CHAPTER

1

Pathogenesis of Cancer

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BIOLOGICAL BASIS OF CANCER

Cancer is the uncontrolled growth and spread of abnormal cells in the body. Cancers (malignant neoplasms) can arise from virtually any tissue and are usually named by the anatomic site of origin. Examples include breast cancer, prostate cancer, colon cancer, and lung cancer, the four cancers responsible for the majority of cancer deaths in many developed nations such as the United States. Cancer cells have the ability to divide continuously, invade other tissues, and spread (metastasize) to other organs through the blood and lymph. These primary features (uncontrolled cell division, invasion of contiguous tissue, and metastasis) distinguish cancerous growths from noncancerous (benign) cellular growths, the latter being self-limiting with no ability to invade tissue or metastasize.

Cancer is further defined according to the cell of origin based on microscopic examination by pathologists. Adenocarcinomas arise from the cuboidal epithelial cells that line the ducts of glandular tissue (e.g., adenocarcinomas of the breast, prostate, colon, or lung). Squamous cell carcinomas arise from the flat pavement cells that cover the skin and line portals of entry or exit in the human body (e.g., squamous cell carcinomas of the oral cavity, upper airways, esophagus, uterine cervix, urinary tract, or anus). Sarcomas arise from connective tissue (fibrosarcoma) or bone (osteosarcoma). Malignant gliomas arise from glial cells that provide vital support to neurons in the central nervous system. Adenocarcinomas, squamous cell carcinomas, sarcomas, and gliomas are solid tumors, as opposed to cancerous growths of immune cells that arise in the bone marrow (leukemias) or the lymph nodes (lymphomas) and circulate in the blood or the lymphatic system.

EVOLUTION OF CANCER

Solid cancerous growths (tumors) develop most often from cells of the epithelial lining of organs of the body (e.g., lungs, colon, breast, prostate), and less often from dividing cells of muscle, bone, and the central nervous system. The evolution of solid tumors is thought to be a long-term process spanning many years and often decades.

The hematopoietic (blood) cancers (leukemias, lymphomas) arise from lymphocytes, granulocytes, or other dividing cells of the bone marrow and the lymphatic system. Leukemias and lymphomas develop much more rapidly than solid tumors, perhaps over the span of a few months or even weeks.

CELL DIVISION (MITOSIS) AND CANCER

Approximately 90% of all cancers are solid tumors that originate in the epithelial lining of various organs of the gastrointestinal tract (oral cavity, esophagus, stomach, liver, pancreas, colon, rectum), the genitourinary tract (kidney, urinary bladder, uterine cervix, vagina), and the reproductive tract (breast, ovary, prostate). Solid tumors called *basal cell carcinomas* can also arise from the stratified (layered) epithelial covering of the skin. Collectively, all epithelial-derived tumors are called *carcinomas*.

In sharp contrast to epithelial-derived carcinomas, only about 8% of malignant neoplasms arise from cells of the immune system (lymphomas and leukemias), less than 2% arise from connective tissues (sarcomas of muscle, bone, and fat), and less than 1% develop in the central nervous system (gliomas and meningiomas). This distinct histologic

pattern of cancer appears to be driven to a large extent by the relatively high cell division rates of epithelial and hematopoietic cell populations compared to connective tissue and the central nervous system. Periods of increased cell division during the life span also increase the likelihood of cancer development; for example, sarcomas of connective tissues arise in conjunction with bursts of cell division in fat and muscle cell populations and the elongation of bones during the early developmental years. Clearly, cancer is more likely to develop in those cell populations with higher rates of cell division. "In general, the greater the replicative activity, the greater the cancer risk" (Robbins & Cotran, 1979).

CANCER AND THE CELL CYCLE

Cancer can be succinctly defined as "deranged cell division." In cancer research, it is thus important to understand fundamental characteristics of the cell division cycle. In humans, normal cell division is an extraordinarily complex process whereby a progenitor (mother) cell forms two replicate daughter cells. In this remarkable miracle of nature, amino acids are efficiently transformed into millions of proteins and enzymes necessary for DNA replication, chromosomal formation, and reproduction of all of the working parts of the newly created cells.

Normal Cell Division and Cell Cycle

The human cell cycle consists of a preparatory phase of nutrient and energy buildup (G1 phase), DNA synthesis and replication of double-stranded DNA (S phase), condensation of DNA into 46 chromosomes and preparation for mitosis (G2 phase), and segregation of chromosomes to create two genetically equivalent daughter cells (mitosis or M phase) (**Figure 1.1**). Cytokinesis takes place after the segregation of chromosomes and is the final event in the cell division cycle. During cytokinesis, the cytoplasm and cytoplasmic organelles (e.g., mitochondria, Golgi apparatus, endoplasmic reticulum, ribosomes, lysosomes) are equally distributed to create two daughter cells with chromosomes, cytoplasm, organelles, nuclear and plasma membranes, and microtubular cytoskeletons that are virtual mirror images.

Progression through the cell cycle is tightly regulated by a family of genes called *cyclins* and the proteins they encode. Cyclins are activated by the docking of hormones, growth factors, and other extracellular *signaling molecules* to cell-surface receptors that trigger events inside the cell, a process known as *signal transduction*. Extracellular signaling molecules that modulate cell division include hormones of the endocrine system (e.g., estrogen, progesterone, testosterone, and thyroxine), paracrine factors that act locally (e.g., growth factors, cytokines, and prostaglandins),

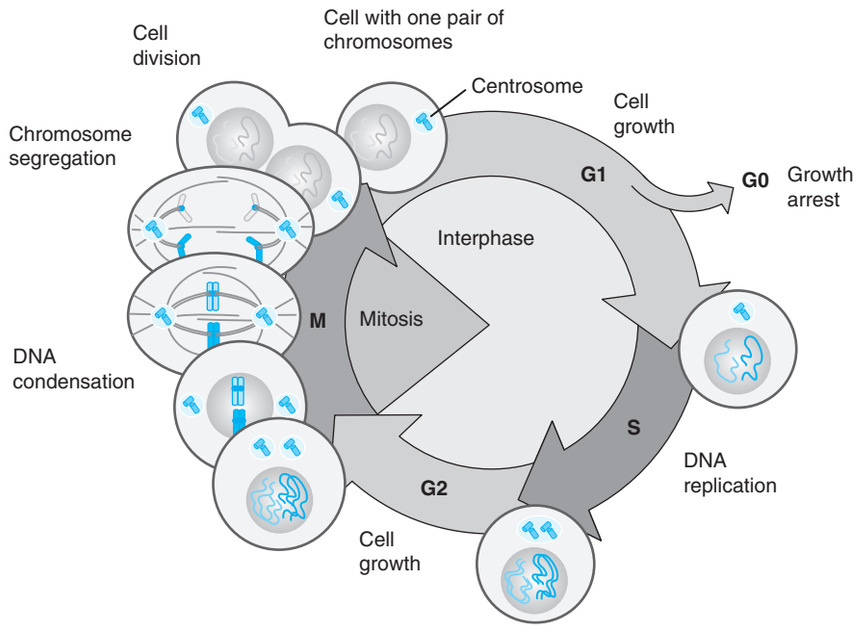


Figure 1.1 Model of Human Cell Cycle.

and autocrine factors released by the cells themselves (e.g., certain interleukins) (Hancock, 2010).

The cyclin proteins bind to cyclin-dependent kinases (CDKs), which modulate the passage of the cell through the sequential phases of the cell division cycle. The cyclins and CDKs function by phosphorylation of signaling proteins that regulate transcription and translation of genes essential for cell division. Furthermore, inhibitors of CDK encoded by specific genes (e.g., p53, RB, p27, APC, CHEK1, CHEK2) function as *gatekeepers* of error-free DNA synthesis by facilitating repair of any DNA coding errors that occurred during the S phase of the cell division cycle (Deninger, 1999).

One very important gatekeeper gene, p53, encodes a protein that checks for errors or damage in newly replicated DNA near the end of the S phase of the cell division cycle. As shown in the schematic of **Figure 1.2**, the p53 protein prompts the enzymatic repair of DNA damage before the cell progresses through the final steps of cell division. If excess DNA damage is detected, the cell undergoes apoptosis (programmed cell death), thereby preventing the proliferation of abnormal cells. In the absence of normal p53 function, cells with DNA damage (or mutations that predispose to cancer development) may continue to grow and divide (Figure 1.2). Because of its important role in eliminating mutant cells and conserving genomic stability, the p53 gene has been deemed the "guardian of the human genome" (Efeyan & Serrano, 2007).

G0 (Functional) Phase of the Cell Cycle

The G0 phase of the cell cycle is the nonreplicating "functional" period of the cell. During G0, newly formed cells undergo differentiation and carry out their designated functions; for example, epithelial cells produce and secrete proteins and glycoproteins, inflammatory cells produce and secrete prostaglandins and cytokines, immune cells produce and secrete antibodies, muscle cells exert contractile energy, skeletal cells provide structural support, fat cells store energy, endocrine cells secrete hormones, endothelial cells maintain blood pressure and water balance, neurons conduct impulses, and so on. The human body consists of trillions of cells that respond synchronously to changes in environmental conditions in order to maintain internal equilibrium (homeostasis) of life-sustaining functions.

LIFE SPANS OF HUMAN CELLS

The life spans and G0 phases of different types of cells are highly variable. In recent studies, isotopic (C^{14}) carbon dating has been used to accurately estimate the life spans of different cell types. These investigations confirm the wide range of normal cell life spans; for example, intestinal epithelial cells live only a few days, skin epithelial cells live a few weeks, lymphocytes live for months, macrophages live for years, muscle and fat cells live for decades, and nerve cells (neurons) live for the entire life span of the human

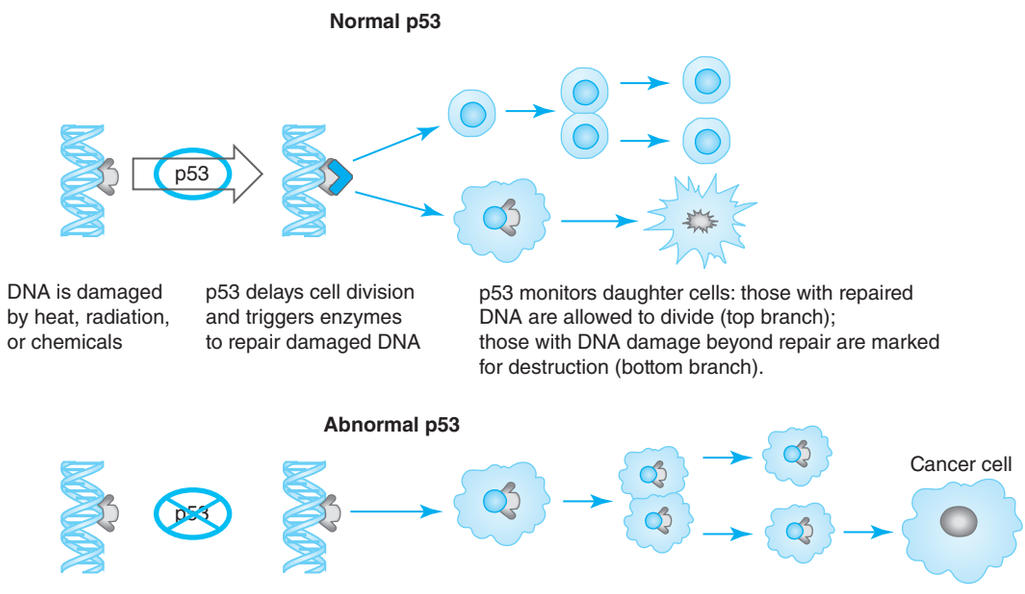


Figure 1.2 Mechanism of Action of p53 in Cell Division.

host (Spalding, Bhardwaj, Buchholz, Druid, & Frisén, 2005).

TURNOVER OF HUMAN CELLS

Populations of cells with relatively short life spans continuously undergo rapid self-renewal and have considerable regenerative capacity. The turnover rate of a given cell population depends on the delicate balance between the rate of programmed cell death (apoptosis) and the rate of cell replacement by cell division (mitosis) of progenitor cells (stem cells). Factors that upset this balance may lead to malignant transformation (Pellettieri & Alvarado, 2007).

Most epithelial cell populations continuously self-renew during adult life and have relatively short turnover times. Under normal physiologic conditions, intestinal epithelium turns over (is replaced) within about 5 days, skin epithelium within about 1 month, and lung epithelium within about 6 months. In general, cell populations that have fast and furious rates of cell turnover are more prone to cancer development than those that turn over more slowly (Blanpain, Horsley, & Fuchs, 2007).

Cells of the immune system are continuously formed from progenitor cells (stem cells) in the bone marrow by a process called *hematopoiesis*. However, mature immune cells ordinarily do not divide unless stimulated by inflammation or infection, and have relatively long life spans (months or years). It is during periods of accelerated cell division that cells of the immune system are most likely to undergo malignant transformation.

Throughout the early years of life, and especially during puberty, the cell populations of bone, muscle, and fat show high mitotic rates in conjunction with rapid growth and development, after which cell populations stabilize and cell division subsides. Indeed, cells of mature connective tissues divide infrequently and tend to have long life spans. Fat cells (adipocytes) and muscle cells divide only occasionally to replace about 5% of their respective cell populations every year. Bone undergoes continuous remodeling throughout life whereby old bone is resorbed by cells called osteoclasts and new bone is formed by osteoblasts; however, this process replaces less than 10% of skeletal cells annually. Some cells do not undergo mitotic division and therefore rarely form malignant neoplasms (e.g., neurons of the central nervous system and cells of cardiac and skeletal muscle) (Spalding et al., 2005).

EVOLUTION OF CANCER IN THE HUMAN HOST

In epithelial cell populations, normal cell division is restricted to stem cells that are in contact with the basement membrane. Mitosis of an epithelial stem cell produces one new stem cell, which remains in contact with the basement membrane and therefore retains replicative potential, and one new daughter cell that loses contact with the basement membrane and matures and differentiates into a functional cell. As newly formed daughter cells migrate to higher layers of stratified epithelium, they eventually undergo programmed cell death, which is called *apoptosis*, and are sloughed off at the surface (Marshman, Booth, & Potten, 2002).

The normal pattern of epithelial cell replacement is distinctly different from carcinomas, where the cancer cells divide indefinitely and fail to undergo apoptosis. The evolution of a solid cancerous tumor proceeds through a continuum of steps, wherein cells undergo visible morphological changes of the nucleus and cytoplasm (Holowaty, Miller, Rohan, & To, 1999; Kumar, Abbas, & Aster, 2014). The following paragraphs provide a brief description of the cell types that appear during the evolution of cancer. The progression of normal epithelial cells to invasive cancer is depicted for the epithelium of the uterine cervix in **Figure 1.3**.

Dysplasia (Premalignant Lesions)

Dysplasia is a distinct benign growth that arises from the epithelial cells lining various organs. In dysplasia, the cell nucleus becomes prominent, the cytoplasm appears swollen and vacuolated, and the cells exhibit increased rates of cell division and disordered maturation.

Dysplasia invariably precedes the development of cancer, and dysplastic lesions that serve as precursors of cancer can be detected at a number of anatomic sites. Examples of premalignant lesions include actinic keratosis of the skin, leukoplakia of the oral cavity, Barrett's esophagus, fibrocystic disease with atypia of the breast, adenomatous polyps of the colon, dysplasia of the prostate, and intraepithelial neoplasia of the uterine cervix. Fortunately, all such dysplastic lesions do not have the ability to invade or metastasize and they are curable by surgical excision or other methods of ablation. Clearly, the early detection and treatment of such lesions is an important component of effective cancer control.

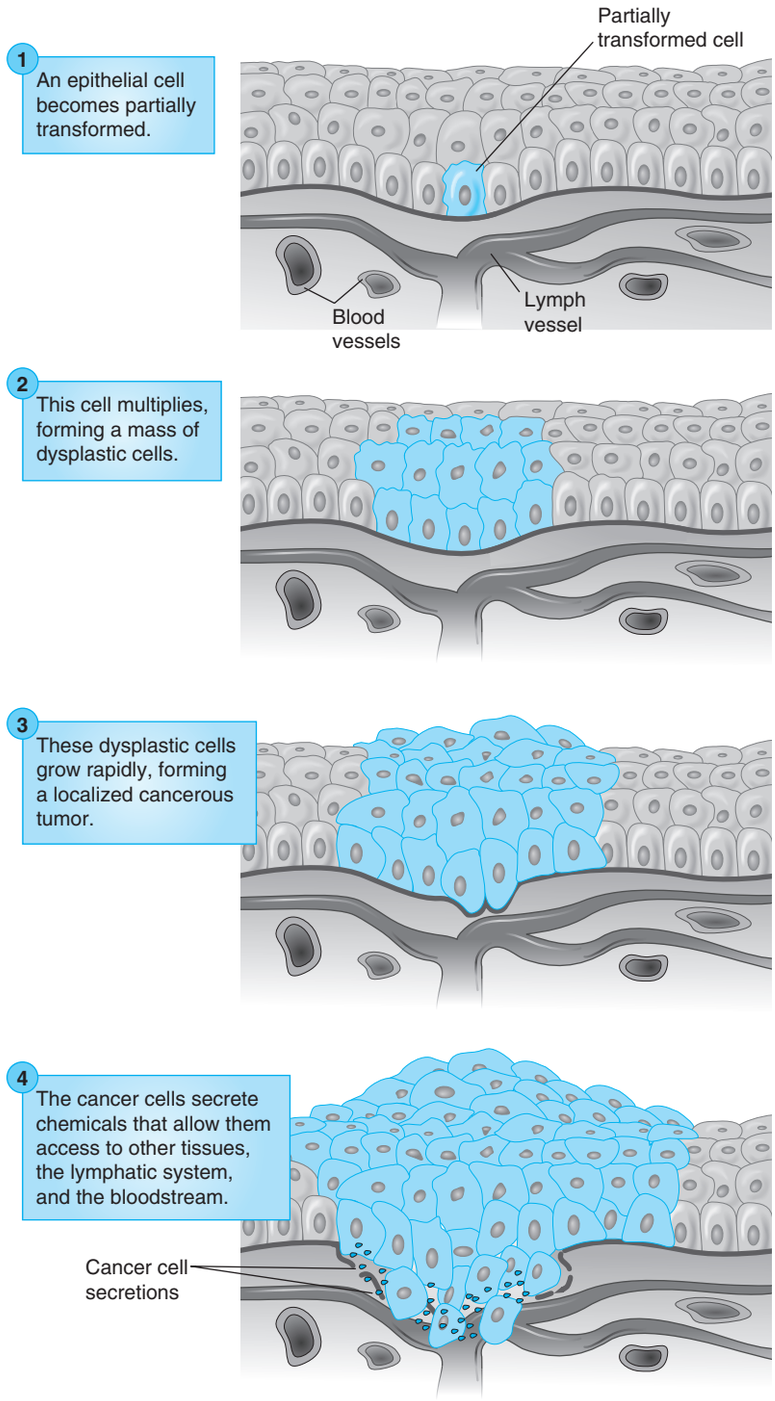


Figure 1.3 Progression of Normal Cervical Epithelium to Invasive Cancer.

Carcinoma in Situ

When solid cancerous tumors arise, they are at first confined to their original location and have not broken through the basement membranes into surrounding tissues. A confined neoplasm of the epithelial cell layer is thus called *carcinoma in situ (CIS)*. Such in situ lesions represent a defined step in cancer evolution. They exhibit all of the features of malignancy except invasiveness and metastasis; namely, the cells of in situ neoplasms manifest unchecked mitosis and proliferation, maturation failure and resistance to death, and disordered organization of the cell population. And yet, the in situ neoplasm is theoretically curable by excision because it has not spread beyond its original location and is contained by the basement membrane.

Invasive Cancer

In contrast to in situ neoplasms, invasive cancers have broken through the basement membrane to spread beyond their original location into contiguous tissues. This is a critical step in the evolution of cancer because surgical excision may no longer be effective as a form of cancer therapy. The breach of the basement membranes by cancer cells requires acquisition of certain new functions (e.g., the secretion of proteolytic enzymes that degrade basement membranes).

Metastatic Cancer

Metastatic disease represents the final step in the evolution of cancer. Cancer cells first invade contiguous tissues, and then spread through lymphatic channels and blood vessels to other anatomic sites. Cancer causes death by metastasizing (spreading) to vital organs such as the liver, brain, and spine. It is within these vital organs that the cancer cells overwhelm the normal cellular constituency, resulting in the collapse of their life-sustaining functions. Early detection prior to the development of invasive cancer and metastasis is therefore vitally important to the successful treatment and survival of cancer patients. The entire process of cancer development often goes unheeded and undetected by the human immune system over a period of many years; hence the term *silent killer* has been aptly applied to describe cancer in its various forms.

THEORIES OF CARCINOGENESIS

Cancerous growths result from a complex process known collectively as *carcinogenesis*. Although numerous theories of carcinogenesis have been proposed, considerable controversy exists as to which one is correct. Nevertheless, there is reasonable consensus that carcinogenesis is definable as a disease of the genes or a "DNA disease." Although genetic changes are ultimately responsible for the uncontrolled cell division and aberrant growth dynamics that characterize all forms of cancer, this does not infer that cancer-predisposing genetic alterations are heritable. In fact, about 95% of malignant neoplasms arise due to nonheritable genetic alterations acquired during the life span rather than inherited cancer-predisposing genes. Two main theories of carcinogenesis are discussed in this text: the *somatic mutation theory* and the *epigenetic theory*.

Somatic Mutation Theory of Cancer

Because a variety of genetic (DNA) mutations have been identified through molecular studies of cancerous tissues (Fearon & Vogelstein, 1990), the process of carcinogenesis is considered by many to result from the accumulation of two or more somatic mutations that impact upon control of the cell cycle or other features of neoplastic development (Knudson, 2001). This idea was first proposed in 1953 by Carl Nordling in Sweden, who stated that "the cancerous cell contains not one but a number of mutated genes. The occurrence of such accumulations of mutations may be expected to increase according to a certain exponent of age as well as according to the increase of cell proliferation" (Nordling, 1953).

In 1971, Alfred Knudson published a study of 48 cases of retinoblastoma, a devastating tumor of the retina that occurs primarily in children under 5 years of age. Based on the time sequence of tumors in these cases, Knudson hypothesized that retinoblastoma arises from two sequential mutational events. In the heritable form, a mutated gene is inherited from one parent and the second occurs in somatic cells of the retina. In the nonheritable form, both mutations occur in somatic cells of the retina (Knudson, 1971). The mutated retinoblastoma gene, Rb1, was later discovered by a team of investigators in Boston (Dryja, Friend, & Weinberg, 1986). The Rb1 gene was the first tumor suppressor gene ever discovered, and Knudson's two-hit mutation theory of retinoblastoma has been generalized as the *somatic mutation theory of cancer*.

Somatic mutations are base pair changes in DNA that occur during the human life span in cells of the body, in contrast to mutations that are "inherited" from parents through the germline. The somatic mutation theory of cancer is based on the premise that cancer arises from a single somatic cell and that tumor progression and development involve the

accumulation of multiple DNA mutations that occur as the abnormal cells continue to replicate over time. It is postulated (but not proven) that with each successive mutation, the evolving clone of cancer cells acquires new properties that favorably impact tumor growth, invasion of contiguous tissues, and eventually metastasis to other anatomic sites.

Although the somatic mutation theory of cancer is the dominant paradigm of carcinogenesis, the emerging molecular evidence is not entirely consistent with this model. For example, many cancers arise without evidence of accumulating somatic mutations, and studies of precancerous and cancerous tissues often fail to disclose either chromosomal aberrations or mutated tumor suppressor genes and oncogenes (Soto & Sonnenschein, 2004). Furthermore, mutational events that are identified in cancerous tissues may have occurred late in tumor development as the result of cancer development, rather than being the cause (Lijinsy, 1989; Loeb, Loeb, & Anderson, 2003; Prehn, 1994).

Epigenetic Theory of Cancer

The existing scientific evidence is inconsistent with the hypothesis that cancer always arises from a single "mutated" cell and progresses due to accumulation of subsequent mutations that confer a survival advantage to cancer cells. An alternative hypothesis, the *epigenetic theory of cancer*, proposes that cancer develops due to activation or deactivation of certain genes that have a major impact on mechanisms of cell survival and cell division, *but in the absence of mutations that alter the DNA sequence* (Momparler, 2003; Verma, Maruvada, & Srivastara, 2004). Any process that modifies the expression of genes that control major mechanisms of cancer development (e.g., mitosis, apoptosis, angiogenesis, mutagenesis, immunosuppression, and metastasis) could obviously fuel the process of cancer development.

In the epigenetic theory there is no change in the underlying base pair sequence of the DNA; rather, nongenetic factors of the cellular microenvironment cause genes to be expressed differently. Epigenetic programming involves chemical reactions such as the addition or removal of methyl groups (CH_3) or acetyl groups (NH_2) to/from DNA or histones that alter the conformation of the DNA and determine whether genes are expressed or suppressed.

The best known example of programmed epigenetic changes in human biology is the process of cellular differentiation that occurs during embryogenesis. During embryogenesis, a single fertilized egg with a fixed genotype undergoes continuous mitosis and cell differentiation, leading to cell lineages consisting of billions of cells with extraordinarily diverse phenotypes (e.g., neurons, blood vessels, muscles, bones, epithelium). Cell differentiation produces such phenotypic diversity through programmed activation and inactivation of different sets of genes. Thus, differentiated cells in multicellular organisms express only the genes that are necessary for their own phenotypic activity. Furthermore, once a cell lineage becomes fully differentiated and mature, its inherent epigenetic pattern of genetic expression is usually preserved over multiple generations of cell division throughout the human life span. Nevertheless, certain molecular factors are capable of disrupting the normal epigenetic phenotype of a cell population, giving rise to mechanisms that initiate and promote the development of malignant tumors. Molecular mechanisms responsible for the genesis of specific forms of cancer are addressed in the next section.

MOLECULAR MECHANISMS OF CARCINOGENESIS

Several molecular mechanisms, acting either alone or in combination, are likely responsible for the initiation and promotion of carcinogenesis in a given individual. These mechanisms can be broadly categorized into six distinct groups: *mutagenesis*, *mitogenesis*, *angiogenesis*, *metastasis, inhibition of apoptosis*, and *immunosuppression* with reduced antineoplastic activity of T and B lymphocytes. A brief overview of each of these mechanisms is given in the following sections. More thorough reviews can be found in the literature (Howe, Subbaramaiah, Brown, & Dannenberg, 2001; Shiff & Rigas, 1999).

Mutagenesis

Germline and Somatic Mutations

Gene mutations can be either inherited from a parent or acquired during a person's lifetime. Mutations that are passed from parent to offspring are called hereditary mutations or *germline mutations* (because they are present in the egg and sperm cells, which are also called germ cells).

Somatic mutations occur in the DNA of individual cells at some time during the life span. Somatic mutations can be caused by environmental factors such as ionizing radiation from the sun or free radical compounds formed by physiologic processes such as lipid peroxidation, or they can result from spontaneous errors that occur during DNA replication in the cell cycle. Acquired mutations in somatic cells (cells

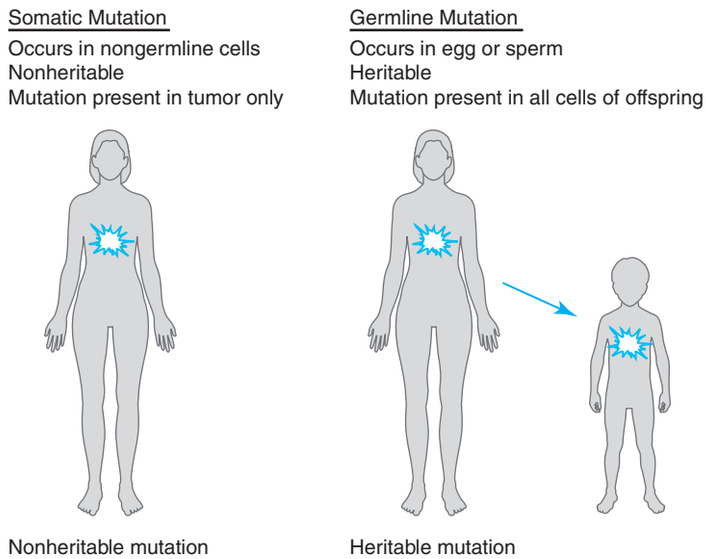


Figure 1.4 Somatic and Germline Mutations.

other than germ cells) cannot be passed on to the next generation (**Figure 1.4**).

Mutations that occur only in an egg or a sperm cell, or those that occur just after fertilization, are called *de novo mutations*. A genetic disorder in which an affected individual has a mutation in every cell but has no family history of the disorder may be attributable to a de novo mutation.

Mutations and Cancer

Accumulated mutagenic damage to DNA is believed to contribute substantially to the etiology of cancer; and it is indeed true that hundreds of genetic alterations have been identified that appear to influence carcinogenesis. Nevertheless, the form and timing of genetic alterations that spur the initiation, growth, and development of neoplastic cell populations remain elusive (Ames, Durston, Yamasaki, & Lee, 1973; Armitage & Doll, 1956; Cairns, 1975; Knudson, 1971; Loeb et al., 2003).

Subtypes of Genetic Alterations

Genetic alterations that have been found to influence carcinogenesis are divisible into distinct subtypes. They include (1) subtle base pair changes that alter gene function, (2) cytogenetically observable alterations in chromosome number (gain or loss of chromosomes) that produce genetic instability, (3) chromosomal translocations that create fusion genes that enhance neoplastic transformation, and (4) gene amplification whereby multiple copies of cancer-promoting genes are overexpressed. It is notable that genetic alterations are a common feature of most forms of cancer but not of normal cell populations (Lengauer, Kinzler, & Vogelstein, 1998).

Causes of Mutagenesis

Mutations may be caused by exposure to both intrinsic and extrinsic factors. Several natural intrinsic physiologic processes generate reactive oxygen species that have mutagenic potential. Chief among them are aerobic respiration (oxidative phosphorylation), anaerobic respiration (glycolysis), lipid peroxidation, and chronic inflammation.

Intrinsic Mutagens. *Aerobic respiration* (*oxidative phosphorylation*) converts glucose to the high-energy compound adenosine triphosphate (ATP) in the mitochondria of the cytoplasm. The process of oxidative phosphorylation, which involves the flow of high-energy electrons through the electron transport chain, continuously generates DNA-damaging reactive oxygen species such as hydrogen peroxide, superoxide, and hydroxyl radicals. Because mitochondria contain haploid DNA unprotected by histones, mitochondrial DNA is highly vulnerable to oxidative damage mediated by persistent exposure to these compounds (Griffiths, Doudican, Shadel, & Doetsch, 2009).

The *Warburg Effect*, discovered by Otto Warburg and colleagues in 1924, describes the switch from oxidation phosphorylation to anaerobic respiration (glycolysis and lactate fermentation) for energy

production in a wide spectrum of cancer cells (Warburg, 1956; Warburg, Posener, & Negelein, 1924). Warburg observed that cancer cells exhibit a high rate of glycolysis even in the presence of oxygen (*aerobic glycolysis*), a process that supplies energy for rapid cancer cell proliferation (Lunt & Vander Heiden, 2011). Although rapid cell proliferation per se may enhance mutagenesis of nuclear DNA, recent studies suggest that the diminution of reactive oxygen species with transition from oxidative phosphorylation to aerobic glycolysis may actually protect the mitochondrial DNA from damage (Ericson et al., 2012).

It is well known that the oxidative biodegradation of lipids (*lipid peroxidation*) in the human system generates reactive electrophilic compounds that have mutagenic potential. For example, malondialdehyde is a naturally occurring product of lipid peroxidation that is both mutagenic and carcinogenic (Marnett, 1999). Lipid peroxidation of polyunsaturated fatty acids is a self-propagating chain reaction that generates a variety of reactive oxygen species that are capable of interacting with DNA to cause mutations (Burcham, 1998).

Chronic inflammation stimulates the release of reactive oxygen species and reactive nitrogen species, which can interact with DNA to cause mutations. Furthermore, spontaneous breakdown of prostaglandins of the prostaglandin cascade produce the mutagenic agent malondialdehyde, which reacts with DNA under physiological conditions to form DNA adducts. Prolonged chemical exposures, persistent foreign bodies, chronic infection, and obesity are all causes of chronic inflammation (Ferguson, 2010).

The normal process of cell division has an intrinsic form of mutagenesis (Burnett, 1974). With every cell division there is incomplete duplication of the chromosomal tips (called telomeres). Successive cell divisions in a cell lineage therefore result in shortening of DNA until a point is reached where the daughter cells are no longer capable of dividing (called the *Hayflick limit*). This intrinsic mutational event is the basis for the *telomere theory of aging*—namely, as an ever-increasing percentage of cells reach their Hayflick limit and are unable to replicate, the defense, maintenance, and repair mechanisms of the body become increasingly impaired (Hayflick, 1985; 2007).

Extrinsic Mutagens Exogenous mutagenic factors include ionizing radiation, infectious agents, and certain chemicals found in some foods, beverages, drugs, and complex mixtures such as tobacco smoke and polluted air. Ionizing radiation carries enough kinetic energy to cause breaks and other damage in double-stranded DNA. Sources of ionizing radiation include cosmic rays from the sun, radon decay products from rock, medical x-rays, fallout from nuclear weapons, and discharges of radioactive waste from nuclear reactors. Light from the sun that reaches the earth is primarily nonionizing because ionizing rays are largely filtered out by ozone and other gases in the atmosphere. However, nonionizing ultraviolet rays may also induce DNA mutations by photochemical reactions (Doll, 1995; Gilbert, 2009).

Various infectious agents have strong mutagenic potential, particularly viruses that use the host DNA for replication (e.g., human immunodeficiency virus [HIV], human papillomaviruses [HPVs], hepatitis viruses, Kaposi's sarcoma virus, and Epstein–Barr virus). Such viruses have the ability to incorporate their genomes into human DNA, thereby causing structural changes and modification of gene expression. For example, integrated viral genes may encode proteins that bind to and inactivate the host tumor suppressor gene products that normally provide checks and balances on the cell cycle and thereby regular cell growth (zur Hausen, 1991). Furthermore, some viruses such as simian virus 40 (SV40) contain *oncogenes* that induce cancerous changes in the host cell (Bocchetta, Miele, Pass, & Carbone, 2003).

Tobacco smoke is a complex mixture of more than 5,000 gases and compounds, many of which are carcinogenic and/or mutagenic. Tobacco smoke contains three major classes of mutagenic substances: polycyclic aromatic hydrocarbons, nitrosamines, and heterocyclic amines. The metabolism of tobacco carcinogens is complex and involves activation versus detoxification by liver enzymes. The carcinogens of tobacco smoke, their uptake and metabolism, and the mechanisms by which they cause DNA mutations and DNA damage have been thoroughly discussed elsewhere (Hecht, 1999).

Food and beverages may also contain mutagenic agents. Notably, thermic reactions during cooking of meat can form mutagenic compounds such as heterocyclic amines and polycyclic aromatic hydrocarbons. Metabolic activation of such compounds by liver enzymes may increase their mutagenic potency. Grains and other foods may be contaminated with molds, fungal organisms, or bacteria that produce toxic substances with mutagenic properties; for example, ingestion of grain contaminated by the mold *Aspergillus flavus* results in exposure to the mycotoxin aflatoxin B1, which is highly mutagenic to hepatocytes. Food preservatives such as sodium nitrite may be metabolized in the stomach to form nitrosamines with mutagenic and carcinogenic properties. Ethanol present in alcoholic beverages has

been classified as a human carcinogen by the International Agency for Research on Cancer (IARC). Dioxins with carcinogenic properties have been identified in foods and beverages. Heavy salt intake may damage the gastric epithelium and promote mutagenesis and carcinogenesis. Finally, excessive consumption of omega-6 polyunsaturated fatty acids may promote mutagenesis and carcinogenesis by enhancing chronic activation of the inflammatory cascade and the formation of reactive oxygen species (Sugimura, 2000).

Mitogenesis

Mitogens are factors that stimulate cell division (mitosis). There are a multitude of such factors that are both intrinsic and extrinsic to the human system. Intrinsic mitogens include hormones of the endocrine system and growth factors and cytokines that exert primarily paracrine effects. Whereas *endocrine* factors (hormones) travel through the blood to exert their effects, *paracrine* factors are secreted into the microenvironment in close proximity to the cell producing them to elicit local cellular responses. A special form of cell signaling involves *autocrine* factors that bind to receptors on the secreting cell to elicit cell division or changes (**Figure 1.5**). Hormones can be either steroids or polypeptides, whereas virtually all growth factors and cytokines are polypeptides.

Endocrine Factors

The human endocrine system is highly complex and consists of multiple secreting and responding organs and tissues (e.g., the central nervous system, the hypothalamic-pituitary axis, the thyroid and parathyroid glands, the thymus, the adrenal glands, the lungs, the gastrointestinal tract, the liver, the pancreas, the kidneys, the bone marrow, gonadal organs of the reproductive tract, and the immune system). A few examples of important endocrine factors with mitogenic activity are presented here.

Steroidal hormones (estrogen, progesterone, testosterone, glucocorticoids, vitamin D_3, and retinoids) are all small lipophilic molecules that easily penetrate both the cellular and nuclear membranes to enter the nucleus, where they bind to their respective receptors that are ligand-dependent transcription factors. These ligand–receptor complexes bind to specific DNA response elements in the promoter regions of genes to regulate gene expression and cell division in target cell populations.

Polypeptide hormones secreted by specific endocrine organs travel through the blood bound to carrier proteins and elicit their responses in distant cell populations. Pituitary hormones with mitogenic impact on their target tissues include growth hormone (GH), thyroid-stimulating hormone (TSH), follicular-stimulating hormone (FSH), luteinizing hormone (LH), and adrenocorticotropic hormone (ACTH). Hormones are released from the pituitary under the influence of releasing hormones secreted by the hypothalamus.

Insulin is a polypeptide hormone secreted by the beta cells in the pancreas that regulates carbohydrate and fat metabolism and induces glucose uptake for energy in muscle cells and storage in fat cells. Insulin also can stimulate the proliferation of fat and smooth muscle cells. Compounds with close homology to insulin, *insulin-like growth factors (IGFs)*, are synthesized and secreted by the liver under the influence

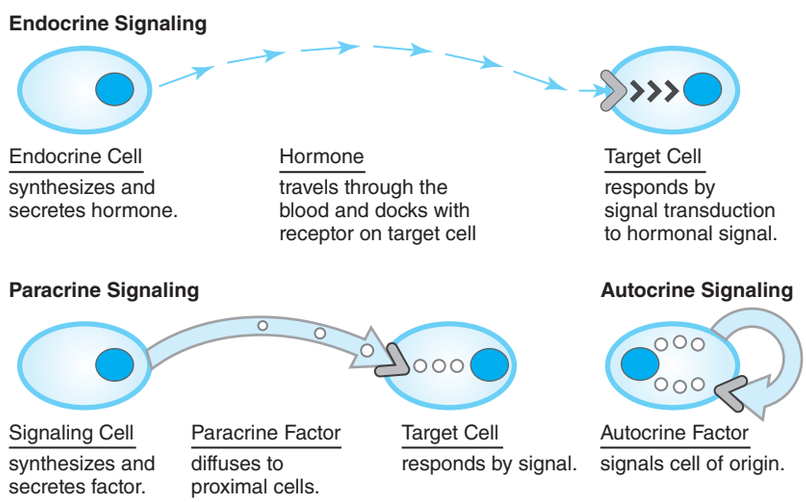


Figure 1.5 Types of Intercellular Signaling.

of pituitary GH. The IGF compounds make up the *IGF axis*, which promotes cell proliferation and the inhibition of cell death (apoptosis) in a variety of tissues (Le Roith, 1997).

Glucagon is a polypeptide hormone secreted by the alpha cells in the pancreas that induces the breakdown of glycogen and release of glucose into the bloodstream by the liver. The effects of glucagon and insulin therefore counterbalance one another in maintaining normal levels of blood glucose. Glucagon and *glucagon-like factors (GLFs)* have been found to stimulate the proliferation of intestinal epithelium (Keiffer & Habener, 1999).

Calcitonin and parathyroid hormone are polypeptide hormones synthesized by the thyroid and parathyroid glands, respectively. These hormones coordinate bone remodeling and maintain calcium homeostasis. Calcitonin stimulates bone formation by osteoblasts whereas parathyroid hormone induces bone resorption by osteoclasts (Carter & Schipani, 2006).

Erythropoietin is a hormone that is essential for red blood cell (erythrocyte) production. This glycoprotein is secreted by the kidneys and induces the differentiation and growth of erythrocytes from progenitor cells in the bone marrow.

Paracrine Factors

A number of paracrine factors called *growth factors* can also stimulate cell division in certain tissues, particularly sites of wound healing and inflammation. Examples include epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factors (TGFα and TGFβ), and platelet-derived growth factor (PDGF); there are many others. Each of these mitogens elicits cellular responses through interaction with specific receptor molecules on cell membranes. For example, PDGF induces proliferation of fibroblasts, smooth muscle cells, and monocytes; EGF is mitogenic for fibroblasts and many types of epithelial cells; FGF induces growth and development of blood vessels; and TGFα has mitogenic effects similar to EGF, whereas TGFβ has growth inhibitory effects in many cell populations.

Cytokines are proteins secreted by immune cells that help orchestrate both the induction and effector phases of all immune and inflammatory responses, including cell proliferation, intercellular communication, and cell death. The term *cytokine* is often used specifically to refer to a wide variety of locally acting immunomodulating agents (e.g., interleukins, interferons, tumor necrosis factor) that facilitate the immune response. Nevertheless, certain cytokines effectively spur the proliferation of other cells. For example, specific interleukins secreted by activated T lymphocytes (IL-2 and IL-4) stimulate the clonal expansion of other immune cells in response to viral infection (Dinarello, 2007).

Prostaglandins and *leukotrienes* are short-lived compounds derived from fatty acids that modulate important physiological functions. Prostaglandins, particularly PGE1 and PGE2, are the principal mediators of the inflammatory response, whereas leukotrienes are involved in allergic and asthmatic reactions. Some prostaglandins have potent mitogenic activity in epithelial cell populations; for example, PGE2, the chief prostaglandin of the inflammatory response, stimulates proliferation of the mammary epithelium (Harris, 2007).

Mitogen-Activated Protein Kinase (MAPK) Cascade

Mitogenic hormones and growth factors stimulate cell division by a process known as *signal transduction* in which the mitogen activates specific receptors on the cell membranes of target cells that, in turn, initiate tightly regulated cascades of intracellular signaling by mitogen-activated protein kinases (MAPKs) (**Figure 1.6**). Human cells contain multiple MAPK pathways, each consisting of specific cytoplasmic protein kinases that are sequentially phosphorylated. Certain of these protein kinases are translocated into the nucleus, where they induce the expression of genes necessary for cell division. Each MAPK pathway consists of a specific profile of protein kinases that ultimately control cell differentiation, cell proliferation, and cell death in a specific tissue. In humans, more than 500 different protein kinase genes and approximately 50 distinct MAPK cascades have now been identified (Manning, Whyte, Martinez, Hunter, & Sudarsanam, 2002; Pearson et al., 2001).

Extrinsic Factors

In general, any form of cellular injury can result in a mitogenic response by the afflicted tissue. Causative

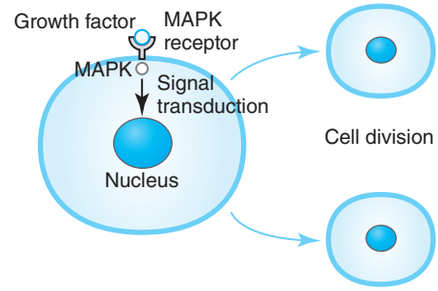


Figure 1.6 Mitogen-Activated Protein Kinase (MAPK) Cascade.

factors of cell injury include microbial infection, physical agents (burns, radiation, trauma), chemical exposures (toxins, caustic substances), ischemia, necrosis, and all types of immunological reactions. All such exposures elicit an immediate inflammatory response of the innate immune system. Although acute inflammation is self-limited and of short duration, chronic inflammation is associated histologically with the persisting presence of immune cells and is nearly always accompanied by the proliferation of blood vessels and involved tissues.

Chronic inflammation is maintained by constitutive overexpression of cyclooxygenase-2 (COX-2), which is the rate-limiting enzyme of the prostaglandin cascade. The COX-2 enzyme efficiently catalyzes the conversion of essential dietary fats (principally arachidonic acid and unconjugated linoleic acid) into prostaglandins. Chronic inflammation and continuous overexpression of COX-2 markedly amplify the biosynthesis of PGE-2, which is the chief prostaglandin of the inflammatory cascade. This short-lived intercellular hormone is capable of inducing the transcription of specific genes in the nucleus of nearby cells. In particular, PGE-2 has been found to stimulate the transcription of genes that have powerful mitogenic effects (Harris, 2007).

Contact inhibition is a special form of growth control by which cells ordinarily do not divide when in close contact with one another. This phenomenon is regulated by the density of the cell population, the access of cells to nutrients, and protein complexes of beta-catenin and cadherins that control cell-to-cell adhesion. In general, an intact *E-cadherin-catenin complex* maintains normal intercellular adhesion and limits cell division, whereas loss of function of this complex permits or enhances cell proliferation and leads to invasion and metastasis by malignant cells (Wijnhoven, Dinjens, & Pignatelli, 2000).

Angiogenesis

Angiogenesis is a normal and vital physiological process by which new blood vessels develop from preexisting vessels. *Vascular endothelial growth factor (VEGF)* is a potent stimulant of de novo blood vessel formation (angiogenesis) in a variety of tissues. Once believed to be present only in the endothelial lining of blood vessels, VEGF has now been discovered in virtually all types of cancers. Indeed, the process of angiogenesis is fundamental to the growth, development, and metastatic spread of cancerous tumors (Folkman, 2006; Folkman & Klagsbrun, 1987).

When a tumor outgrows its blood supply, cancer cells secrete VEGF to signal nearby blood vessels, which respond by sprouting new vessels that grow into the tumor (**Figure 1.7**). This sprouting process involves the secretion of proteolytic enzymes by the

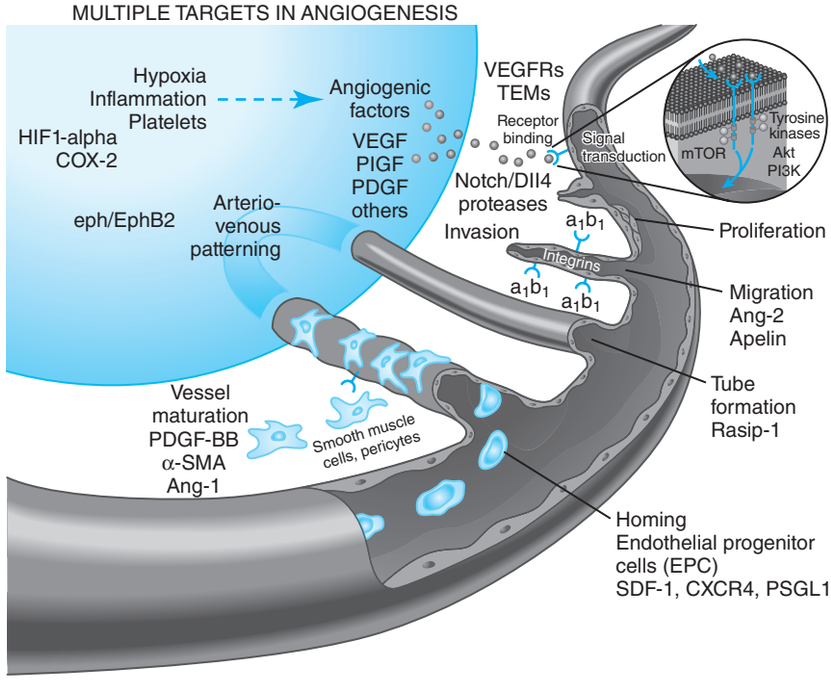


Figure 1.7 Angiogenesis Cascade in Tumor Growth and Development.

parent vessel to enable the formation of new capillaries. As a consequence, the new blood vessels are immature and "leaky," thereby providing a doorway for cancerous cells to enter the bloodstream and spread to other organs (Folkman, 2006).

Suppression of Apoptosis

Apoptosis, or controlled cell death, is an important regulatory mechanism for the maintenance of homeostasis in cell populations. Dysfunctional apoptosis results in immortalization of cells, a key feature of cancer cells. Apoptosis is regulated by an intrinsic pathway that originates inside the cell and an extrinsic pathway that originates outside the cell.

The intrinsic pathway involves mitochondrial release of cytochrome c and activation of caspase 9 and other enzymes that destroy the cell. Intrinsic apoptosis is triggered when the expression of two nuclear genes, Bcl-2 and BAX, favors BAX. The extrinsic pathway involves activation of death receptors on the cell membrane by tumor necrosis factors alpha and beta, and other epigenetic factors. This results in activation of caspase 8 and other enzymes that destroy the cell (Lowe & Lin, 2000).

Telomeres are sequences of DNA that cap the ends of eukaryotic chromosomes. In normal cell populations, as mentioned earlier, telomeres are lost from the chromosomal tips with each successive cell division, and eventually cell division ceases and apoptosis occurs. However, in abnormally proliferating cells, a special reverse transcriptase enzyme called *telomerase* replicates the terminal DNA sequence of chromosomes and restores telomeres to the chromosomal tips (**Figure 1.8**). Telomerase activity is amplified in cancer cells that proliferate indefinitely, but is virtually undetectable in normal somatic cells that undergo programmed apoptosis. Maintenance of telomere integrity by telomerase therefore protects cancer cells from apoptosis, and reciprocally, inhibition of telomerase and loss of telomeres elicits an apoptotic response. For this reason, apoptotic factors that selectively target cancer cells may be of value in cancer therapy (Mondello & Scovassi, 2004).

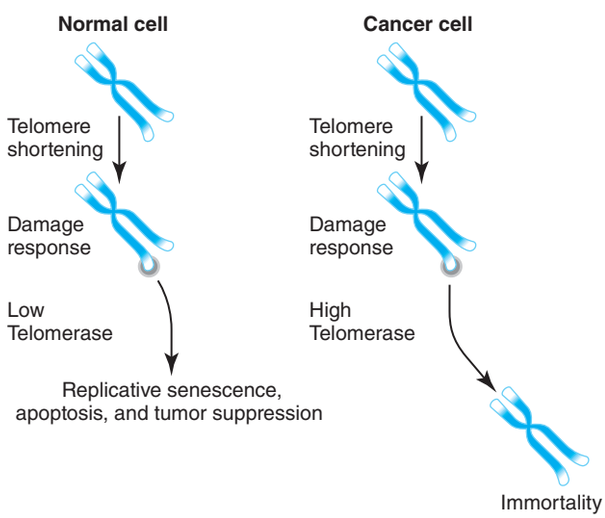
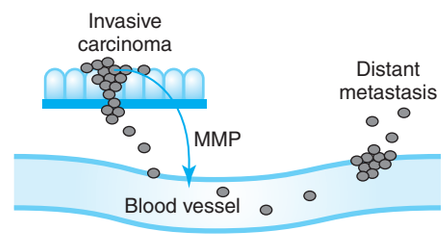


Figure 1.8 Telomeres and Apoptosis.

Metastasis

Metastatic spread of cancer occurs by direct invasion of proximal tissues, lymphatic invasion, and hematogenous dissemination. Epithelial carcinomas develop from cells that are anchored by the basement membrane, a thin collagenous membrane that serves as a mechanical barrier to invasion of underlying tissues by malignant cells. Obviously, a key initial step in the metastatic spread of epithelial tumors is penetration of the basement membrane by cancer cells. Proteolytic enzymes called *matrix metalloproteinases (MMPs)* secreted by cancer cells facilitate penetration of the basement membrane. These zinc-dependent enzymes are important for normal tissue remodeling and repair and are capable of degrading collagen, proteins, and other components of the basement membrane. By secreting MMPs, cancer cells can break through the basement membrane as well as other cell membranes and gain access to underlying tissues, lymphatics, and blood vessels, thereby facilitating cancer metastasis (Deryugina & Quigley, 2006; Itoh & Nagase, 2002) (**Figure 1.9**).

The HER-2/neu oncogene is a member of the epidermal growth factor receptor (EGFR) family. It is an important mediator of cancer cell growth and metastasis. When the HER-2/neu receptor protein is



Cancer cells breach the basement membrane, enter the bloodstream, and invade distant tissues by secreting *matrix metalloproteinases* (MMPs) that biodegrade cell membranes.

Figure 1.9 Matrix Metalloproteinase (MMP) Activity in Metastatic Cancer.

activated, multiple other factors are activated that promote tumor development and metastatic spread of cancer cells. For example, VEGF is secreted to promote the sprouting of new blood vessels (angiogenesis) and MMPs are produced that degrade cell membranes and basement membranes and are thus associated with tumor invasiveness, metastasis, and poor survival. Overexpression of HER-2/neu is now widely used by clinicians as a biomarker of poor prognosis and metastasis for patients with invasive breast cancer, and the HER-2/new receptor is a molecular target for breast cancer therapy (Coussens et al., 1985; Olayioye, 2001).

Immunosuppression

Immunosuppression is a characteristic feature of cancer patients that correlates with disease promotion and progression. Prostaglandins, particularly PGE-2, are important modulators of immunosuppression. Pockaj et al. (2004) found that increased levels of PGE-2 suppress the immunocompetence of helper T-cells and dendritic cells in newly diagnosed breast cancer patients. Specifically, elevated levels of PGE-2 were associated with reduced secretion of anti-tumor factors by T-cells (interferon gamma, tumor necrosis factor alpha, and interleukins IL-2 and IL-12) and loss of immunocompetence in dendritic cells (reduced secretion of stimulatory molecules, loss of antigen-sensitizing function, reduced phagocytic activity, and lack of maturation potential). Defective T-cell and dendritic cell function due to COX-2–driven PGE-2 biosynthesis is therefore an important mechanism by which tumors evade immunosurveillance.

Inflammogenesis of Cancer

One microenvironmental process capable of inducing untoward epigenetic changes in gene expression leading to cancer development is *chronic inflammation*. More than 150 years ago the famous German pathologist, Rudolph Virchow, suggested that chronic inflammation leads to cancer development by increasing uncontrolled cellular proliferation (Balkwill & Mantovani, 2001; Virchow, 1863). In 1992, molecular biologists discovered that the process of inflammation is primarily under the control of an inducible gene called cyclooxygenase-2 (COX-2) (Herschman, 2002). The COX-2 gene is normally silent in noninflamed tissue but is readily "turned on" by a variety of inflammatory environmental stimuli known to cause cancer including tobacco smoke, reactive oxygen species (ROS), polyunsaturated fatty acids, radiation, certain infectious bacteria and viruses, hypoxia, endotoxins, and many other agents (Harris, 2007; Harris, Beebe-Donk, & Alshafie, 2007). Overwhelming molecular evidence has linked induction of the COX-2 gene and overexpression of certain molecules of inflammation (called prostaglandins) to all of the essential features of carcinogenesis (mutagenesis, mitogenesis, angiogenesis, reduced apoptosis, metastasis, and immunosuppression). Indeed, carcinogenesis often evolves as a progressive series of highly specific cellular and molecular changes in response to induction of constitutive overexpression of COX-2 and the prostaglandin cascade in the *inflammogenesis of cancer* (**Figure 1.10**).

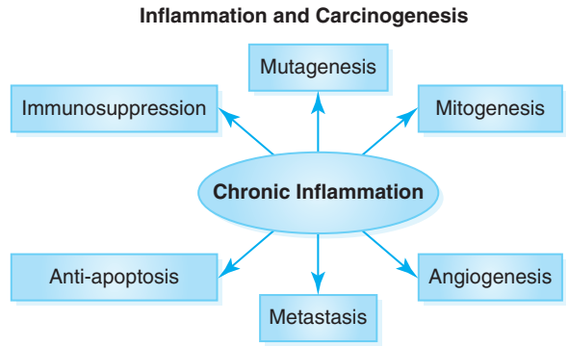


Figure 1.10 Chronic Inflammation and Carcinogenesis.

REFERENCES

Ames, B. N., Durston, W. E., Yamasaki, E., & Lee, F. D. (1973). Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. *Proceedings of the National Academy of Sciences*, *70*(8), 2281–2285.

Armitage, P., & Doll, R. (1956). The age distribution of cancer and a multi-stage theory of carcinogenesis. *British Journal of Cancer*, *8*, 1–12.

Balkwill, F., & Mantovani, A. (2001). Inflammation and cancer: Back to Virchow? *Lancet*, *357*, 539–545.

Blanpain, C., Horsley, V., & Fuchs, E. (2007). Epithelial stem cells: Turning over new leaves. *Cell*, *128*, 445–458.

Bocchetta, M., Miele, L., Pass, H. I., & Carbone, M. (2003). Notch-1 induction, a novel activity of SV40 required for growth of SV40-transformed human mesothelial cells. *Oncogene*, *22*(1), 81–89.

Burcham, P. C. (1998). Genotoxic lipid peroxidation products: Their DNA damaging properties and role in formation of endogenous DNA adducts. *Mutagenesis*, *13*(3), 287–305.

Burnett, M (1974). *Intrinsic mutagenesis*. Lancaster, England: Medical and Technical Publishing Company.

Cairns, J. (1975). Mutation selection and the natural history of cancer. *Nature*, *255*, 197–200.

Carter, P. H., & Schipani, E. (2006). The roles of parathyroid hormone and calcitonin in bone remodeling: Prospects for novel therapeutics. *Endocrine, Metabolic and Immune Disorders—Drug Targets, 6*(1), 59–76.

Coussens, L., Yang-Feng, T. L., Liao, Y. C., Chen, E., Gray, A., McGrath, J., … Francke, U. (1985). Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science*, *230*(4730), 1132–1139.

Deninger, P. (1999). Genetic instability in cancer: Caretaker and gatekeeper genes. *Ochsner Journal*, *1*(4), 206–209.

Deryugina, E. I., & Quigley, J. P. (2006). Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Review*, *25*(1), 9–34.

Dinarello, C. A. (2007). Historical review of cytokines. *European Journal of Immunology*, *37*(Suppl 1), S34–S45.

Doll, R. (1995). Hazards of ionizing radiation: 100 years of observations on man. *British Journal of Cancer*, *72*(6), 1339–1349.

Dryja, T. P., Friend, S, & Weinberg, R. A. (1986). Genetic sequences that predispose to retinoblastoma and osteosarcoma. *Symposium on Fundamental Cancer Research*, *39*, 115–119.

Efeyan, A., & Serrano, M. (2007). p53: Guardian of the genome and policeman of the oncogenes. *Cell Cycle*, *6*(9), 1006–1010.

Ericson, N. G., Kulawiec, M., Vermulst, M., Sheahan, K., O'Sullivan, J., Salk, J. J., & Bielas, J. H. (2012). Decreased mitochondrial DNA mutagenesis in human colorectal cancer. *PLoS Genetics*, *8*(6), e1002689.

Fearon, E. R., & Vogelstein, B. A. (1990). A genetic model for colorectal tumorigenesis. *Cell*, *61*(5), 759–767.

Ferguson, L. R. (2010). Chronic inflammation and mutagenesis. *Mutation Research*, *690*(1–2), 3–11.

Folkman, J. (2006). Angiogenesis. *Annual Review of Medicine*, *57*, 1–18.

Folkman, J., & Klagsbrun, M. (1987). Angiogenic factors. *Science*, *235*(4787), 442–447.

Gilbert, E. S. (2009). Ionising radiation and cancer risks: What have we learned from epidemiology? *International Journal of Radiation Biology*, *85*(6), 467–482.

Griffiths, L. M., Doudican, N. A., Shadel, G. S., & Doetsch, P. W. (2009). Mitochondrial DNA oxidative damage and mutagenesis in Saccharomyces cerevisiae. *Methods in Molecular Biology*, *554*, 267–286.

Hancock, J. T. (2010). *Cell signaling* (3rd ed.). New York: Oxford University Press.

Harris, R. E. (2007). Cyclooxygenase-2 (COX-2) and the inflammogenesis of cancer. *Subcellular Biochemistry*, *42*, 63–126.

Harris, R. E., Beebe-Donk J., & Alshafie, G. A. (2007). Cancer chemoprevention by cyclooxygenase 2 (COX-2) blockade: Results of case control studies. *Subcellular Biochemistry*, *42*, 193–212.

Hayflick, L. (1985). The cell biology of aging. *Clinics in Geriatric Medicine*, *1*(1), 15–27.

Hayflick, L. (2007). Entropy explains aging, genetic determinism explains longevity, and undefined terminology explains misunderstanding both. *PLoS Genetics*, *3*(12), e220.

Hecht, S. S. (1999). Tobacco smoke carcinogens and lung cancer. *Journal of the National Cancer Institute, 91*(14), 1194–1210.

Herschman, H. R. (2002). Historical aspects of COX-2. Cloning and characterization of the

cDNA, protein and gene. In R. E. Harris (Ed.), *COX-2 blockade in cancer prevention and therapy* (pp. 13–32). Totowa, NJ: Humana Press.

Holowaty, P., Miller, A. B., Rohan, T., & To, T. (1999). Natural history of dysplasia of the uterine cervix. *Journal of the National Cancer Institute*, *91*(3), 252–258.

Howe, L. R., Subbaramaiah, K., Brown, A. M. C., & Dannenberg, A. J. (2001). Cyclooxygenase-2: A target for the prevention and treatment of breast cancer. *Endocrine-Related Cancer*, *8*, 97–114.

Itoh, Y., & Nagase, H. (2002). Matrix metalloproteinases in cancer. *Essays in Biochemistry*, *38*, 21–36.

Kieffer, T. J., & Habener, J. F. (1999). The glucagon-like peptides. *Endocrine Reviews*, *20*, 876–913.

Knudson, A. G. (1971). Mutation and cancer: Statistical study of retinoblastoma. *Proceedings of the National Academy of Sciences*, *68*, 820–823.

Knudson, A. G. (2001). Two genetic hits (more or less) to cancer. *Nature Review Cancer*, *1*(2), 157–162.

Kumar, V., Abbas, A. K., & Aster, J. C. (2014). *Robbins & Cotran pathologic basis of disease* (9th ed.) Philadelphia, PA: Mosby & Saunders.

Lengauer, C., Kinzler, K. W., & Vogelstein, B. (1998). Genetic instabilities in human cancers. *Nature*, *396*, 643–649.

Le Roith, D. (1997). Insulin-like growth factors. *New England Journal of Medicine*, *336*, 633–640.

Lijinsy, W. (1989). A view of the relation between carcinogenesis and mutagenesis. *Environmental and Molecular Mutagenesis*, *14*(Suppl 16), 78–84.

Loeb, L. A., Loeb, K. R., & Anderson, J. P. (2003). Multiple mutations and cancer. *Proceedings of the National Academy of Sciences*, *100*, 776–781.

Lowe, S. W., & Lin, A. W. (2000). Apoptosis in cancer. *Carcinogenesis*, *21*(3), 485–495.

Lunt, S. Y., & Vander Heiden, M. G. (2011). Aerobic glycolysis: Meeting the metabolic requirements of cell proliferation. *Annual Review of Cell and Developmental Biology*, *27*, 441–464.

Manning, G., Whyte, D. B., Martinez, R., Hunter, T., & Sudarsanam, S. (2002). The protein kinase complement of the human genome. *Science*, *298*(5600), 1912–1934.

Marnett, L. J. (1999). Lipid peroxidation-DNA damage by malondialdehyde. *Mutation Research*, *424*(102), 83–95.

Marshman, E., Booth, C., & Potten, C. S. (2002). The intestinal epithelial stem cell. *Bioessays*, *24*, 91–98.

Momparler, R. L. (2003). Cancer epigenetics. *Oncogene*, *22*(42), 6479–6483.

Mondello, C., & Scovassi, A. I. (2004). Telomeres, telomerase, and apoptosis. *Biochemistry and Cell Biology*, *82*(4), 498–507.

Nordling, C. O. (1953). A new theory on the cancer-inducing mechanism. *British Journal of Cancer*, *7*, 68–72.

Olayioye, M. A. (2001). Update on HER-2 as a target for cancer therapy: Intracellular signaling pathways of ErbB2/HER-2 and family members. *Breast Cancer Research*, *3*(6), 385–389.

Pearson, G., Robinson, F., Beers Gibson, T., Xu, B. E., Karandikar, M., Berman, K., & Cobb, M. H. (2001). Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions. *Endocrine Review*, *22*(2), 153–183.

Pellettieri, J., & Alvarado, A. S. (2007). Cell turnover and adult tissue homeostasis: From humans to planarians. *Annual Review of Genetics*, *41*, 83–105.

Pockaj, B. A., Basu, G. D., Pathangey, L. B., Gray, R. J., Hernandez, J. L., Gendler, S. J., & Mukherjee, P. (2004). Reduced T-cell and dendritic cell function is related to cyclooxygenase-2 overexpression and prostaglandin E2 secretion in patients with breast cancer. *Annals of Surgical Oncology*, *11*, 328–339.

Prehn, R. T. (1994). Cancers beget mutations versus mutations beget cancers. *Cancer Research*, *54*(5), 296–300.

Robbins, S. L, & Cotran, R. S. (1979). Neoplasia. In Robbins S. L., & Cotran R. S., *Pathologic basis of disease* (pp. 141–187). Philadephia, PA: W. B. Saunders Company.

Shiff, S. J., & Rigas, B. (1999). The role of cyclooxygenase inhibition in the antineoplastic effects of nonsteroidal anti-inflammatory drugs (NSAIDs). *Journal of Experimental Medicine*, *190*, 445–450.

Soto, A. M., & Sonnenschein, C. (2004). The somatic mutation theory of cancer: Growing problems with the paradigm? *Biosessays*, *26*(10), 1097–1107.

Spalding, K. L., Bhardwaj, R. D., Buchholz, B. A., Druid, H., & Frisén, J. (2005). Retrospective birth dating of cells in humans. *Cell*, *122*(1), 133–143.

Sugimura, T. (2000). Nutrition and dietary carcinogens. *Carcinogenesis*, *21*(3), 387–395.

Verma, M., Maruvada, P., & Srivastara, S. (2004). Epigenetics and cancer. *Critical Reviews in Clinical Laboratory Sciences*, *41*, 5–6.

Virchow, R. (1863). Aetiologie der neoplastischen Geschwulst/Pathogenie der neoplastischen Geschwulste. In: *Die Krankhaften Geschwulste* (pp. 57–101). Berlin, Germany: Verlag von August Hirschwald.

Warburg, O. (1956). On the origin of cancer cells. *Science*, *123*(3191), 309–314.

Warburg, O., Posener, K., & Negelein, E. (1924). Über den Stoffwechsel der Carcinomzelle. *Biochemische Zeitschrift*, *152*, 309–344.

Wijnhoven, B. P. L., Dinjens, W. N. M., & Pignatelli, M. (2000). E-cadherin-catenin cell-cell adhesion complex and human cancer. *British Journal of Surgery*, *87*, 992–1005.

zur Hausen, H. (1991). Viruses in human cancers. *Science*, *254*(5035), 1167–1173.