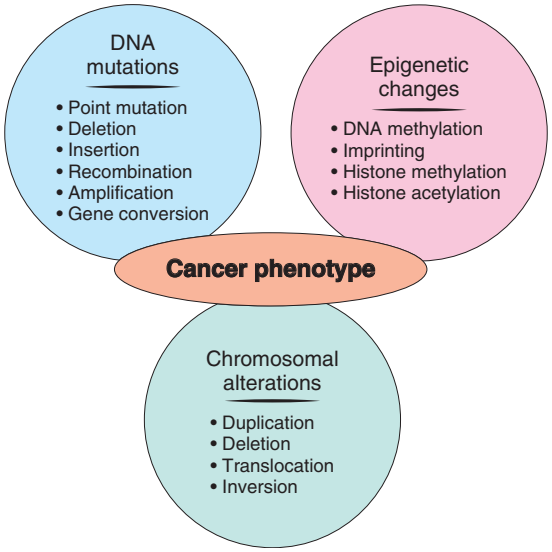


Figure 6-33 Heritable Alterations Contributing to Carcinogenesis. Many genetic changes caused by extrinsic and intrinsic DNA-damaging agents, normal physiologic processes, and aging alter the amino acid sequences of encoded proteins and the levels at which these proteins are expressed. It is these interacting alterations that are ultimately responsible for the neoplastic phenotype. (Redrawn with permission from Dr. D.F. Kusewitt, Health Sciences Center, University of New Mexico.)

Genetic Changes in Cancer

As illustrated in Fig. 6-33, DNA is susceptible to many types of chemical and physical alterations. Some of these alterations are caused by injurious endogenous and exogenous agents. In addition, DNA alterations also occur as part of normal processes of genome replication, repair, and rearrangement.

Point Mutations

DNA damage alone does not constitute mutation. However, when a DNA strand containing unrepaired or misrepaired damage is used as a template for the synthesis of a complementary DNA strand, DNA polymerases may insert an incorrect base in the newly synthesized DNA strand. The altered base sequence is reproduced in all subsequently synthesized DNA. This process is known as *mutation fixation*; at least one and sometimes two rounds of replication are required for mutations to become fully fixed in the genome.

If a point mutation occurs in an exon or at a splice site of a protein-coding gene, it may lead to an altered amino acid sequence in the gene product. A mutation located in a noncoding region of a gene may affect the level of gene transcription or the stability of the transcribed RNA, thus resulting in an altered level of expression of the encoded protein. Altered protein expression in turn may contribute to neoplastic transformation, tumor growth, invasion, and metastasis.

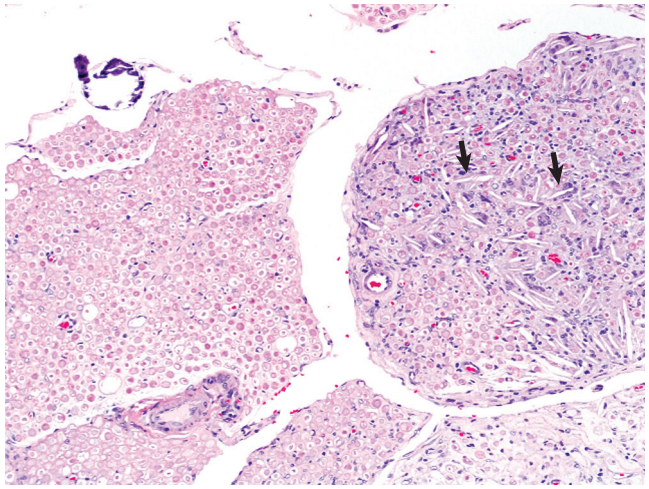
DNA Strand Breaks

Single- and double-strand breaks in DNA are caused by physical and chemical agents and viruses; they may also occur during normal physiologic processes such as recombination of immunoglobulin genes and T lymphocyte receptor genes. Although single-strand breaks are usually readily repaired, they sometimes trigger gene

Neurologic Syndromes. Paraneoplastic neurologic disease in veterinary cancer patients is usually related to hypercalcemia, hypoglycemia, or hyperviscosity and is often manifest as seizure activity. Primary peripheral nervous system disease has also been reported. In many dogs with cancer there is microscopic evidence of peripheral neuropathy (E-Fig. 6-1); however, clinical signs of disease, such as areflexia, muscle weakness, reduced muscle tone, or paralysis, are much less commonly reported. Myasthenia gravis is occasionally seen in veterinary cancer patients, usually in association with a mediastinal tumor such as thymoma. The underlying defect in myasthenia gravis is a failure of nerve impulse transmission at neuromuscular junctions. Clinical signs include muscle weakness, reduced exercise tolerance, and dysphagia. Many neurologic paraneoplastic syndromes in human beings are immune mediated, and the pathogenesis is likely to be similar in animals as well.

Cutaneous Syndromes. There are only a few reports of cutaneous manifestations of paraneoplastic disease in dogs and cats. Clinical signs of flushing, alopecia, or necrolytic dermatitis have been reported to be associated with a variety of tumor types. The syndrome of nodular dermatofibrosis in German shepherd dogs is a heritable disorder characterized by multiple benign-appearing fibrous nodules in the skin in conjunction with bilateral renal cystadenocarcinomas.

Other Syndromes. Mast cell tumors are very common in dogs. Release of excess histamine from the neoplastic mast cells can cause paraneoplastic gastrointestinal ulceration and hemorrhage. Similarly, gastrin-secreting tumors in dogs and cats can cause gastroduodenal ulceration, abdominal pain, vomiting, and blood loss.



E-Figure 6-1 **Peripheral Neuropathy, Lumbar Spinal Cord, Mixed-Breed Dog with a Jejunal Adenocarcinoma and Abdominal Carcinomatosis.** Nerve roots are markedly expanded due to variable axonal swelling, inflammation, and demyelination, or stripping of myelin sheaths from axons, which resulted in lameness and bilateral hind limb muscle atrophy in this patient. Tumor-associated demyelination and resultant cholesterol cleft formation *(arrows)* in the nerve roots (demyelinating radiculopathy) is thought to occur as an immune-mediated response to tumor antigens from masses arising in other organs, as in the intestine in this case, that mimic antigens expressed normally in neural tissues. (Courtesy Dr. E.M. Brannick, College of Agriculture and Natural Resources, University of Delaware.)

conversion, which is the replacement of a gene or part of a gene by DNA derived from a closely related gene. Gene conversion is one mechanism by which animals routinely generate diversity in large families of related genes, for example, genes encoding MHC antigens. Double-strand breaks produce unprotected, recombinogenic DNA ends and often lead to major chromosomal anomalies, including deletions and translocations. Clearly, such large-scale chromosomal changes have the potential to alter the gene expression repertoire of a cell in a dramatic fashion.

Insertions and Deletions

Insertions, or additions, of DNA bases into the genome may be as small as a single base or larger than a viral genome. *Deletions* involve loss of a DNA segment and range in size from one base pair to an entire chromosome arm. Heterozygous deletions occur on only one chromosome, whereas homozygous deletions occur on both chromosomes. Small deletions or insertions of one or two base pairs cause a shift in the reading frame during protein synthesis, a process termed *frameshift mutation*. Such mutations may alter the protein-coding sequences downstream from the site of deletion, eliminate or create splice sites, or generate premature stop codons, resulting in modified or truncated proteins (see Chapter 1).

Retroviral genomes replicate only after they insert into the animal genome, and these large insertions can interrupt the coding sequence of animal genes, abrogating their expression or leading to the production of abnormal gene products. On the other hand, juxtaposition of viral promoter elements adjacent to cellular coding sequences of the host can lead to dysregulated, often markedly increased, expression of cellular genes that drive tumorigenesis. Perhaps the best-studied example of insertional activation of host animal genes by a retrovirus is the mouse mammary tumor virus, which can integrate "upstream" of a variety of cellular genes to enhance their expression, leading ultimately to the formation of mammary adenocarcinomas.

Amplifications

Genomic *amplification* results in the presence of more than one copy of a DNA sequence. The amplified region can involve large segments of a chromosome and encompass millions of base pairs. Alternatively, the amplified region may be very small and contained within a portion of a single gene, such as the internal tandem duplication of the c-kit gene in canine mast cell tumors.

Unscheduled amplification of DNA segments is a poorly understood process by which multiple rounds of localized DNA replication produce hundreds or thousands of copies of DNA segments up to several megabases in length. Expansion or contraction of small regions of tandemly repeated DNA sequences can occur as the result of DNA polymerase slippage during replication.

Aneuploidy

Many cancer cells have abnormal numbers of chromosomes, a condition termed *aneuploidy*. Aneuploidy often results from deletion or duplication of one or multiple chromosomes or chromosome segments. Alterations in chromosome number are largely the result of mistakes in chromosome segregation caused by multipolar spindles, centrosome amplification, kinetochore malfunction, or abnormal cytokinesis. Cytogenetic analysis can determine the copy number of each chromosome. *Monosomy* is the term used when only one copy of a chromosome is present, instead of the usual two. *Trisomy* is the term used when three copies of a chromosome exist. For example, one-quarter of canine lymphomas show trisomy of chromosome 13. In mice, trisomy of chromosome 15 occurs in almost all T lymphocyte lymphomas and leukemias, suggesting that overexpression of a gene or genes on this chromosome plays an important role in tumor development.

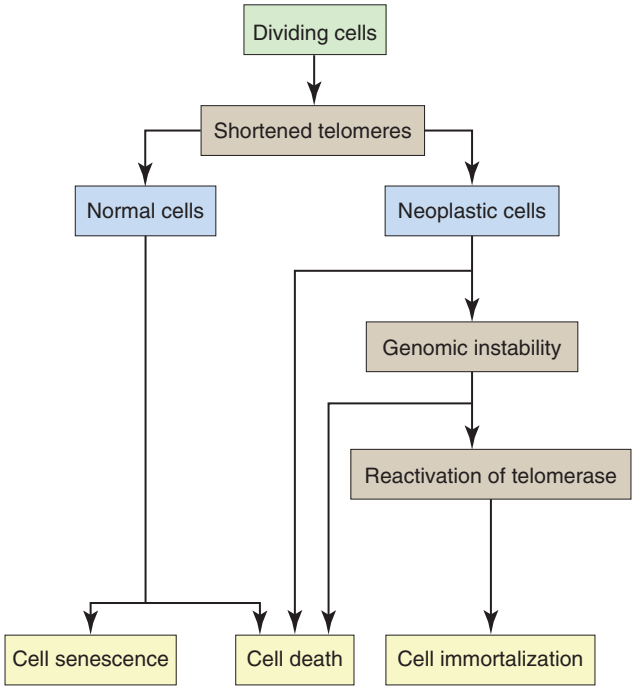


Figure 6-34 Cellular Responses to Telomere Shortening. This figure illustrates the difference between normal and neoplastic cells in their response to telomere shortening. In normal cells telomere shortening leads to cell death or senescence. In neoplastic cells, however, telomere shortening may lead to genomic instability, telomerase reactivation, and cell immortalization. (Redrawn with permission from Dr. D.F. Kusewitt, Health Sciences Center, University of New Mexico.)

Chromosomal Instability

The number and arrangement of chromosomes, termed the *karyotype*, of many tumor cells are extremely abnormal. These alterations are the result of chromosomal instability. In tumors with notable chromosomal instability, each cell may have a different karyotype and exhibit a remarkable array of duplications, deletions, and translocations. *Translocations* occur when pieces of two separate chromosomes break off and reattach inappropriately. As a result of the abnormal position of genes on the rearranged chromosomes, many cell processes are markedly disturbed.

Chromosomal instability often occurs when normal processes of DNA repair are disrupted. Dysfunctional telomeres also contribute to chromosomal instability (Fig. 6-34). Telomeres are DNA sequences that make up the ends of the chromosomes and help protect the DNA from damage. The precise mechanisms by which an intact DNA damage response and normal telomerase activity maintain chromosomal integrity are unclear.

In some cases, specific chromosomal abnormalities are associated with specific disease entities. The best-studied example of this is a reciprocal translocation between chromosomes 9 and 22 that yields an abnormal chromosome called the Philadelphia chromosome and results in chronic myeloid leukemia in human beings. This translocation fuses portions of the *BCR* and *ABL1* genes; the fusion gene thus produced encodes an abnormal protein that is responsible for the neoplastic transformation of myeloid cells.

Germline Mutations and Cancer Syndromes

Germline mutations affecting oncogenes or tumor suppressor genes are heritable. These mutations are transmitted to offspring and are present in all cells of affected progeny. Human families and genetically related animals with germline mutations that result in the development of a specific spectrum of tumor types are said to have a *cancer syndrome*. Inherited cancer syndromes due to germline mutations account for less than 10% of tumors in human beings. Characteristics of these heritable familial cancers include an early age of onset, formation of bilateral tumors in paired organs like the kidneys, occurrence of multiple primary tumors in unpaired organs like the colon, and a family history of cancer. Cancer syndromes generally show an autosomal dominant pattern of inheritance. However, some cancer syndromes have a recessive mode of inheritance. In such syndromes the affected individual must inherit the genetic defect from both parents. For instance, the mutant *ter* gene carried by strain 129/Sv-ter mice confers high susceptibility to testicular teratoma when present in the homozygous state but not when carried in the heterozygous condition.

Well-known inherited cancer syndromes in human beings include germline mutations of p53 in Li-Fraumeni syndrome associated with multiple tumor types, mutations of *NF1* and *NF2* that lead to neurofibromatosis, mutations of *BRCA1* and *BRCA2* associated with breast and ovarian cancers, and mutations in *MEN1* and *RET*, which are linked to multiple endocrine neoplasia. A well-documented veterinary cancer syndrome is the disease with the unwieldy name "hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis" that occurs in the German shepherd dog. This disease is characterized by bilateral and multifocal renal tumors, uterine leiomyomas, and nodules in the skin (dermatofibrosis). The gene responsible has been mapped to a locus homologous to the human *BHD* locus; mutations in *BHD* cause a phenotypically similar human disease.

Acquired Somatic Mutations and Sporadic Cancers

In contrast to germline mutations, acquired *somatic mutations* are restricted to individual cells and the progeny of these cells. Such somatic mutations are responsible for sporadic tumors in the general population. Somatic mutations accumulate over time; thus the risk for cancer increases with age (Fig. 6-35). Somatic genetic alterations are caused by both intrinsic metabolic processes and extrinsic mutagens.

Epigenetic Changes in Cancer

In addition to the genetic changes that occur in cancer cells, there are also many epigenetic changes. The term *epigenetic* refers to a heritable change in gene expression in somatic cells resulting from something other than a change in the DNA sequence. Epigenetic alterations have recently come to light as being major players in tumor biology. The most frequently studied epigenetic changes are DNA cytosine methylation and histone modifications. These epigenetic modifications can enhance or suppress gene expression and can be transmitted to daughter cells during cell division. Although DNA methylation and histone modifications are carried out by normal cellular enzymes, the activity and specificity of these enzymes can be altered by exogenous agents such as carcinogens. Although epigenetic changes are usually stable and are readily transmitted from tumor cells to their progeny, they can be modulated or reversed by pharmacologic agents. This response makes them attractive targets for therapeutic intervention designed to restore gene expression to its normal state.

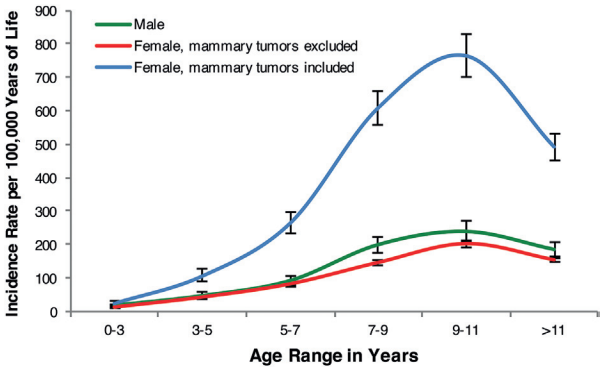


Figure 6-35 Cancer Incidence by Age in Dogs. This figure shows the incidence of tumors per 100,000 years of life for male *(green line)* and female *(red and blue lines)* dogs. The tumor incidence in female dogs is shown both for all tumors *(blue line)* and for all tumors with mammary tumors excluded *(red line)*. The difference between the incidence of all tumors in females dogs and the incidence of tumors exclusive of mammary gland tumors in females indicates the very large contribution of mammary gland tumors to overall tumor incidence in female dogs, especially if intact. For both sexes the tumor incidence increases with age until the age of 11 years. (Data courtesy Dr. D.F Merlo, National Cancer Research Institute, Genoa, Italy [Merlo DF, et al: *J Vet Intern Med* 22:976-984, 2008].)

DNA Methylation

DNA methylation involves the addition of a methyl group to carbon 5 of cytosine on cytosines located immediately 5′ to guanine (CpG dinucleotide). Methylation is essential for regulating gene expression in normal cells and is carried out by various methyltransferase enzymes. In general, hypomethylation of genes, particularly of promoter regions, leads to gene activation, whereas hypermethylation results in gene silencing. Cancer cells have lower levels of methylation in the genome, termed *global hypomethylation*, with a paradoxical increase in gene-specific methylation, termed *hypermethylation*, of clusters of CpG sites located in the promoter or first exon of genes (Fig. 6-36). Aberrant promoter methylation has been found in every type of human cancer studied.

Histone Modification

DNA is wound around histones to form chromatin (see E-Fig. 1-22). Loosely packed chromatin termed *euchromatin* is said to be in an open configuration; in this configuration, DNA is accessible to transcription factors. Chromatin in a closed, compact configuration is termed *heterochromatin*; in this state, DNA is inaccessible to transcription factors. Posttranslational histone modifications, such as acetylation, methylation, and phosphorylation, alter the transcription of associated DNA. These posttranslational histone modifications form the "histone code" that plays an important role in determining which genes are expressed and the level at which these genes are expressed. For example, the addition of a negatively charged acetyl group to certain lysine residues in a histone tail results in a weaker bond between the DNA and the histone. This histone acetylation results in a more relaxed chromatin configuration, making the DNA more accessible to transcription factors and thereby increasing transcription of the associated gene (Fig. 6-37).

Imprinting

Genomic imprinting refers to allele-specific expression of certain genes whereby only the maternal or paternal allele is expressed. This monoallelic expression is controlled in part by DNA methylation, but this regulation is sometimes lost in cancer. The loss of imprinting can allow a double dose of a growth-promoting gene product.

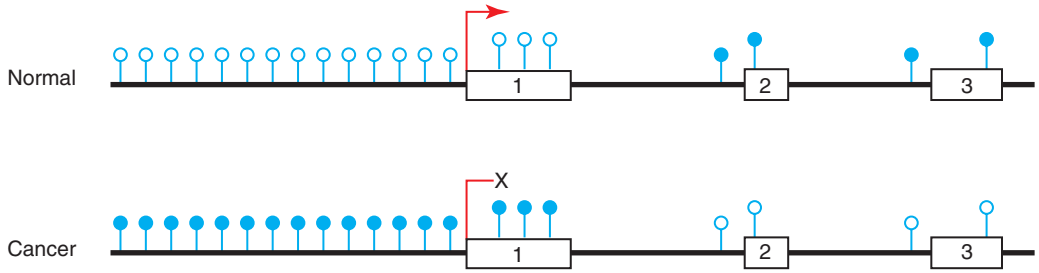


Figure 6-36 CpG Island Methylation. In most normal tissues the dense clusters of CpG sites in the 5′ regions of genes (CpG islands) are unmethylated *(open lollipops)*, whereas those in the body of the gene are methylated *(filled lollipops)*. The reverse is often seen in cancer where 5′ CpG islands become hypermethylated, and there is concurrent hypomethylation of CpG sites in the body of the gene. Unmethylated 5′ CpG islands are associated with active transcription *(arrow)*, whereas methylated 5′ CpG islands are associated with transcriptional repression *(x)*. The net effect is a heritable change in patterns of gene expression. (Redrawn with permission from Dr. L.J. Rush, College of Veterinary Medicine, The Ohio State University.)

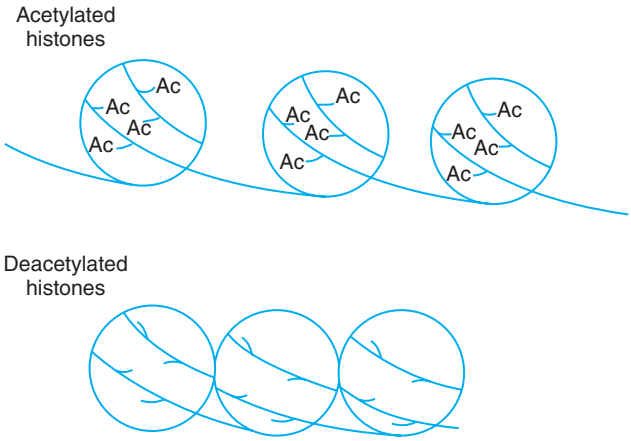


Figure 6-37 Histone Acetylation. DNA is wound around histones. The presence of acetyl groups *(Ac)* on histone tails is associated with relaxed chromatin, which allows gene transcription. Removal of acetyl groups by histone deacetylases results in a closed chromatin configuration that prevents gene transcription. (Redrawn with permission from Dr. L.J. Rush, College of Veterinary Medicine, The Ohio State University.)

For example, insulin-like growth factor-2 (IGF-2) is an imprinted gene that is expressed from only the paternal allele in most normal tissues. If a cancer cell undergoes relaxation of imprinting, the methylation-mediated silencing of the maternal allele is lost, enabling biallelic expression and higher than normal levels of this growth-promoting gene product.

Noncoding RNAs and Cancer

Although protein-coding genes constitute only approximately 2% of the mammalian genome, at least 90% of the genome is transcribed into RNA. Thus the vast majority of RNA transcripts do not encode proteins. Two classes of these *noncoding RNA* transcripts, short (less than 200 nucleotides long) and long (200 to several kilobases in length) noncoding RNAs, play important roles in regulating the transcription, stability, and translation of messenger RNA (mRNA) from protein-coding genes. Through these activities, noncoding RNAs modulate a variety of biologic processes, including normal growth and differentiation. Dysregulated expression of some noncoding RNAs contributes significantly to cancer development.

MicroRNAs (miRNAs) are the most thoroughly studied subclass of noncoding RNAs. These small noncoding RNA molecules post-transcriptionally regulate, usually by blocking, the expression of other genes. MiRNA genes are found throughout the genome, both within other known coding genes and within intergenic regions. MiRNA genes are transcribed into large precursor RNAs that undergo extensive enzymatic processing both within the nucleus and after export into the cytoplasm. Mature miRNAs are 18 to 25 nucleotides long and bind target mRNAs that have a complementary sequence. Once bound, miRNAs either trigger degradation of their target mRNAs or prevent translation of these mRNAs into proteins.

Approximately 1000 miRNA genes are present in the human genome, and each miRNA can regulate translation of approximately 200 target mRNA species. Overall, miRNAs control translation of perhaps one-third of all protein-coding genes in the human genome. The pattern of miRNA expression is extensively dysregulated in cancers by a wide variety of genetic and epigenetic mechanisms. Altered patterns of miRNA expression in turn create extensive changes in cellular processes related to neoplasia, including proliferation, apoptosis, invasiveness, and genomic stability. Intensive research effort is currently being focused on understanding how altered patterns of miRNA expression can be exploited for diagnostic and therapeutic purposes.

Molecular Determinants of Cancer

Although tumor cells may display a wide variety of genetic alterations, usually only a few of these alterations, termed *driver mutations*, are predominantly responsible for tumor development. Driver mutations frequently involve tumor suppressor genes or oncogenes. However, it is rare that a single driver mutation is responsible for cancer; instead multiple genetic and epigenetic changes collaborate to transform a normal cell into a tumor cell and to allow transmission of the neoplastic phenotype. The molecular changes that occur during cancer development are summarized in Fig. 6-38.

Oncogenes

Proto-oncogenes are normal cellular genes that regulate cell growth and differentiation. They often encode products such as growth factors and their receptors, cell cycle regulators, DNA-binding proteins, transcription factors, protein kinases involved in signal transduction, and others. When "activated" by overexpression or mutation, proto-oncogenes are termed *oncogenes*. Oncogenes drive proliferation and render the cell unresponsive to normal growth inhibitory signals, ultimately resulting in tumor formation.

There are a number of ways in which proto-oncogenes can be activated. The gene can be amplified, so that a signal to transcribe the gene results in the production of many more copies of mRNA than usual. Oncogenes can undergo mutations that cause constitutive activation of the encoded protein. In these cases the protein product is always "turned on" and is unresponsive to inhibitory

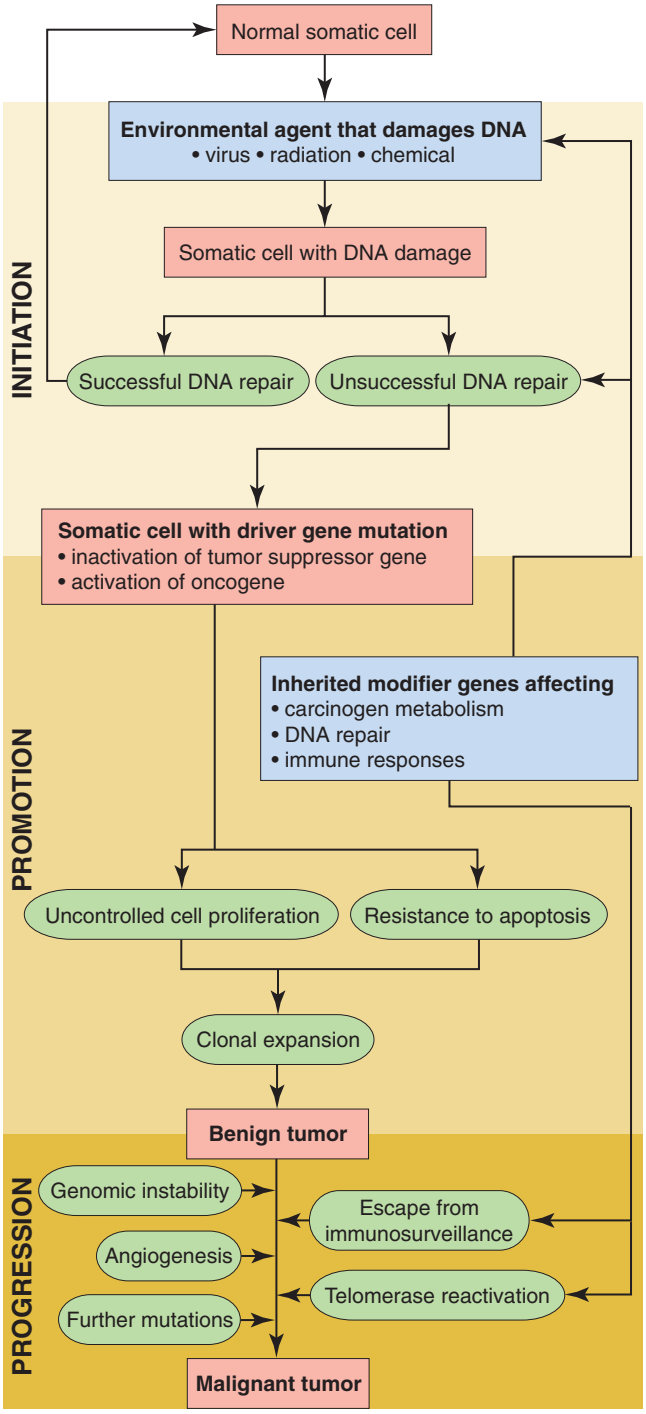


Figure 6-38 The Molecular Basis of Cancer. This diagram highlights multistep development of cancer. Although mutations in driver genes due to environmental DNA-damaging agents may initiate cancer, inherited modifier genes make significant contributions to tumor susceptibility and rate of growth. During the stage of tumor promotion, clones of initiated cells capable of continued cell proliferation and resistant to apoptosis emerge. A wide variety of additional genetic and epigenetic changes convert a benign tumor into an increasingly aggressive malignant tumor. (Courtesy Dr. D.F. Kusewitt, Health Sciences Center, University of New Mexico; and Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

signals. This scenario is common for tyrosine kinase receptors, such as the epidermal growth factor receptor (EGFR). Activating mutations in genes encoding these receptors result in constitutive kinase activity even in the absence of appropriate triggers or ligands. Tumor cells may also synthesize large amounts of both tyrosine kinase receptors and their activating ligands, forming a growth-promoting autocrine loop.

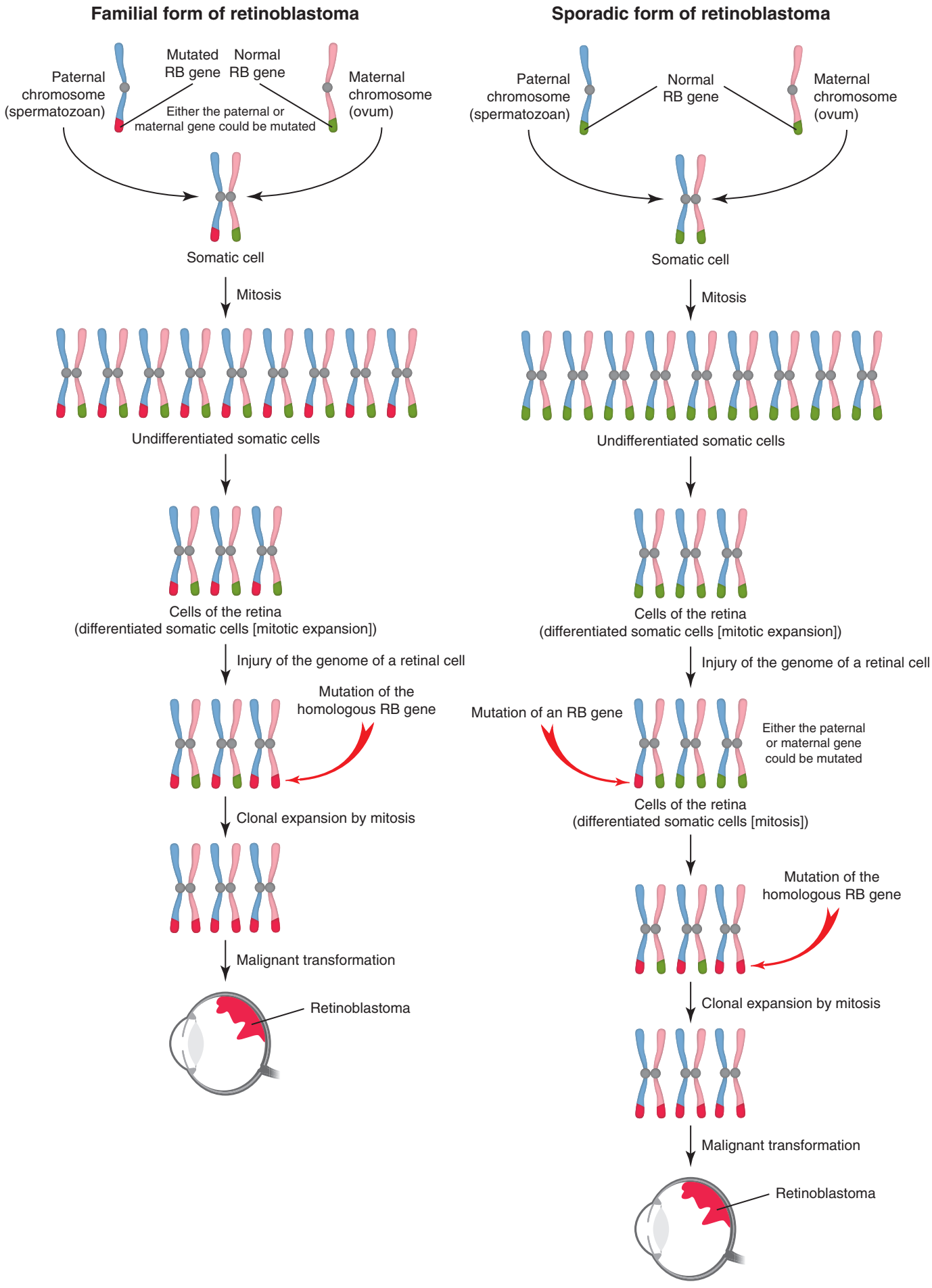
The prototype of signal transduction oncogenes are the *ras* genes, which encode the RAS family of guanosine triphosphate (GTP)-binding proteins (G proteins) (Fig. 6-39). In normal cells, RAS proteins transmit growth stimulatory signals from growth factor receptors to the nucleus, ultimately activating transcription of genes that regulate cell proliferation. RAS is normally located on the cytoplasmic side of the cell membrane and is closely associated with farnesyl transferase. Inactive RAS binds guanosine diphosphate (GDP). Upon receiving a stimulatory signal from an activated growth factor receptor, RAS exchanges GDP for GTP. RAS bound to GTP is the active form, which triggers the RAS–RAF–mitogen-activated protein kinase (MAPK) signaling cascade and results in transcription of genes that promote cell division. The activation of RAS is normally short lived, because RAS has an intrinsic guanosine triphosphatase (GTPase) activity that hydrolyzes GTP to GDP and converts RAS to its inactive state. In many cancers, RAS mutation renders RAS activation independent of upstream growth factor receptor activation or abrogates RAS GTPase activity. RAS family members, the farnesyl transferase membrane anchor, and other components of the downstream MAPK signal transduction pathway are all attractive molecular targets for therapeutic intervention in cancer patients.

Tumor Suppressor Genes

The designation of *tumor suppressor gene* was originally given to genes that inhibited cell proliferation. Over time the class of tumor suppressor genes has expanded to include many different types of cancer-related genes that, when inactivated through genetic or epigenetic means, allow uncontrolled cell proliferation and tumor growth. Suppressor genes include genes that control cell cycle, apoptosis, DNA repair, and other fundamental pathways.

The pivotal concept of tumor suppressor genes was advanced by Alfred Knudson in 1971, based on his observations of children with familial and sporadic retinoblastoma, an uncommon tumor arising in the retina. According to Knudson's *"two-hit" hypothesis*, both alleles of a tumor suppressor gene must undergo mutation, a genetic "hit," for cancer to develop. When only one allele is inactivated, the remaining tumor suppressor allele prevents uncontrolled cell proliferation and tumor development. In inherited cancer syndromes a person is born with a germline mutation in one allele of the tumor suppressor gene in all cells of the body (E-Fig. 6-2). The second hit is acquired as a somatic mutation of the remaining tumor suppressor allele in a single cell. When both copies of the tumor suppressor gene are inactivated in the cell, a tumor arises from this cell. In contrast, development of a sporadic tumor in those born with two normal tumor suppressor alleles requires the much more unlikely event that a single cell sustains two hits, one on each allele of the tumor suppressor gene.

Loss of a tumor suppressor gene allele can occur by a variety of mechanisms, including point mutation in the allele, deletion of the allele or the chromosomal segment where it resides, deletion of the entire chromosome containing the allele, or mitotic recombination resulting in replacement of the normal allele by the mutant allele. In addition, DNA methylation is an alternative, epigenetic method of silencing tumor suppressor genes.



E-Figure 6-2 Pathogenesis of Retinoblastomas (RBs). (Courtesy Dr. D.F. Kusewitt, Health Sciences Center, University of New Mexico; and Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

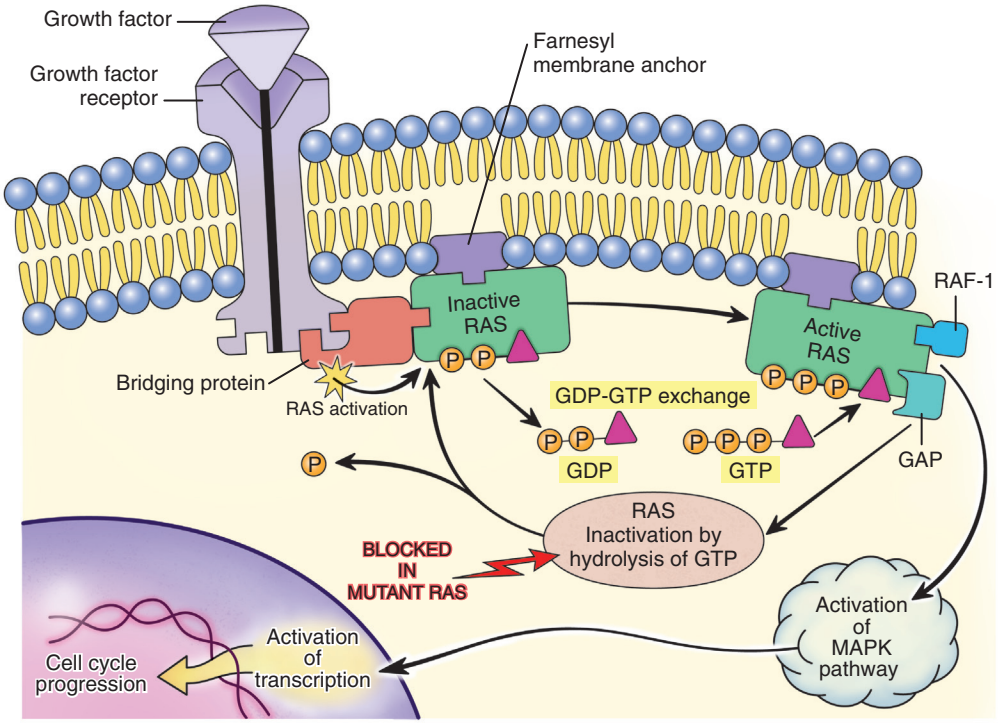


Figure 6-39 Model of RAS Action. When a normal cell is stimulated through a growth factor receptor, inactive RAS is activated to an active state by exchanging guanosine diphosphate (GDP) for guanosine triphosphate (GTP). Activated RAS in turn activates RAF-1 to stimulate signaling through the mitogen-activated protein kinase (MAPK) pathway, ultimately leading to transcription of genes that drive cell cycle progression. In normal cells, activated RAS is inactivated via guanosine triphosphatase (GTPase)–activating protein (GAP), which stimulates the GTPase activity of RAS, thus terminating signaling through the RAS-RAF-MAPK pathway. However, in cancer cells, mutant RAS proteins cannot be inactivated in this manner; thus they stimulate continual cell cycle progression. Anchoring of RAS to the cell membrane by the farnesyl moiety is essential for its action.

Although the classic definition of a tumor suppressor gene dictates that both alleles must be inactivated, recent evidence suggests that for certain genes inactivation of only one copy, a condition termed *haploinsufficiency*, is sufficient for tumor growth. Haploinsufficiency can contribute to tumor development by a number of mechanisms. One mechanism is a simple gene-dosage effect in which half the normal amount of a protein is insufficient to maintain the normal homeostatic balance in the cell. Alternatively, a mutation in one allele can give rise to a dominant-negative protein that blocks the function of the normal protein produced by the remaining normal allele.

Many tumor suppressor genes are key components of the cell cycle. One of the most widely studied tumor suppressor genes is *p53* (Fig. 6-40). It is inactivated, most commonly by mutation, in more than half of human cancers. It is a DNA-binding protein that regulates transcription of numerous genes and plays a critical role in cell cycle arrest and induction of apoptosis after DNA damage. Intracellular levels of p53 are rapidly elevated in response to DNA damage. Enhanced expression leads to increased transcription of p53 target genes, such as p21, which stop the cell cycle, allowing an opportunity for DNA repair. If DNA repair is unsuccessful, p53 directs cell death by activating BCL2-associated X protein (BAX), an important element of the apoptotic cascade. Therefore loss of functional p53 can have devastating consequences for maintaining integrity of the genome. Without p53, DNA damage goes unrepaired, the cell proceeds through division, and genetic changes become fixed in the genome. For these reasons, p53 has been called the "guardian of the genome."

Modifier Genes

In some cancer syndromes, such as the Li-Fraumeni syndrome and familial adenomatous polyposis in human beings, tumor risk is very markedly increased because of changes in a single driver gene; however, there are also a variety of tumor modifier genes that alter cancer susceptibility to a lesser extent. These so-called *modifier genes* or *quantitative trait loci* (QTL) alter the incidence and progression of tumors with driver mutations. In the absence of a driver mutation, modifier genes typically have no phenotype of their own. Indeed, many modifier genes represent polymorphic genes, such as those encoding drug-metabolizing enzymes or DNA repair enzymes, occurring naturally in the population. These cancer modifier genes only modestly alter the phenotype produced by cancer driver genes but may have a significant impact on cancer susceptibility. Their effects are substantially modified by interactions with each other and with the environment. For instance, sun exposure is the major etiologic factor in squamous cell carcinoma of the ears in white cats, but the lack of pigmentation in these cats contributes to their susceptibility to tumor development; thus genes that determine skin pigmentation are modifier genes for this cancer. Similarly, susceptibility to chemically induced skin cancer in mice is highly dependent upon the genetic background of the mouse strain. At least 13 skin cancer susceptibility genes have been identified that account for this strain-dependent variability. In the dog, a variety of cancer susceptibility patterns, presumably due to differences in modifier genes, have been identified (Table 6-3).

In many cases, tumor modifier genes are identified due to the association of their polymorphic variants with cancer susceptibility,

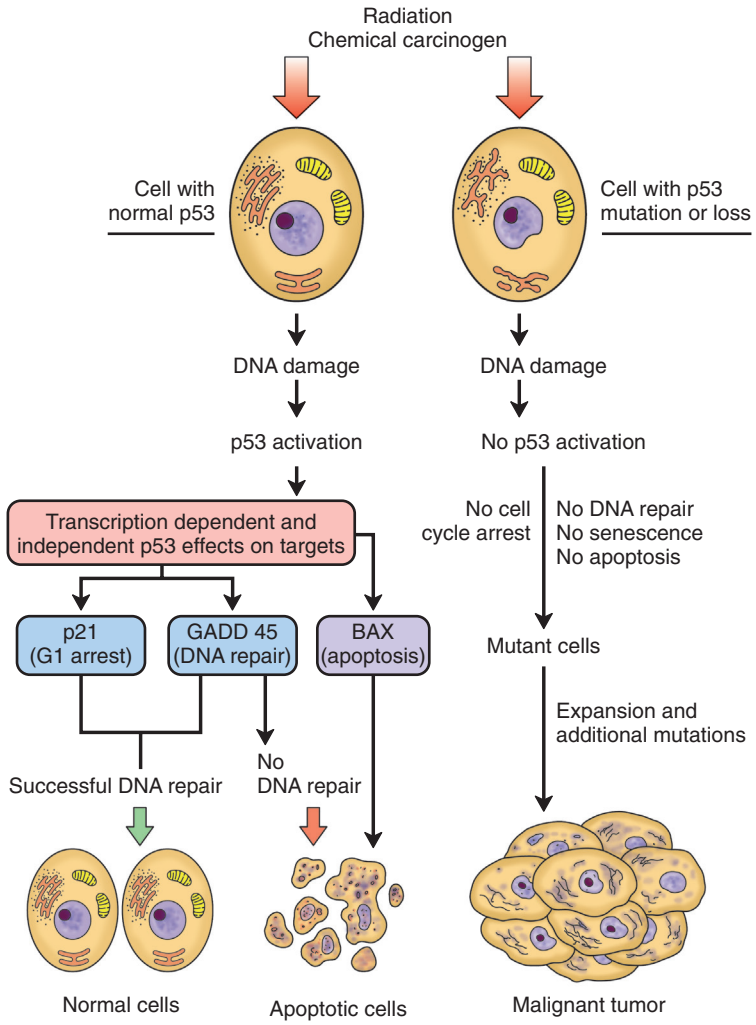


Figure 6-40 p53 and Maintenance of Genome Integrity. DNA damage activates normal p53. Activated p53 acts via both transcription-dependent and transcription-independent pathways to cause G_1 arrest via p21 and induction of DNA repair via growth arrest and DNA damage-inducible 45 (GADD45). Successful DNA repair allows cells to proceed through the cell cycle. However, if DNA repair fails, BCL2-associated X protein (BAX) promotes apoptosis. In contrast, DNA damage to cells with loss or mutation of p53 does not induce cell cycle arrest or DNA repair. The genetically damaged cells proliferate, accumulate mutations, and may eventually give rise to tumors.

and this association is determined by whole genome sequencing of large populations. Further studies are then required to determine the characteristics of the gene and the manner in which it influences tumor penetrance.

Defects in DNA Repair

Failure of DNA repair enzymes to function effectively results in DNA mutations and genomic instability. If these mutations inactivate tumor suppressor genes or activate oncogenes, the cell may develop an uncontrolled proliferative capacity. Specific types of DNA repair mechanisms have evolved to repair specific DNA lesions. *Mismatch repair* enzymes, such as MLH1 and MSH2 proofread DNA, much like the spell-check function on a computer, to locate and fix single nucleotide mismatches that occur on a regular basis during normal DNA synthesis. For example, if an adenine is mistakenly paired with a guanine during DNA replication, this error will be recognized and corrected. Some carcinogens create bulky DNA lesions. For example, ultraviolet (UV) light leads to cross-linking of pyrimidine residues and formation of pyrimidine dimers. Such lesions are repaired by *nucleotide excision repair*, which requires a large cohort of DNA repair proteins. This process is similar to the cut-and-paste function of a computer in that the DNA lesion is excised and the correct nucleotides are replaced. Additional DNA repair genes in human beings include such genes as *ATM*, *BRCA1*, and *BRCA2*.

As discussed earlier, intracellular p53 levels rise in response to DNA damage from any number of agents and stop the cell cycle to give the cell time to carry out repair processes. Failure of DNA repair can lead to mutation fixation with subsequent rounds of cell division. When function of the DNA repair gene itself is lost, through mutation, promoter methylation, or deletion, the result is an exponential increase in mutations throughout the genome, resulting in widespread genomic instability and, ultimately, increased cancer susceptibility.

Multistage Carcinogenesis

Some tumor types demonstrate an orderly morphologic progression through premalignant to malignant to invasive and metastatic disease. Molecular genetic investigations of these various stages have made important contributions to our understanding of cancer biology. The molecular events that occur in the development of familial adenomatous polyposis, a form of human colorectal cancer,

Table 6-3 Cancer Susceptibility in Dogs

Tumor Site	Tumor Type	Susceptible Breeds
Hematopoietic system	Lymphoma	Boxer
	Histiocytic sarcoma (malignant histiocytosis)	Bernese mountain dog, flat-coated retriever
Brain	Various gliomas	Boston terrier, boxer, bulldog
Chemoreceptor organs (aortic and carotid bodies)	Chemodectomas	Brachycephalic breeds (Boston terrier, boxer, bulldog, and others)
Skin	Mast cell tumor	Boxer, bulldog, retriever
Vasculature	Hemangiosarcoma	German shepherd, golden retriever, boxer
Mammary gland	Various	Boxer, Brittany spaniel, dachshund, English setter, Labrador retriever, pointer, springer spaniel
Nose and sinuses	Various	Airedale, collie, Scottish terrier
Oropharynx	Various	Boxer, cocker spaniel, golden retriever
Ovary	Carcinoma	Pointer
Pancreas	Carcinoma	Airedale terrier, poodle, boxer
	Insulinoma	Fox terrier, standard poodle, German shepherd, boxer
Thyroid	Carcinoma	Beagle, boxer, retrievers
Skeleton	Osteosarcoma	Giant breeds, boxer, Danish dog, German shepherd, Rottweiler
Testis		Boxer, collie, German shepherd
Urinary bladder	Carcinoma	Beagle, collie, Scottish terrier

Modified from McCullen JM, Page R, Misdorp W: An overview of cancer pathogenesis, diagnosis, and management. In Meuten DJ, editor: *Tumors in domestic animals,* ed. 5, Hoboken, NJ, 2010, John Wiley and Sons, with additional material from Meuten DJ, editor: *Tumors in domestic animals,* ed. 5, Hoboken, NJ, 2010, John Wiley and Sons, and Dobson JM: Breed-predispositions to cancer in pedigree dogs. *ISRN Vet Sci* 2013:941275, 2013.

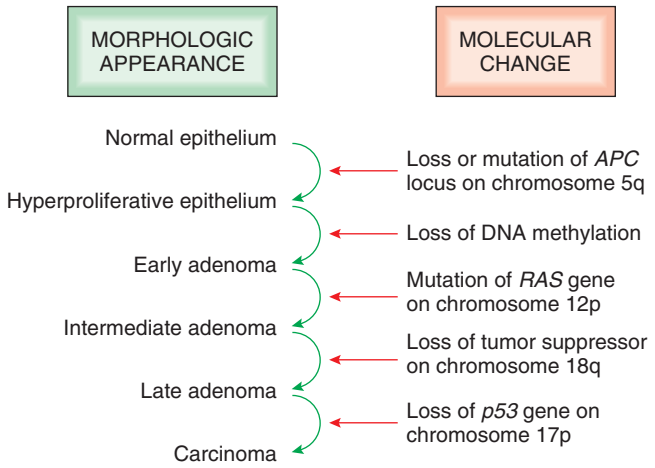


Figure 6-41 Evolution of Human Colorectal Cancers. Although adenomatous polyposis coli (*APC*) mutation is an early event and loss of *p53* occurs late in the process of tumorigenesis, the timing for the other changes may show variations. Note also that individual tumors may not have all of the changes listed. (Adapted from Vogelstein B, Kinzler KW: Colorectal tumors. In Vogelstein B, Kinzler KW, editors: *The genetic basis of human cancer*, New York, 2002, McGraw-Hill.)

provide an excellent example of the genetic evolution that underlies progressive morphologic changes in cancer (Fig. 6-41). The initiating event is loss or mutation of the adenomatous polyposis coli (*APC*) tumor suppressor gene, leading to the formation of an adenoma. This event is followed by an activating mutation of a RAS oncogene and loss of genetic material harboring additional tumor suppressor genes. Ultimately, a malignant carcinoma emerges.

Therapeutic Implications

Cancer is often treated using cytotoxic drugs or radiation therapy, neither of which discriminates between normal and tumor cells. Nonselective cell killing is responsible for many of the deleterious side effects of cancer treatment. Understanding the molecular basis of cancer is crucial for developing interventional strategies to kill neoplastic cells while leaving healthy cells unaffected. Examples of molecularly targeted therapies used in human beings include imatinib mesylate (Gleevec), which inactivates the BCR/ABL oncogene in chronic myeloid leukemia, and afatinib (Gilotrif), which targets the oncogenic EGFR in lung cancer. Specific molecular defects can also be exploited for early detection or early intervention at a stage when the tumor may be more responsive to treatment. Mutations in the *BRCA1* gene are associated with a high risk for development of breast and ovarian cancer in women. Identification of the carrier status gives women the option of prophylactic mastectomy or oophorectomy. Mutations and other molecular defects can also be used to stratify patients for treatment or prognostic purposes. Indeed, with the advent of whole genome sequencing, which allows identification of all mutations in a tumor, considerable emphasis in the human cancer field is now being placed on identifying and targeting specific driver mutations. The era of truly individualized cancer therapy has arrived.

Mechanisms of Carcinogenesis

Intrinsic Factors

As a by-product of ordinary cell metabolism, a variety of DNA-damaging metabolites, such as reactive oxygen species and organic acids, are produced. Additionally, in the course of many rounds of replication, DNA changes are introduced as a result of copying errors made by DNA polymerases. Illegitimate recombination and inappropriate nucleotide addition, activities carried out by normal cellular enzymes, can also lead to changes in DNA. Chromosomal abnormalities arise as a result of decreased telomere length, altered telomerase activity, and mistakes in chromosome segregation. The DNA lesions induced by these processes can result in mutations in critical cancer-related genes and ultimately in neoplasia.

Extrinsic Factors

Extrinsic factors that interact with DNA to cause cancer include chemical and physical environmental agents and oncogenic viruses. *Mutagens* are agents that create DNA damage that gives rise to mutations, whereas *carcinogens* are agents that cause cancer. Many mutagens are also carcinogens. However, there are carcinogens with unknown mechanisms of action; such carcinogens may or may not be mutagens.

Chemicals

A very wide variety of chemicals can cause cancer in animals. As an example, ptaquiloside, a toxin found in bracken fern, causes bladder cancer in cattle. Moreover, the susceptibility of mice and rats to chemically induced cancers is exploited for safety testing during drug development. *Direct-acting* chemical carcinogens are effective in the form in which they enter the body, but most carcinogens are procarcinogens that require metabolic activation by cellular enzymes, such as cytochrome P450 in hepatic microsomes, to form ultimate carcinogens. Such procarcinogens are thus termed *indirect-acting carcinogens*. Despite their varied composition, the effective form of most carcinogens binds covalently to DNA to form DNA adducts.

As discussed previously, experimental carcinogenesis studies have been critical in elucidating the stepwise development of cancer. Moreover, these studies have clearly defined the contribution of initiating versus promoting agents to cancer development (Fig. 6-42). In multistage tumorigenesis models, such as the skin carcinogenesis model in mice, the initiator must be administered before the promoting agent. Furthermore, the initiator is ineffective without subsequent application of a promoter. Often multiple, closely spaced promoter treatments are required to drive tumor emergence.

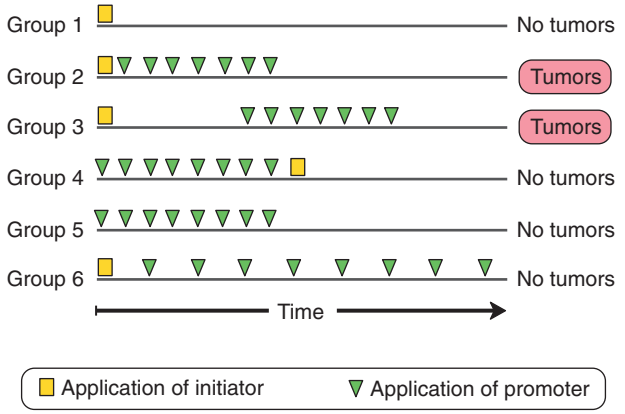


Figure 6-42 Experiments Demonstrating Initiation and Promotion Phases of Chemical Skin Carcinogenesis in Mice. Tumors arose only if application of an initiator was followed by multiple applications of a promoter. For group 2, application of the promoter was repeated twice weekly for several months. For group 3, application of the promoter was delayed for several months, and the promoter was then applied twice weekly. When the promoter was applied monthly rather than twice weekly (group 6), it did not effectively promote tumor emergence. In the absence of initiator (group 5) or promoter (group 1) application or if promoter treatment occurred before initiator application (group 4), no tumors developed. For these studies the initiator employed was a polycyclic hydrocarbon, and the promoter used was croton oil; however, similar results are seen with a variety of initiator and promoter combinations.

Radiation

Unlike chemicals, all forms of radiation are *complete carcinogens*, that is, they are able both to initiate and, with continued exposure, to promote tumorigenesis. For example, in both human beings and animals, secondary tumors may arise at sites previously treated for cancer by radiation. Direct DNA damage caused by ionizing radiation consists primarily of single- and double-strand breaks and base elimination. Absorption of UV radiation by DNA results in the formation of hallmark pyrimidine dimers, which are potentially mutagenic. Ionizing radiation and UV radiation, to a lesser extent, also generate reactive oxygen species from many cellular molecules. These highly reactive molecules cause many types of DNA damage, including altered bases, strand breaks, and DNA-protein cross-links. Because UV radiation is a component of sunlight, sun exposure can cause cancer in nonpigmented and relatively hairless areas in animals, such as the ears of white cats and the conjunctiva of Hereford cattle.

Viruses

Viruses that cause cancer are termed *oncogenic viruses*. Oncogenic viruses important in veterinary medicine are listed in E-Table 6-2). Oncogenic viruses employ a remarkable array of direct and indirect mechanisms to induce cancer.

Dominant Oncogene Mechanism. The genomes of many rapidly transforming oncogenic viruses include a dominant oncogene that drives tumor development. A virus may actually acquire an oncogene from the host animal cell, by incorporating a cellular proto-oncogene into the genome of the infecting virus and subsequently transmitting that oncogene to new animal cells. Once the oncogene becomes part of the viral genome, its expression is no longer subject to normal cellular controls. Uncontrolled production of oncoproteins from the viral oncogene drives cell proliferation and, ultimately, carcinogenesis. Examples of animal-derived oncogenes include the *fes*, *fgr*, *abl*, *fms*, and *kit* genes acquired by oncogenic sarcoma and leukemia retroviruses of cats. Viruses may also contain oncogenes not derived from the host target cell genome. For example, papillomavirus genomes include endogenous *E6* and *E7* genes that encode proteins that inhibit the tumor suppressor proteins p53 and pRb, respectively.

Insertional Mutagenesis Mechanism. Viruses that do not possess their own oncogenes can instead activate the expression of target cell oncogenes by a process called *insertional mutagenesis*. The insertion of viral DNA in these sites results in unregulated production of target cell–encoded oncoproteins responsible for carcinogenesis. For example, most tumors caused by the avian leukosis virus exhibit only a few sites of viral insertion near host proto-oncogenes, notably the *c-myc* gene, where viral promoters drive unregulated production of target cell–encoded oncoproteins.

Hit-and-Run Mechanism. In the two mechanisms discussed previously, the viral genome or portions of the genome persist in the host target cell. However, some viruses also cause tumors merely by transient residence in target cells. Bovine papillomavirus uses such a *hit-and-run* mechanism of cell transformation. In these instances the presence of the virus is necessary to initiate carcinogenesis, but the virus is typically no longer detectable in the tumor itself. The precise mechanism by which this might occur has not been elucidated.

Indirect Mechanisms. Viruses may also stimulate tumorigenesis by suppression of the animal's immune system or by stimulation

E-Table 6-2 Oncogenic Viruses of Animals

Classification	Virus	Species Affected	Associated Tumors
Retrovirus	Mouse mammary tumor virus	Mouse	Mammary adenocarcinomas
	Avian sarcoma-leukosis virus complex	Fowl	Various sarcomas, carcinomas, lymphomas, leukemias
	Avian reticuloendotheliosis virus complex	Fowl	Lymphomas, leukemias
	Mouse leukemia and sarcoma viruses	Mouse	Various sarcomas, carcinomas, lymphomas, leukemias
	Feline leukemia virus	Cat	Leukemias, lymphomas
	Bovine leukosis virus	Cattle	Leukemias, lymphomas
	Primate leukemia and sarcoma viruses	Primate	Fibrosarcomas, leukemias
	Mason-Pfizer monkey virus	Primate	Fibrosarcomas
	Feline immunodeficiency viruses	Cat	Lymphomas
	Maedi-visna virus	Sheep	Pulmonary carcinomas
Hepadnavirus	Hepatitis B group	Primate, rodent, duck	Hepatocellular carcinomas
Papovavirus	Polyoma	Mouse, raccoon	Various carcinomas, sarcomas
	Simian virus 40 (SV40)	Primate	Sarcomas (in rodents)
	Papilloma	Many species	Papillomas, carcinomas
Adenovirus	Types 2, 5, 12	Human	Sarcomas (in hamsters)
Herpesvirus	Frog herpesvirus	Frog	Adenocarcinomas
	Marek's disease	Fowl	Lymphoproliferative disease
	Herpesvirus ateles Herpesvirus saimiri	Primate	Lymphomas, leukemias
Poxvirus	Various	Various	Fibromas, papillomas

Modified from Wyke J: Viruses and cancer. In Franks LM, Reich NM, editors: *Cellular and molecular biology of cancer*, New York, 2003, Oxford University Press.

of target cell proliferation. The herpesvirus that causes Marek's disease, a T cell lymphoma of poultry, is an example of a virus that suppresses the ability of the host to eliminate transformed cells; this suppression is believed to be due to cytolysis of B and T lymphocytes during the early lytic phase of viral infection. As a second example, the genome of the Shope fibroma virus, a poxvirus, encodes a homologue of the epidermal growth factor (*EGF*) gene, which drives host cell proliferation, thus promoting tumor development.

Cancer in Animals

Animal Models of Cancer

Animal models have been and remain critically important tools for understanding the cause of human cancer and for testing cancer therapeutic agents. Animal models of cancer include both experimentally induced and naturally occurring tumors. In experimentally induced cancer models, administration of carcinogenic substances or transplantation of human cancer cells results in *de novo* development of cancer in test animals. Naturally occurring models of cancer rely on the spontaneous development of tumors in the test animal.

Experimentally Induced Tumors

A major advantage of experimental model systems is the rapid and reproducible induction of cancer in a very large proportion of experimental animals. Rodents, mice in particular, are often used for such studies. Mice are small and relatively inexpensive to maintain, reproduce rapidly, and have genetics that are highly defined and readily manipulated. The mouse genome has been sequenced in its entirety and detailed comparative maps of the human and mouse genomes have been developed. Many inbred mouse strains, each consisting of genetically identical or syngeneic individuals, are available. Genetic homogeneity of mice standardizes responses and thus reduces the numbers of animals required for research studies. However, mice have several inherent shortcomings as models of human cancer. The genetic homogeneity of inbred mice does not reflect the high degree of genetic diversity within the general human population. Experimentally induced tumors in mice rarely metastasize, whereas metastasis is an important cause of morbidity and mortality in human beings. Finally, the short life span and small size of mice make them less than ideal for long-term testing of tumor therapies.

A number of inbred mouse strains have been developed that are particularly suited to specific needs in cancer research. Nude mice and other profoundly immunodeficient mouse strains accept tumor or normal tissue grafts from other species and provide an environment in which these xenografts can be maintained, manipulated, and studied. Sencar and hairless mice are highly susceptible to tumors of keratinocyte origin and have thus been employed for many skin carcinogenesis studies. Inbred mice have been used extensively to determine the carcinogenicity of chemical and physical agents and to test the safety and efficacy of anticancer therapeutics. Studies in mice, particularly studies of chemically induced skin cancer, have been critical in defining the stages of carcinoma progression. Differences in strain susceptibility to different experimentally induced cancers have been exploited to identify modifier genes that dramatically affect tumor incidence.

With the advent of effective means for creating genetically engineered mice, specific genes of interest can be introduced into or inactivated in the mouse genome. An exogenous gene introduced into the mouse genome is generally termed a *transgene*. A mouse lacking a functional normal gene is referred to as a *knockout* for that gene. Moreover, the timing, location, and level of gene expression in genetically engineered mice can now be precisely controlled, thus allowing gene expression to be turned on or off in particular tissues as required for specific studies. Gene expression modulated in this fashion is termed *conditional* gene expression. Genetically engineered mice have been essential for identifying the mechanisms by which specific genes act to retard or enhance tumor development, growth, and spread.

Naturally Occurring Tumors

Several naturally occurring cancers in animals, including avian leukosis, bovine lymphoma, and feline leukemia, have provided invaluable information about the cause, transmission, and prevention of virally induced cancers. However, virally induced cancers do not appear to account for a large proportion of human cancers.

Recently, the dog has become the focus of increasing attention as a useful animal model of human cancers. Sequencing of the canine genome and comparative alignment with human and murine genomes enhance the usefulness of dogs as a naturally occurring cancer model. The annual incidence rate for cancer in dogs is 381 per 100,000; this is comparable with the cancer incidence in human beings. With the large number of pet dogs in this country, many cancer cases are thus available for entry into clinical trials. Like human beings, dogs are outbred. Moreover, dogs share a common environment with human beings and are exposed to many of the same carcinogens. As in human beings, many canine tumors metastasize widely. Because tumors in dogs progress more rapidly than human tumors, studies can be completed within a reasonable time frame. On the other hand, the time course of tumor development is sufficiently long to allow meaningful comparison of response times in different treatment groups. Because dogs are relatively large, they provide abundant tumor tissue for diagnostic and experimental purposes. In addition, many therapeutic approaches that are difficult to test in small rodents can readily be examined using larger dogs. Clinical trials in dogs are much easier to initiate and much cheaper to carry out than comparable studies in human beings. Many dog owners are enthusiastic participants in clinical trials that may benefit their pets. Tumor types for which dogs are particularly good models of human cancer include osteosarcoma and lymphoma.

Tumor Diagnosis and Prognosis

Taken together, the tumor type, grade, stage, and completeness of excision are used by the veterinary clinician to develop the most appropriate treatment plan for the patient. As we learn more about the molecular pathogenesis of certain tumors, the need for specialized diagnostic tests will certainly increase, because targeted molecular therapies will only be effective if the target is present in the animal's tumor. A full description of molecular techniques used in cancer diagnosis is beyond the scope of this chapter. However, it is certain that their use will become more widespread and commonplace in veterinary medicine.

Histopathologic Diagnosis

A definitive diagnosis of cancer is frequently obtained by standard histopathologic evaluation of tumor biopsy specimens or cytologic studies of tumor aspirates. Biopsy specimens for histopathologic evaluation are analyzed by routine hematoxylin and eosin (H&E) staining, whereas cytologic samples are typically stained with Wright's or Diff-Quik stains. Cells are scrutinized for features of malignancy, including abnormal morphologic features, high mitotic index, presence of abnormal mitoses, high nuclear to cytoplasmic ratio, and evidence of invasion or metastasis. The degree of differentiation is also routinely evaluated. Malignant neoplasms are frequently poorly to moderately differentiated, and some may be so anaplastic that the cell of origin cannot be determined. The

presence of cellular products, such as osteoid in osteosarcomas, may provide clues to the identity of the cell of origin for the tumor.

Immunohistochemistry for specific cell markers may be used to aid in the diagnosis of some tumors. For example, immunohistochemistry is commonly used to determine if a lymphoma originates from B or T lymphocytes (E-Fig. 6-3). This knowledge may be useful to the clinician in designing treatment or delivering a prognosis. The type of intermediate filaments present in an undifferentiated malignancy can indicate if the tumor is of epithelial (positive for cytokeratin staining) or mesenchymal (positive for vimentin staining) origin. Some neoplasms, such as mesotheliomas and synovial cell sarcomas, are often positive for both cytokeratin and vimentin. Carcinomas that have undergone EMT will have areas positive for cytokeratin or vimentin, and transition areas may be positive for both. An exhaustive list of antibodies is beyond the scope of this chapter, but immunohistochemical staining is becoming a widely used tool that assists pathologists in providing a more complete diagnosis in cases in which routine H&E evaluation does not provide a definitive diagnosis.

Histochemical stains can also aid in diagnosis. Poorly differentiated canine mast cells may have granules that are not clearly visible by H&E staining. Staining with toluidine blue often highlights the granules and confirms the diagnosis in otherwise challenging cases.

Other Diagnostic Techniques

Clonality Assays. Sometimes it is difficult to distinguish benign lymphoid hyperplasia from lymphoma by morphologic features alone. Most neoplasms are believed to be clonal, that is, they are ultimately derived from a single transformed cell. Thus, establishing that a lymphocyte population is clonal gives more weight to a diagnosis of a malignancy. Clonality can be assessed by analyzing the lymphocytes for T or B lymphocyte receptor rearrangement, using the polymerase chain reaction (PCR). If the entire lymphocyte population has a single rearrangement, the proliferation is clonal, and most likely neoplastic. Conversely, if each lymphocyte has a different receptor rearrangement, this indicates a polyclonal proliferation, which is more consistent with lymphoid hyperplasia. However, the presence of a clonal population of lymphocytes does not, by itself, guarantee lymphoma. Some nonneoplastic conditions, such as canine ehrlichiosis, can give rise to clonal lymphocyte populations. Therefore results must be interpreted in conjunction with clinical signs and other clinicopathologic data.

Cytogenetic Analysis. Cytogenetic analysis can be a useful tool for diagnosis, determining the presence of residual disease after treatment, and stratification of high- and low-risk patients. The discovery of recurrent chromosomal abnormalities and translocations, particularly in leukemias and lymphomas, will aid in diagnosis and understanding the pathogenesis of these diseases, as well as the design of targeted therapies.

Pedigree Analysis. Identification of genes involved in inherited cancers can be accomplished through the detailed analysis of well-described pedigrees, particularly in certain cancer-prone breeds. As is the case in human beings, the elucidation of these genes is important not only in the diagnosis and screening of high-risk animals but also in providing insight into the pathogenesis of sporadic tumors.

Molecular Diagnostic Techniques. Recently, new techniques have been developed that permit global gene expression analysis of tumors. Microarrays, which allow the measurement of thousands of mRNA transcripts simultaneously, are already available for a wide range of species. New techniques of high-throughput sequencing can determine the identity and abundance of all mRNAs in a tumor. High-throughput sequencing can also be used to sequence the entire genome of a tumor to identify potentially oncogenic mutations. Studies using these new techniques are likely to identify significant changes in gene sequence and gene expression, which can be used to facilitate diagnosis and therapy.

Grading

A tumor *grade* is assigned by a pathologist to provide some indication of how similar or dissimilar the neoplastic cells are to their normal counterparts. The underlying assumption is that this grade provides some indication about biologic behavior. This assumption is not universally true, however, and experience has demonstrated that tumor stage (see next section) is sometimes a more useful prognostic measure.

All grading schemes evaluate the degree of differentiation of tumor cells. The tumor grade classifications usually include well-differentiated (very similar to normal cells), moderately differentiated (somewhat similar to normal cells), and poorly differentiated (anaplastic) cells. These categories translate to low, medium, and high grade, or grades I, II, and III, respectively. Other criteria that may be included in grading schemes include the mitotic index, defined as the number of mitotic figures per 400× field (usually the average of 10 fields); the extent of tumor necrosis; tumor invasiveness; and overall tumor cellularity. Grading schemes vary depending on the tumor type. In an ideal scheme, grading criteria are easily identified on H&E-stained tumor sections, and the grade is strongly linked to prognosis or response to therapy. The criteria employed should be periodically reevaluated in light of new discoveries and diagnostic capabilities.

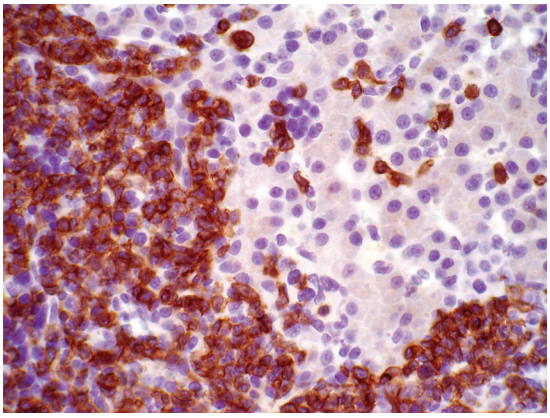
Staging

Tumor *stage* gives an indication of the extent of tumor growth and spread in the animal. In general, staging guides the clinician in developing a therapeutic plan and offering an estimate of prognosis to the client. One of the most widely used schemes is the TNM system, which is based on the size of the primary tumor (T), degree of lymph node involvement (N), and extent of metastasis (M). Within each category a number is assigned based on clinical, diagnostic, and histopathologic evaluations. A designation of T0 is given to carcinoma in situ, whereas T1 to T4 indicate increasing size of the primary tumor. N0 indicates the absence of detectable lymph node involvement, whereas N1 to N3 indicate progressive involvement. Similarly, M0 signifies no detectable metastasis, whereas M1 and M2 indicate metastasis to one and two organs, respectively.

Overall, TNM staging provides a standard measurement by which the natural course of disease and impact of treatment modalities can be compared. However, there is some variability in tumor staging at different institutions. This variability often reflects the availability of more sophisticated imaging modalities, such as computed tomography (CT) and magnetic resonance imaging (MRI), as well as more sensitive techniques of histologic detection, such as immunohistochemistry for cytokeratin to detect micrometastases in lymph nodes of carcinoma patients.

Surgical Margins

With *incisional biopsies* the intent is merely to get enough tissue to make a diagnosis, whereas *excisional biopsies* are performed with the intent of complete removal of the tumor mass to effect a cure. Microscopic evaluation of surgical margins to confirm that the tumor has been completely excised has long been a valuable service



E-Figure 6-3 **Lymphoma, Liver, Dog.** Note the neoplastic lymphocytes invading the liver. The malignant cells are immunoreactive to CD3 *(brown)*, indicating a T lymphocyte neoplasm. The surrounding hepatocytes are not labeled. Immunohistochemistry. (Courtesy Dr. D.F. Kusewitt, Health Sciences Center, University of New Mexico.)

provided by the diagnostic pathologist (see Figs. 17-34, 17-35, and 17-36). Residual malignant cells at the surgical site may warrant a second surgical procedure. However, evaluation of margins is not always straightforward. It is often difficult for the pathologist to properly orient the gross specimen with respect to lateral, deep, and superficial margins. Using sutures or different colors of ink along with proper annotations is helpful in indicating which margin is which. It may also be difficult to distinguish true surgical margins from those produced at trimming. Inking of margins by the surgeon at the time of removal is often recommended to distinguish real margins from those created after sample removal. More importantly, having clean surgical margins on a histologic slide does not guarantee that the patient is free of tumor. Neoplasms are three-dimensional lesions and only a portion of the mass is examined in any one section. So although the margins examined may be free of neoplastic cells, in other areas the neoplastic cells may extend to the surgical margin. In addition, multifocal or *multicentric* lesions may not be submitted to the pathologist. Lastly, lymphatic or hematogenous spread may not be evident on the section or sections examined. Submission of regional lymph nodes is often helpful in determining if the tumor has spread.

Suggested Readings

Suggested Readings are available at www.expertconsult.com.

Suggested Readings

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