PART I

The Cancer Problem

CHAPTER

1

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Biology of Cancer

INTRODUCTION

Research into the biology of the cancer cell has led to better understanding of the signaling pathways and the molecular machinery that drives the ability of cancer cells to proliferate, resist attrition, and invade surrounding and long distance tissues. As the altered machinery of the cancer cell becomes more defined, better targeted therapies may be developed.[1] It is important that nurses in the various oncology settings have the understanding and ability to translate this molecular information into their patient care.

The double helix was identified in the 1950s. Emerging genetics knowledge was not included in high school biology textbooks until the late 1970s. The average age of the newly diagnosed patient is 66, born in 1949. Most of these new patients had a limited education in biology, cellular biology, and certainly genetics. In addition, most of the current oncology nurses are in their late 40s and early 50s, creating a similar lack of molecular knowledge, especially in regard to genetics.[2] Oncology nurses today need to embrace the challenge to understand the complexity of the biology of malignant cells, know how to translate this new and robust knowledge into cancer care, and be able to simply explain this multifaceted information to their patients and families.[3] The goal of this chapter is to provide simple descriptions of complex concepts for those nurses new to oncology, whether they are looking for a new challenge or just beginning their careers in cancer nursing.

MODELS AND THEORY OF CANCER DEVELOPMENT

The pathology of cancer demonstrates various phenotypes due to heterogeneity created from diverse cell types plus genetic or epigenetic differences among the cancer cells; put simply, each cancer is different.[4] There are two commonly recognized models of cancer development: clonal evolution and development of the cancer stem cell (CSC). Both models suggest a path to metastatic disease. A third model of tumorigenicity proposes that plasticity exists between the non-CSCs and CSC compartments such that non-CSCs can reacquire a CSC phenotype. The theory of inflammation suggests the association of inflammation with the development of cancer. It addresses the many populations and subpopulations of immune cells in and around the tumor that seem to have an unsubstantiated rationale for their presence other than sustaining the malignant process.

CLONAL MODEL

The clonal model suggests that initial damage to the DNA results in benign tumor growth.[4] Over time, during multistep tumorigenesis, heritable genetic and epigenetic changes accumulate to an ever greater extent, encouraging transformation of normal cells into a lineage of malignant cells. The accumulation of multiple genetic mutations confers beneficial traits that further alter the heterogeneity of cells, supporting clonal expansion. Because the genetic variants accumulate more malignant characteristics that benefit their growth, these cells proliferate more rapidly at the expense of the less fit clones. Consequently, examination of the late-stage tumor reveals much heterogeneity in its makeup.[5]

CANCER STEM CELL MODEL

The focus of the second model is the mutationally corrupt heterogeneous cancer stem cell. In this model, cancer cells show a hierarchy of development, such that a few stem cells have the ability to produce progeny, but demonstrate limited proliferation potential.[6] As the cells divide and proliferate, only one subpopulation has the capability of becoming tumorigenic, with the consequent ability to drive growth and expansion of the malignancy. The successful survival and proliferation of these CSCs depends on their ability to undergo self-renewing mitotic divisions where at least one of the progeny maintains its ability to multiply, virtually forever. Ultimately, this subpopulation of cells within a tumor is responsible for the growth and progression of the malignancy.[4]

Three classes of connective tissue support the CSC function in a variety of tumor types. The first class, consisting of the endothelial cells, pericytes, and perivascular cells, organizes CSCs in the microenvironment into vascular niches within the primary tumors and metastatic sites.[7–9] Cancer-associated fibroblasts (CAFs) constitute the second class of connective tissue induced by cancer cells. These CAFs switch to an unusual aerobic form of glycolysis, secreting lactate (along with the tumor cells) plus pyruvate to be used as energy to fuel cancer cell proliferation.[4,6] Finally, the third class includes the columnar cells important for water absorption.

As the new tumor is established, the growing heterogeneous populations cause progressive enlargement of the tumor while the CSCs move into the resting phase of the cycle (G_0). Cells in this phase of the cell cycle remain sequestered and are known to be resistant to cancer treatment. The other non-CSCs remain in the chronically dividing phases of the cell cycle (G_1, S, G_2, and M), in which they proliferate. Because they are dividing rapidly, they can be destroyed by a variety of treatments or the inherent immune system. Years later, the "resting" CSC can move back into the active phases of the cell cycle (G_1, S, G_2, and M). They do not slip out of the cycle into the G_0 resting phase, however; consequently, there may be continuous

proliferation and exacerbation of a cancer that was believed to be in remission or cured.[4,10,11]

PLASTICITY MODEL OF CANCER STEM CELLS

A newer model embraces the CSC theory, but suggests there is plasticity between the non-CSCs with low potential to become tumors and the CSCs with tumorigenic capability. The CSC model suggests these cells move in a one-way fashion through division to either replenish the CSC pool or become non-CSCs with very low tumorigenic potential. New research indicates that some carcinomas may be able to convert their non-CSCs into aggressive de novo CSCs, in a bidirectional conversion.[4]

INFLAMMATION THEORY

The inflammation theory of cancer development proposes that a variety of infectious agents and their relationships with inflammatory cells plus chronic inflammation are the primary cause of cancer, including the proliferation, survival, and ultimate migration of cancer cells. This theory also stresses the inclusion of factors from the innate immune system, including selectins, chemokines (such as NF-kappa-B), and their receptors, that have the ability to encourage invasion, migration and metastasis.[12,13]

HALLMARKS, DRIVERS, AND ENABLERS OF CANCER

The microenvironment associated with the various models of cancer development plays an important role in malignant growth and development; however, there are known hallmarks of cancer that are associated with the aberrant changes. In addition, other functions have been identified that drive and foster tumorigenesis.[14]

The functions of the cell that drive cancer development are increased proliferation, decreased attrition, and invasion of tissue. The acquired hallmarks of cancer include core changes to the cell such as the ability to sustain proliferative signaling, evasion of growth suppressors, replicative immortality, resistance of cell death, deregulation of defective DNA repair, angiogenesis, and evasion of immunity. Genetic instability, inflammation, angiogenesis, and fibrosis foster the malignant transformation. The changes associated with these elements provide an organizing structure to better understand the dysregulation of the cell associated with malignant transformation. These malignant changes vary with the type of cancer and the organ affected.[15,16]

Hallmarks are the traits or characteristics of tumor cells that sustain oncogenic tasks. The instability of the genome, inflammation, angiogenesis, and fibrosis enable tumor progression by fostering maintenance of gene mutations through defective DNA repair, recruitment of normal cells while molding the tumor, and alterations made to the microenvironment.[1,16,17]

HALLMARK: GENETIC INSTABILITY

Vogelstein[18] was the first to note that "all cancer is genetic." This statement is based on the central dogma that DNA is transcribed into RNA, and all RNA is translated into protein, even in a malignancy. This dogma persists even though matters have become more complicated over time with the inclusion of more types of RNA **(Table 1-1)**.[19–24] Research into malignancies has revealed much about defective DNA

TABLE 1-1

Types and Functionality of RNA

RNA	Function
Messenger RNA (mRNA)[20]	Orders nucleotides into amino acids for protein expression
Ribosomal RNA (rRNA)[20]	Provides structural support for the protein
Transfer RNA (tRNA)[20]	Brings amino acids to site of protein synthesis
MicroRNA (miRNA)[21,22]	Short noncoding RNA that inhibits mRNA so there is decreased gene expression
Piwi-interacting RNA (piRNA)[21,23]	Epigenetic and post-transcriptional gene that silences germline cells, especially spermatogenesis
Small interfering RNA (siRNA)[21,24]	Gene silencing
Short hairpin RNA (shRNA)[24]	Short sequence of RNA that makes a tight hairpin turn to be used to silence targeted gene expression; usually delivered via plasmid or bacterial vectors

Source: Data from Ghildiyal and Zamore[20]; Ritchier et al[21]; Siomi et al[22]; Whitehead et al[23]; Wang et al.[24]

repair and genetic instability and how these processes are associated with the biology of cancer.

DEFINITIONS OF INDIVIDUAL DNA

Most DNA (99.9%) is the same among individuals. A change in the DNA or RNA that occurs in a population and is associated with normal variation is identified as a polymorphism (0.1%).[10] Disease-causing changes to DNA are identified as mutations. Such disease-causing mutations can occur in the germline (inherited DNA originally from the egg and sperm or gametes) or in the somatic cells (DNA found in every cell of the body except the sperm and the egg).[25,26]

CATEGORIES OF GENES ASSOCIATED WITH CANCER

Mutations can occur in normal genes that direct cell growth (proto-oncogenes), such as *ras* or *v-src*, enabling them to be activated into cancer-causing genes (oncogenes), which have the trait of ongoing cell proliferation.[27] Mutations can also occur in tumor suppressor genes,[28] including *p53*, *Rb*, *WT1*, *BRCA1*, *BRCA2*, *VHL*, *APC*, *NF-1*, *NF-2*, and *MTS-1*. When reading the literature, genes are typically identified by *italics* (*p53*), while the proteins of the genes are not italicized (p53).

SOURCES OF CANCER-CAUSING CHANGES IN DNA

Normal cell processes cannot continue when a disease-causing mutation is present. Programmed cell death (apoptosis) will not occur if tumor suppressor genes damage the cell so that it becomes immortalized. As mutations accumulate in these two types of regulatory genes, proliferation also increases; thus immortalization of the cells works together with this process to allow development of a malignancy. Other types of cancer-causing changes in genes include point mutations, deletions, duplications, insertions, translocations, chromosomal aberrations, incorporation of viral segments of genetic material, and epigenetic inactivation.[29]

PASSENGER AND DRIVER MUTATIONS

The current literature describes both passenger and driver mutations. Driver mutations (e.g., *TP53*) are directly associated with oncogenesis and are selected in the microenvironment of the cancer for clonal advantage. Passenger mutations are found in the same tumors but do not offer any clonal advantage and do not contribute to the development of the cancer type; thus they are viewed as "along for the ride."[30,31] Different cancers may have a variable mixture of driver and passenger mutations, yet still demonstrate the presence of similar phenotypes. The drivers in this combination of mutated gene sets can be targeted for treatment, either individually or in a staggered approach.[30–32]

TYPES OF DNA REPAIR

Most normal cells have driver and passenger mutations that could be restored to their pre-damage levels through the normal DNA repair mechanisms; this is obviously important for cancer-free survival. These DNA repair systems include (1) the nucleotide repair (NER) groups, which address mutations associated with xeroderma pigmentosum; (2) the mismatch repair (MMR) genes, which accompany the inherited predisposition to colorectal cancer (CRC); (3) DNA crosslink repair (Fanconi anemia genes); and (4) the well-known DNA repair genes exemplified by the breast cancer genes (*BRCA1/2*). To date, approximately 130 genes have been linked to DNA repair.[33]

EPIGENETIC MECHANISMS

Epigenetic mechanisms can affect the DNA repair genes. While the changes noted previously affect the primary DNA sequence of the repair systems, epigenetic changes occur "above and over" the DNA; consequently, they do not affect the basic structure, but rather have the capacity to determine when and where genes might be expressed (turned on) by altering the chromatin.[34,35]

Chromatin is the material within a chromosome that consists of protein and DNA.[36] The major proteins of chromatin are the histones, which are responsible for compacting the primary DNA by wrapping it tightly (like thread on a spool) so it fits within the nucleus of the cell. These continuous strands of DNA and histones appear like "beads on a string" (euchromatin). When multiple histones wrap tightly together (heterochromatin), they prevent transcription and contain inactive genes.[35–37]

Both the DNA and the histone proteins may become modified with addition or removal of chemical groups, the core of epigenetics. Methyl groups can be added to or removed from DNA, while phosphate, acetyl, or ubiquitin groups can be added to or removed from the histones. Most commonly, methyl groups are added to the core DNA and acetyl groups to the histones.[35,37,38]

The nucleosome is a composition of eight separate histone molecules, with two loops of DNA wrapped around each group of eight histones.[35,37] Because histones are proteins, they can be modified after translation by attachment of acetyl, phosphate, or ubiquitin groups to their "tails."[39] Epigenetic readers are types of enzymes that promote

addition and release of methyl, acetyl, and lysine methyl chemical groups to the "tail"; they are used in transcription. No chemical group additions will close the histones, so no epigenetic reading occurs with tightly packed histones that might prevent DNA expression. Instead, attachment of the chemical groups to the tail causes loose packing, which promotes epigenetic reading and DNA expression with protein growth. The chemical groups cause changes that alter the shape of the chromatin in specific places on the genome, making some areas more available to gene expression. These types of epigenetic modifications are now being explored as targets of cancer therapy for malignancies such as chronic myelogenous leukemia (CML), gastrointestinal (GI) stromal tumors, and breast cancer.[40] Such epigenetic changes can be reversed, but there is also a possibility that they may be transmitted to daughter cells.[40,41]

A frequent finding with cancer is methyl groups added to the DNA, where clusters of guanine (G) follow cytosine (C); these so-called CpG islands cause gene expression to be suppressed. Normally, the CpG islands occur in areas of the DNA where gene expression is initiated (promoter). In healthy cells, they are mostly unmethylated, allowing tumor suppressor genes and proto-oncogenes to be expressed normally, and permitting apoptosis and controlled limited cell proliferation to take place.[37,40-42]

Mutations in oncogenes and tumor suppressor genes coexist in the development of a cancer.[3] The phenotypes associated with these mutations vary among many types of cancer.[43] In colon cancer, for example, each person's tumor is as distinctive as a snowflake, with a unique set of mutated genes accumulating in an unpredictable order, both drivers and passengers.[3] Somatic mutations found in some human tumors are reviewed in **Table 1-2**.[2,39–41]

ENABLER: LOSS OF HETEROZYGOSITY

When both alleles are identical, they are referred to as *homozygous*. If one of the alleles has an inherited mutation of a tumor suppressor gene (with loss of genetic material), it is termed *heterozygous* because the alleles are different. Because there is a normal allele, the function of the gene and its protein product is maintained. Once the remaining allele is mutated, however, the gene and its product (protein) will lose normal functioning. This further alteration and loss of genetic material is labeled *loss of heterozygosity* (LOH). The loss of an entire chromosome, translocation of a piece of the chromosome, reduplication of a piece of chromosome that already has an abnormal gene, or development of a point mutation in the second functioning allele is included in the LOH definition.

Cancer susceptibility genes such as oncogenes and tumor suppressor genes can develop LOH. Basic research is identifying an increasing number of tumor suppressor genes that, when mutated, are closely associated with the development and progressions of human cancers.[44–46]

DRIVER: CANCER SUSCEPTIBILITY GENES

Probably the best-known cancer susceptibility gene is the tumor suppressor gene *TP53* (located on 17p13), which commonly has mutations, frequently involving deletions of genomic material. This gene is associated with a wide variety of cancer types, including lung, breast, esophageal, liver, and bladder cancer; ovarian carcinoma; brain tumor; and malignancies of the immune and hematopoietic systems. Because it contributes to the signaling circuitry between genes, *TP53* is designated "champion of the genome" and is believed to be involved in approximately 50% of sporadic human cancers; thus *TP53* is the most common genetic target for mutations leading to cancers. When inherited as a germline mutation, it is transmitted in an autosomal dominant fashion, a hallmark of hereditary cancer syndromes.[45]

Phosphatase and tensin homolog (*PTEN)* is part of a family of genes collectively called the protein tyrosine phosphatases (PTP). *PTEN* produces a ubiquitous protein that acts as a tumor suppressor to help regulate cell-cycle division: It issues a "stop" order to prevent progression through G_1 and trigger the cell to begin the process of apoptosis. Research suggests the protein also participates in migration, adhesion, and angiogenesis, in addition to playing a role in stabilizing the genome.[47,48] The PTEN enzyme antagonizes the PI3K signaling pathway and negatively regulates the mitogen-activated protein kinase (MAPK) pathway, thereby placing constraints on cell propagation.[49] The PI3K/AKT/mTOR pathway comprises an intracellular circuitry that performs an important function by carrying signals to the nucleus to ensure regulation of the cell cycle. Phosphatidylinositol-3-OH kinase (PI3K) activates phosphorylation to nudge AKT and localize it on the cell membrane.[50] When cell glucose is elevated in the normal cell, such that plentiful energy is available for metabolism, the cell will be more likely to proliferate; conversely, less glucose availability causes quiescence due to the p21 tumor suppressor protein.[51] The AKT signal has many effects, including activation of mTOR; mTOR (mechanistic target of rapamycin) promotes expression of the protein kinase that regulates cell growth, cell proliferation, cell motility and survival, protein synthesis, and transcription.[52] When associated with a malignancy, the PI3K/AKT/mTOR pathway is hyperactive, enabling cellular proliferation and decreasing apoptosis. Mutations in genes or in proteins expressed by genes are associated with this important signaling pathway and have been the target of multiple therapies.[53]

The epidermal growth factor receptor gene (*EGFR*) is a tumor suppressor gene. This gene expresses a transmembrane glycoprotein, a member of the protein kinase

TABLE 1-2

Somatic Mutations Associated With Various Types of Cancer

Regulatory Gene Types	Normal Function	Cancer Type(s)
Oncogenes		
BRAF	Produces protein for the RAS/MAPK pathway to regulate proliferation of cells, differentiation, migration, and apoptosis	Somatic mutations causing melanoma, colon, rectum, ovarian, and thyroid cancer
Ras family (*H-Ras*, *N-Ras*, *K-Ras*)	Actin cytoskeletal integrity, cell proliferation, differentiation, and apoptosis	Pancreatic cancer
HER2/neu	Epidermal growth factor	Breast cancer
MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*)	Maintain fidelity of DNA during replication	Colorectal cancer
Myc	Regulatory gene that codes for a transcription factor and regulates global chromatic structure by regulating histone acetylation	Burkitt's lymphoma
hTERT	Maintains telomere ends, so it is associated with immortalization of cells	Not specific
Src	Participates in engagement of various members of tyrosine kinase families Part of immune response signaling pathways	Colon cancer, breast cancer, prostate cancer, metastasis
BCL2	Helps regulate apoptosis (anti-apoptosis)	Chronic lymphocytic leukemia, B-cell non-Hodgkin lymphoma, breast cancer, follicular lymphoma
Tumor Suppressor Genes		
APC	Acts as a tumor suppressor Helps control cell division, attachment to other cells in tissue, and whether a cell moves within or away from a tissue	Colon, familial adenomatous polyposis syndrome
BRCA	DNA repair, transcription, and ubiquitination	Breast, ovarian, prostate, melanoma, and pancreatic cancer
MEN1	Role in repression of telomerase expression Chromatin regulation	Multiple endocrine neoplasia type 1: parathyroid cancer, carcinoid tumors, pancreatic cancer, endocrine tumors such as pituitary tumor
TP53	Conserves stability of the genome and apoptosis Inhibits angiogenesis Activates DNA repair proteins	Colon, breast, and lung cancer; leukemias, lymphomas, and sarcomas; Li-Fraumeni syndrome
PTEN	Promotes apoptosis Negatively regulates the AKT/PKB signaling pathway	Cowden syndrome, breast cancer, thyroid cancer, head and neck cancer, glioma type 2, prostate cancer, and endometrial cancer

Source: Data from Beamer et al[2]; Rodriguez-Paredes and Esteller[39]; Hojfeldt et al[40]; Laird.[41]

superfamily, that penetrates the plasma membrane of the cell and binds with epidermal growth factor to link extracellular clues to appropriate intracellular responses. As the extracellular ligand binds to the receptor, it triggers dimerization and phosphorylation, both of which are important to activate the stress-associated NF-kappa-B (NF-κB) signaling cascade, ultimately causing transcription of DNA. This protein is also involved with regulation of the immune response, especially cytokine production and cell survival. When mutated, the *EGFR* gene is associated with non-small cell lung cancer (NSCLC).[54,55]

Officially known as "B-Raf proto-oncogene, serine/threonine kinase," the *BRAF* gene transmits extracellular signals to the cell's nucleus.[56] The protein expressed by this gene is part of the RAS/MAPK pathway and is essential for normal prenatal development. Like many other

proto-oncogenes, it affects proliferation, differentiation, migration, and apoptosis. When mutated, *BRAF* is associated with cancers of the colon and rectum, ovary, thyroid, and skin (melanoma). The most commonly mutated BRAF protein is V600E. It is found in many cancers and is especially active in persons born with giant congenital melanocytic nevus (GCMN), a large patch of darkly pigmented skin that is considered an especially high risk factor for cancer development due to continuous and uncontrolled cell growth of melanocytes after birth. Somatic missense mutations for *BRAF* have been identified in 66% of malignant melanomas.[57,58]

The oncogene known as the *KRAS* gene is officially named "Kirsten rat sarcoma viral oncogene homolog." The *KRAS* gene produces a protein, K-Ras, which is primarily responsible for regulating cell division through the intracellular RAS/MAPK signaling pathway. This protein travels from the cytoplasm to the nucleus of the cell, where it participates in specialized functions such as proliferation, differentiation, and senescence. Other genes in the Ras family include *HRAS* and *NRAS*.[58,59]

The official gene symbol for the "v-myc avian myelocytomatosis viral oncogene homology" is *MYC*. Like many proto-oncogenes associated with cancer, *MYC* is important for progression through the cell cycle, apoptosis, and cellular transformation. MYC proteins function as transcription factors to regulate transcription of specific target genes. *MYC* gene mutations are associated with hematopoietic tumors, leukemias, and lymphomas, including Burkitt's lymphoma.[60,61]

The "cyclin-dependent kinase inhibitor 2A" gene (*CDKN2*) is also known as *p16* (*INK4*). It produces two major tumor suppressor proteins: p16 (INK4, INK4A) and p14 (ARF). These proteins regulate the p53 cell-cycle regulatory pathways, causing cell-cycle arrest in the G_1 and G_2/M phases; p14 (ARF) produces similar effects in the retinoblastoma pathway (RB1). Mutations in either the p16 or p14 protein allow continued movement through the G_1 and G_2/M phases and promote proliferation of the cell. *CDKN2A* mutations are found in pancreatic cancer and gastrinomas, while mutations in *p16* are associated with leukemias and bladder cancer. Melanoma seems to be associated with either *CDKN2A* or *p16* mutations.[62,63]

HALLMARK: DEFECTIVE DNA REPAIR AND MAINTENANCE

The mutator gene phenotype collects increasing numbers of mutated genes left unrepaired due to poor proofreading or insertion of incorrect nucleotides (G, C, T, or A). Efficient at acquiring mutations in the form of both clonal and random mutations, mutator genes (also known as "driver" mutations) allow thousands of mutations to occur, compared with the lower rates seen with normal cells.[43] Of concern, the normal repair mechanisms of direct reversal of DNA damage, excision repair, and post-replication repair overlook the DNA damage, leading to incorrect messages in the DNA sequences, with consequently increased probabilities of mutations and growth advantage.[43]

ENABLER: INDUCING INFLAMMATION

In 1986, it was noted that tumors could be densely populated with both innate and adaptive arms of the immune system, and could also mirror inflammatory conditions.[64] Today, technology has enhanced the ability to locate immune cells in most neoplastic lesions and identify the level of penetration, which can range from subtle infiltration to gross inflammatory processes. Initially it was believed these immune responses reflected an attempt by the immune system to eliminate the tumors. More recently, it has been documented that antitumor responses to many types of cancer occur.[1] By the turn of the 21st century, researchers had realized that the tumor-associated inflammatory response was able to foster tumorigenesis and progression. It is now known that the innate immune response has influence at the intersection of inflammation and cancer progression.[65–68]

When *p53* is mutated, it may cause binding of a tumor suppressor protein, located in the cytoplasm, to promote TNFα signaling for activation of macrophages and regulation of other immune cells.[69] This mutation affects two other pathways downstream: NF-κB, to alter the control of DNA transcription, and JNK, to diminish apoptosis.[70,71] Inflammation also has the capability to supply bioactive molecules to the tumor microenvironment, including growth factors that sustain proliferative signaling; survival factors that limit cell death; pro-angiogenic factors; extracellular matrix–modifying enzymes that facilitate angiogenesis, invasion, and metastasis; and inductive signals that lead to activation of the epithelial–mesenchymal transition and other hallmark-facilitating pathways.[65–67,72]

Much research literature now links inflammation associated with chronic disease and observed at the earliest stages of neoplastic progression with nurturing of a budding neoplasia into a dangerous malignancy.[67,73] It is well known that inflammatory cells release a variety of chemicals, such as reactive oxygen species (ROS), which include oxygen ions, superoxides, and peroxide. These chemicals react with molecules containing oxygen; they can be mutagenic to cells in close proximity and can accelerate malignant transformation.[1] The immune cells responsible for this enabling trait are described in the "Hallmark: Evasion of Immunity" section.

ENABLER: INDUCING ANGIOGENESIS

Limitations in the oxygen supply for tumor cells induce growth strategies for blood vessels other than just those seen with embryogenesis. The mutations, mechanical stresses, inflammatory processes, and hypoxia all slant the tumor cells' growth gradient toward the pro-angiogenic side of the equation.[74] Specifically, these factors induce the growth of a heterogeneic and disorganized network of dilated vessels lined with irregularly shaped endothelial cells that lack the close and continuous contact of normal cells. Hypoxia is reinforced by the abnormal vessels and cells, thereby stimulating production of vascular endothelial growth factor (VEGF). With the increased availability of VEGF, the endothelial cells are instructed, via D114/notch signaling, to grow vessel sprouts that lack normal pericytes to regulate normal blood flow; these defective blood vessels lead to poor vessel function and enhanced tumor hypoxia.[75] In the face of these defects, the need for oxygen is enhanced, promoting even more growth of abnormal vessels without the capability to supply an already oxygen-deficient endothelium. A vicious cycle without potential for rescue is created.[76] Of note, this process causes the cancer cell to have poor sensitivity to chemotherapy and resistance to radiation.[77]

HALLMARK: MICROENVIRONMENT

ENABLER: CANCER STEM CELLS

The oncogenic and tumor suppressor mutations that define cancer as a genetic disease are present within the cancer cells and have the capability to initiate and drive tumor progression. Although these mutations were originally believed to be present only in hematologic cancers, advances in technology have enabled the researcher and the practitioner to obtain information about the intratumor heterogeneity and the subclasses of neoplastic cells, known as cancer stem cells (CSCs), found within tumors. CSCs have the ability to proficiently seed new tumors, complete with new and normal markers associated with the tissue of origin.[1,78,79] The origin of CSCs is unknown; indeed, it seems to vary from one tumor type to another. It is unclear whether multiple different populations of cell types are present, or whether some multistep progression yields specific cell types at certain stages of tumor progression.[1]

ENABLER: EPITHELIAL–MESENCHYMAL TRANSITION

As briefly noted previously, the epithelial–mesenchymal transition (EMT) allows tumors to acquire CSC traits, especially self-renewal and antigenic phenotypes like those of normal stem cells and CSCs.[80–82] These specific traits may allow cells to be shed from primary tumors and may support the self-renewal capability of cancer cells that is important for clonal expansion at distant sites.[83]

CANCER STEM CELL PLASTICITY

Also noted previously, the phenotypic plasticity of cells within tumors produces bidirectional conversion between CSCs and non-CSCs, enabling a dynamic fluctuation in the availability of CSCs. Because this process might enable CSCs to accumulate after decades of apparent remission, it suggests a rationale for resistance to chemotherapy or exacerbation of disease after long-term remission/latency.[80,84–86] Inherent in this rationale is the threat that the CSCs might be more treatment resistant and have the ability to regenerate once treatment has been stopped.[1]

Phenotypic plasticity also suggests that functionally distinct subpopulations might form within the tumor that support tumor growth. Two examples are epithelial carcinoma cells, which are converted into mesenchymal, fibroblast-like cancer cells that assume the duties of cancer-associated fibroblasts (CAFs), and glioblastoma cells, which have been found to transdifferentiate into endothelial-like cells to form tumor-associated neovasculature.[87–89] Tumor progression with a variety of cell subpopulations might, therefore, be due to rapid genetic diversification that outperforms the capability of tumor elimination by treatment.[1]

DRIVER: TELOMERASE

Cancer stem cells are known to have increased levels of telomerase and thus have an extended life enhanced by the protected telomeres at the ends of the chromosome. This enzyme also protects the cell from apoptosis.[90]

ENABLER: IMMUNE RESPONSE CELLS

Immune cells are also found within the microenvironment of the tumor. They can take the forms of both tumor-antagonizing and tumor-promoting leukocytes, which are found in most cancers. Since the 1990s, it has become apparent that immune cells are present in cancer and that they promote tumor progression. In fact, given the amount of chronic inflammation identifiable within tumor formations, some literature describes tumors as "wounds that never heal."[64,91] These "wounds" harbor tumor-promoting inflammatory cells that include macrophage subtypes, mast cells, neutrophils, and T and B lymphocytes. Collectively, these cells induce and sustain the development of tumors and proliferation of cancer cells. As

the malignant cells gain access to the margins of the tumor, research shows these differentiated immune cells promote tissue invasion and support the dissemination and seeding of the malignant cells.[92–96]

Partially differentiated myeloid progenitors have also been identified in tumors.[95] Specifically, a group of tumor-infiltrating myeloid cells that express the macrophage marker CD11b and the neutrophil marker Gr1 have been shown to exert immunosuppressive effects by paralyzing cytotoxic T lymphocytes (CTLs) and decreasing natural kill (NK) cell activity.[96,97] These tumor-promoting cells are designated as myeloid derived suppressor cells (MDSCs).[98–100]

ENABLER: SIGNALING MOLECULES

In addition to the presence of inflammation-associated immune cells, certain signaling molecules stimulate and support the cancer cell activities. These include tumor growth factors (e.g., epidermal growth factor [EGF]), VEGF, chemokines, and cytokines that have the ability to enhance inflammation, once again supporting the ever-changing nature of cancer. Factors that promote the degradation of the cellular matrix (e.g., metalloproteinases [MMPs], cathepsin proteases, and heparanase) are also produced and influence further cell changes that support the evolving life of the malignant cell.[1,67,95]

HALLMARK: DEREGULATED GROWTH AND MITOGENESIS

The continuous proliferation and deregulated growth of cells is the best-known, albeit poorly understood, characteristic of cancer cells. Normal cells are controlled by multiple growth factors and other chemical signals that direct entry into and out of the phases of the cell cycle.

Limitless replication is also associated with the expression of an enzyme that prevents the destruction of the telomeres—namely, telomerase. Because telomeres protect chromosomes, cells with increased telomerase are known to be associated with longer telomeres and greater longevity of cell life. Shorter telomeres, by comparison, are associated with a shorter life span. Cancer stem cells are known to have increased levels of telomerase, with their extended life span being enhanced by the protective telomeres at the chromosome ends. Telomerase also assists in protecting the cell from apoptosis, thereby preventing the death of malignant cells.[101]

Mitogenic (growth) signals to cancer cells are acquired through a number of mechanisms. For example, production of growth factors (ligands) that attach to cell membrane receptors may stimulate the growth of cancer cells. Usually these ligands are tyrosine kinase receptors (TKRs), which branch into multiple intercellular signaling pathways. As the TKRs are stimulated outside the cell, the signals cross the cell membrane and continue on intracellular pathways that are critical for cell-survival factors. Specifically, two kinases—phosphatidylinositol-3-OH kinase (PI3K) and mitogen-activated protein kinase (MAPK)—are targeted. Because there are more than 20 families of TKRs, once they are phosphorylated to the active state, they affect a variety of tissue needs. If the TKR signals become stuck in the "on" position, unregulated cellular proliferation occurs; this process is a precursor for cellular transformation. **(Table 1-3)**.[102–107]

Signals can also originate from cancer cells and travel to normal cells, all within tumor-associated connective tissue. The normal cells then respond to the ligands by supplying various growth factors to the malignant cells for supplementation of their proliferation requirements.[1,14] These RTK-ligands and pathways are viewed as mechanisms for current and potential treatments. Because malignant cells produce ectopic hormones, growth factors, and cytokines, an increase in RTK-ligand levels could potentially confer resistance to inhibitors of an oncogenic kinase.[108]

TABLE 1-3

Tyrosine Kinase Receptors and Their Activity Status

RTK Class	Type	Acronym	Function (Increased)	Function (Decreased)
I[107]	Epidermal growth factors	EGFR, HER2, HER3, HER4	Malignancies (e.g., breast, pancreatic)	Alzheimer's disease, multiple sclerosis
II[107]	Insulin receptors	InsR, IGF-1, InsRR	Malignancies (e.g., breast, prostate)	Diabetes
III[107]	Platelet derived	PDGFRa, PDGFR, Kit/SCFR, Fit3/fit2b, CSF1r/Fms	Breast cancer	Reduces hypertension

(continues)

TABLE 1-3

Tyrosine Kinase Receptors and Their Activity Status (*continued*)

RTK Class	Type	Acronym	Function (Increased)	Function (Decreased)
IV[104,107]	Vascular endothelial growth factor	VEGFR1/Fit1, VEGFR2/KDR, VEGFR3/Fit4 Epithelial proliferation and angiogenesis	Metastatic disease	Acute graft-versus-host disease, myopic choroidal neovascularization → retinal detachment
V[102,103,107]	Fibroblast growth factor receptor	FGFR1, FGFR2, FGFR3, FGFR4 Angiogenesis and wound healing Immunoglobulin superfamily due to immunoglobulin-like extracellular domains	Malignancies (e.g., pancreatic, esophageal, prostate)	Hypophosphatemia in renal disorders
VI[107]	Hepatocyte GF	Wound healing (angiogenesis) Metastasis	Renal cell cancer, hypertension, arteriosclerosis, myocardial infarction, rheumatoid arthritis	Unknown
VII[107]	Trk receptor family Nerve growth factor	NGF/neurotrophin receptor TrkA Nervous system		
VIII[107]	Epithelial receptor family	EphA1-8, EphA10, EphB1-4, EphB6	Melanoma, breast, prostate, pancreatic, gastric, esophageal, and colon cancer; hematopoietic tumors	
IX[107]	Axl receptor family—located close to the *BCL3* oncogene at 19q13.1–q13.2	Axl, Mer, Tyro3 Stimulation of cell proliferation Mediation of cell aggregation	Chronic myelogenous leukemia, colon cancer, and melanoma; breast cancer metastasis	
X[107]	LTK receptor family	LTK, ALK Cell growth and proliferation		
XI[107]	TIE receptor family	Tie1, Tie2 Angiopoietin		
XII[102,106]	ROR receptor family	Ror1, Ror2 Cell motility Regulation of skeletal and neuronal development, cell migration, and cell polarity Modulation of Wnt signaling		
XIII[107]	DDR receptor family(discoidin domain receptor)	DDR1 (CD167a), DDR2 6p21.3 location	Malignancies (e.g., breast, ovarian, esophageal, pediatric brain)	
XIV[105,107]	RET receptor family	*Ret* proto-oncogene	Multiple endocrine neoplasia type 2 (MEN2A, MEN2B, familial medullary thyroid carcinoma), pheochromocytoma, parathyroid hyperplasia	
XV[107]	KLG receptor family *PTK7* gene	Signal transduction Cell adhesion molecule	Colon carcinomas	
XVI[107]	RYK receptor family	No phosphorylation occurs at this receptor	Leukemia	
XVII[107]	MuSK receptor family	Neuromuscular junction and synapse		

Source: Data from Forrester[102]; Imel and Econs[103]; Sawada et al[104]; Green et al[105]; Moline and Eng[106]; Lemmon and Schlessinger.[107]

Deregulation of the growth factor receptor signaling can occur by increasing the levels of receptor proteins displayed on the surface membrane of the cancer cell. Increased levels of receptor proteins cause the malignant cells to become hyperresponsive to the amount of growth factor available. Sometimes the receptor molecule has a structural alteration that facilitates ligand-independent firing, again causing deregulated growth.[1]

HALLMARK: METABOLIC PROGRAMMING

Early scientists believed alterations in metabolic reprogramming initiated the transformation of the normal cell into the malignant cell.[109] Today it is well known that alterations in the tumor suppressors and oncogenes have intimate connections with metabolic pathways. Changes in these pathways are now recognized to be secondary results, however, rather than primary causes of carcinogenesis.[110–114] In addition, early science theory suggested aerobic glycolysis occurred instead of mitochondrial respiration.[111–113] Work in the laboratory has shown that many cancers retain mitochondrial respiration for energy production while also relying on aerobic glycolysis.[114]

As in the normal cell, the need for energy in the cancer-causing cell can be addressed through the increased use of glucose transport molecules, such as GLUT1, to bring glucose into the cytoplasm or provide glucose that can be converted into lactic acid.[115] When they have glucose available to them, cancer cells have the capability to reprogram their metabolism depending on the oxygenation status of the tissues. Oxygenated cancer tissue will metabolize one molecule of glucose to yield 36 adenosine triphosphate (ATP) molecules, like a normally oxygenated cell, but also convert 10 *more* glucose molecules to 20 molecules of lactic acid through metabolism. Because lactic acid is converted to ATP at a 1:1 ratio, a total of 56 molecules of ATP become available in the oxygenated cancer cell through this process. During poorly oxygenated conditions, the cancer cell will convert 13 glucose molecules to 26 ATP molecules. In the time that a normal cell is able to metabolize 36 ATP molecules for use by the cell, the oxygenated cancer cell generates 56 ATP molecules while the anaerobic cancer cell generates 26 such molecules (equivalent to the production of the normal cell).[109–111,116,117] **Figure 1-1** illustrates the calculations of ATP production in cancer cells versus normal cells. These simplified, yet important calculations are the rationale for the use of positron emission tomography (PET) scanning to detect the higher levels of glucose in the tumor than in the adjacent tissues.[118] Put succinctly, even in nonhypoxic conditions cancer cells can produce lactic acid from glucose.[117] In addition to the energy needed for a normal cell, these mechanisms can make a net increase of approximately 13% more energy available to the malignant cell.[119]

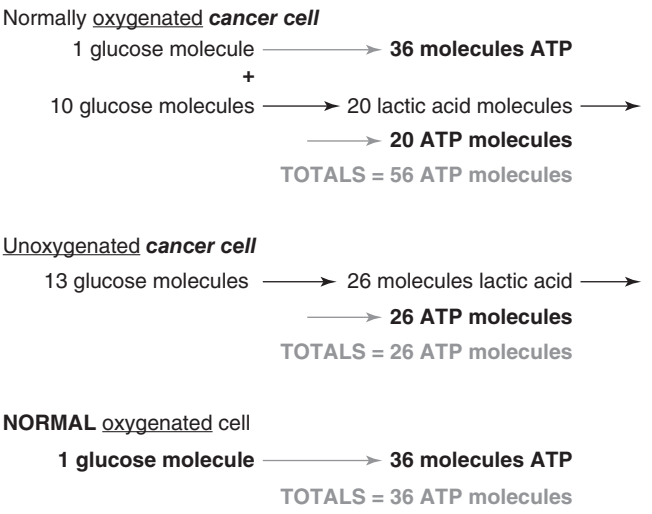

FIGURE 1-1

ATP production in cancer cells versus normal cells.
Source: Data from Warburg[110,111]; Keilin[116]; Racker.[117]

In tumors, 66% of the glucose consumed is changed to lactic acid; by comparison, in healthy cells there is no net production of lactic acid. Cancer cells can "recycle" lactic acid under aerobic conditions. This ability to recycle lactic acid may be altered depending on the proximity of the cancer cell to arterial versus venous blood. Metabolism occurring in close proximity to arterial blood may be more rapid due to the movement of oxygen associated with the oxygenation gradient, whereas metabolism closer to venous blood may be limited by diffusion.[114,120] This concept is the basis for the popular understanding that tumor cells' interior necrosis results from the presence of fewer and highly fragile blood vessels to carry oxygenated blood.

Oxidative phosphorylation (OXPHOS) is the metabolic pathway of the mitochondria involved in respiration and ultimately in ATP generation after oxidation of nutrients. The oxidation–reduction (redox) reaction link between metabolism and tumorigenesis was confirmed when mutations of oncogenes were identified for three mitochondrial metabolic enzymes: fumarate hydratase (FH), succinate dehydrogenase (SDH), and isocitrate dehydrogenase 2 (IDH2).[114,121] Two cancers associated with the mutated SDH enzyme are the rare hereditary paragangliomas and pheochromocytomas.[114,122] Mutations of FH, which is involved with the citric acid cycle downstream of SDH, result in familial leiomyoma, renal cell carcinoma, and uterine fibroids. As the damaged FH and SDH levels rise, another enzyme is inhibited, causing modifications of hypoxia-inducing factor alpha (HIF-α) and its degradation in the presence of oxygen. Tumor development ensues as HIF-α levels become elevated.[123]

Somatic mutations of the proteins IDH1 (cystolic) and IDH2 (mitochondrial) are also associated with OXPHOS mutations. Approximately 80% of low-grade gliomas and 30% of karyotypically normal acute myelogenous leukemias demonstrate these IDH1 and IDH2 mutations.[124,125] Both IDH1 and IDH2 are part of an enzyme cascade that, when mutated, alters the homeostasis of a substrate for enzymes that methylate DNA and histones. Modification of these components of the epigenome enhances tumorigenesis, yet mutations in OXPHOS genes affect only a limited number of cancer types.[114,126,127]

In human cancers, somatic mutations may occur in the mitochondrial DNA (mtDNA). Recently, heteroplasmy (i.e., a mixture of mutant and normal mtDNA within a single cell) in cancer-causing cells versus normal cells has been the focus of research. When mutations in embryonic cells were compared to mutations in the same regions of cancer cells, the protein coding regions were found to make up 33% and 85% of these areas, respectively. These results suggest that endogenous mutagenic events occur normally and that the somatic mutations of mtDNA are enriched, perhaps conferring a selective advantage for survival and growth.[128] Another study identified heterogeneity across tumor types but found a significantly higher number of mutations in the gastrointestinal and hepatobiliary systems, emphasizing the fragility of the mismatch DNA repair system in the GI tract.[129]

It is now believed that changes in cancer cell metabolism extend beyond those addressing the increased anabolic needs of a growing and dividing cell. Some evidence suggests that variations in both oncogenes and tumor suppressor genes cause reprogramming of metabolic enzymes that can then affect the differentiation of the cell.[6] The glycolytic fueling process seen with the metabolic reprogramming of the cancer cell is associated with activated oncogenes (*AKT, RAS*, and *MYC*) as well as mutated tumor suppressor genes (*TP53*).[130–132] It is well known that these altered genes are also associated with cell proliferation and avoidance of cell death.

The first oncogene found to be associated with altered glucose metabolism was *MYC*.[132] It activates glycolytic enzyme genes and glucose transporter genes as well as the splicing factors that produce PKM2—that is, the enzyme pyruvate kinase that catalyzes the last step in glycolysis.[131] Other research suggests that *MYC* can stimulate the aerobic glycolysis associated with HIF-1α, even though it is more commonly associated with anaerobic glycolysis.[133] The *Ras* oncogene stimulates glucose uptake. Depending on the cell type, the *Src* oncogene may also be involved in the activation of HIF-1α to promote glycolysis.[114]

The tumor suppressor genes also have the capability to alter metabolic programming. The *p53* tumor suppressor, known as the "guardian of the genome," acts as the first line of defense against glucose uptake by preventing the expression of the GLUT1.[118,134] Loss of *p53* sensitizes the tumor cell to treatment with drugs that affect metabolism (e.g. metformin)—an interesting option for further cancer treatment research into the metabolic pathways.

During the regulation of metabolism, *p53* communicates with other cancer-associated signaling pathways linked with metabolism, such as *MYC*, *HIF1*, and *Akt*. **Figure 1-2** depicts how the oncogenes *Myc*, *Akt*, and *Ras* signal an increase in glycolysis through the activation of enzymes in the different pathways.[113,117] HIF can inhibit oxygen consumption and increase glycolysis. At times the *Src* oncogene will target HIF in some tumor types. Finally, the tumor suppressor *p53* can both inhibit glycolysis and promote mitochondrial respiration.[114,118] The hypoxic conditions found in cancer cells also mobilize the glucose transporters with HIF-1α and HIF-2α to "turn on" glycolysis.[130–132,135]

Although normal cells and cancer cells utilize similar metabolic pathways, the malignant cells are "addicted" to using both glycolysis (with fermentation of glucose to lactate secretion) and mitochondrial respiration, even in the presence of oxygen, to provide energy (ATP) to the rapidly growing cancer cell.[136] It is now recognized that this altered metabolic programming phenotype of the cancer cell is due to the mutations and altered gene expression of tumor suppressors and oncogenes.[137]

HALLMARK: SUPPRESSION OF APOPTOSIS

DRIVER: DECREASED CELL ATTRITION

In the normal cell, apoptosis is induced by a number of pathways, both intrinsic and extrinsic. One mechanism is a DNA-damage sensor that works via *TP53*. This tumor suppressor gene increases production of apoptotic proteins, such as NOXA, when significant numbers of DNA breaks and other chromosomal abnormalities are detected. Insufficient signaling due to inadequate interleukins (from lymphocytes) or insulin-like growth factor (from epithelial cells) will prompt apoptosis. Hyperactive signaling by oncoproteins like Myc is another common trigger. Interruption of any of these mechanisms will lead to suppression of apoptosis and prolonged cell life, with the accumulation of severe or irreparable genetic abnormalities ultimately leading to tumorigenesis.

ROLE OF POTASSIUM, SODIUM, AND CALCIUM IONS

While potassium has a role in cell proliferation, differentiation, regulation of cell volume, and maintenance of membrane potential, several of the potassium channels fill

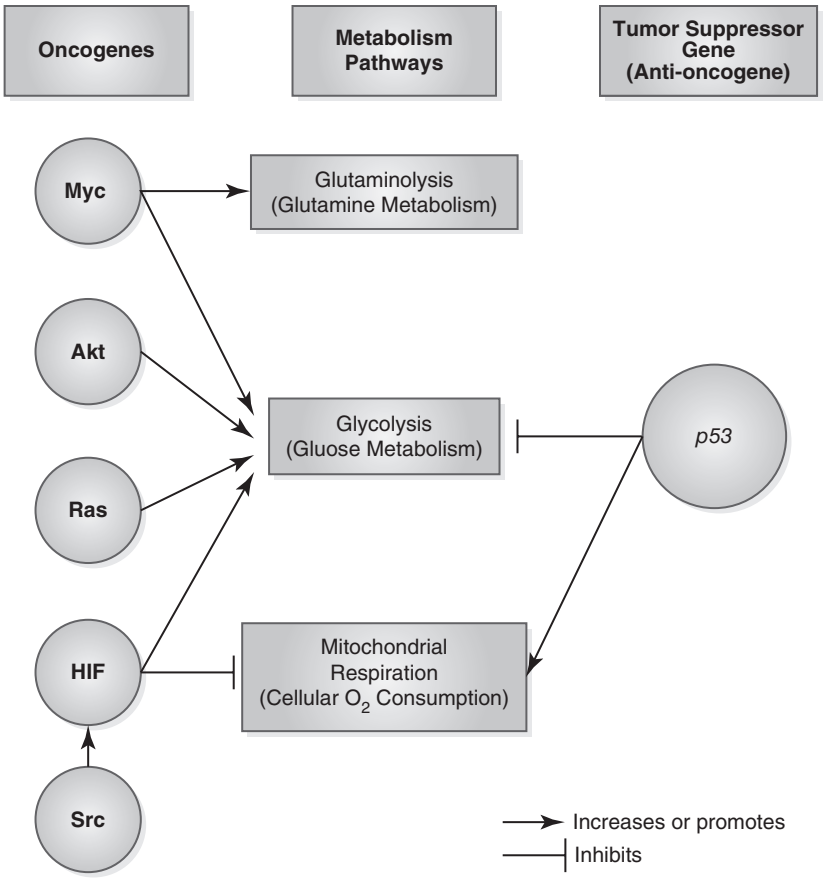

FIGURE 1-2

Role of oncogenes and the *p53* tumor suppressor gene in metabolism.

Source: Data from Koppenol et al[114]; Cheung and Vousden.[118]

critical roles during apoptosis.[138] Positively charged sodium and calcium channels of normal cells contribute to apoptosis.[138,139] Alterations of these ionic channels across the cell membrane affect the status of the cell. Loss of potassium, for example, causes loss of cell volume and cell shrinkage. Such a loss of intracellular potassium can activate the caspase cascade and other apoptotic changes. Conversely, intracellular levels of sodium increase early in the cell death process.[129,138,139] Movement of chloride, an anion conductor, has been shown to increase or decrease apoptosis.[140]

After the initial caspase activation triggers apoptosis, the second pathway of apoptosis-inducing factors such as cytochrome C and other mitochondria-derived activators stimulates the release of more caspases. Resistance to these mechanisms of apoptosis occurs once the cell detaches from its neighboring cells and becomes metastatic. Many other signaling pathways that are as yet unknown are also suspected to play roles in this process.[139,141]

One type of indolent (slow-growing) non-Hodgkin lymphoma, known as follicular lymphoma, demonstrates the effects of loss of apoptosis. This lymphoma accounts for approximately 20% of all non-Hodgkin lymphomas. It is frequently associated with a rearrangement of the *BCL2* gene. Continued proliferation of the follicular cells is caused by increased expression of the bcl2 protein due to this genetic mutation; the greater production of the bcl2 protein hinders apoptosis, making it difficult for the follicular lymphoma to be destroyed.[142]

Multiple pathways and diverse apoptotic mechanisms need to be overcome for a cell to attain malignant status. It is hypothesized that restoration of apoptosis may provide an approach to cancer therapy, perhaps through a means as simple as potassium therapy.[141,142] Sequential application and combinations of anticancer drugs with natural ingredients have been shown to enhance cell death by gene enhancement and rewiring apoptotic signaling networks.[32,143]

HALLMARK: EVASION OF SENESCENCE

Senescence is a stress response that blocks cell proliferation and prevents tumor progression in normal cells. Research in animal models and humans shows that premalignant senescence needs to be overcome if the cell is to evolve into a malignant state.[144] If the capacity for senescence is impaired, this stress response can be "forced" into action by bypassing an important oncogenic pathway or restoring damaged tumor suppressors. These mechanisms that can reverse or clear senescence are potential avenues for development of new drugs, prognostic markers, and targeted therapies for personalized medicine.[144]

Hayflick and Moorhead[145] first identified that normal cells have a finite number of cellular divisions that they can experience before they rest, "long term," from cycling. Senescence is now believed to be due to a lack of reaction to growth factor stimulation, unsustainable metabolic activity, and changes in the morphology of the cell, including diminished length of telomeres associated with a lack of RNase H and reduced or inactivated telomerase activity.[145–147]

ONCOGENES AND TUMOR SUPPRESSOR GENES

Activation of oncogenes or loss of tumor suppressor genes such as *PTEN*, *RB1*, *NF1*, and *INPP4B* can also trigger senescence.[148] When these tumor suppressors check for stress signals in the normal cell, they can sense other proteins responsible for signaling the need for production of different proteins necessary to trigger senescence. Mutation of the tumor suppressor genes can prevent senescence, allowing malignant activation in the cell to occur. Senescent tumor cells can be eliminated through pro-senescence therapy, including efficient removal of these tumor cells by the immune cells.[149]

NONCODING RNAS

Research that targets noncoding RNAs is beginning to enhance our understanding of senescence. For example, miR-20a has the ability to promote senescence induction through the downregulation of lymphoma-related factor (LRF, also known as ZBTB7A).[150]

MICRORNAS

Multiple miRNAs have been identified as having regulatory functions important to genetic control mechanisms and are known to be deregulated in cancer. Approximately 18 to 25 nucleotides in length, these microRNAs regulate the expression of a variety of genes.[150,151] Many of them have been shown to have specific roles in various tumor phenotypes—a characteristic that makes them attractive targets for personalized medicine. For example, miR-17 and miR-21 are upregulated in colon, lung, stomach, prostate, and pancreatic tumors, while miR-155 is upregulated in breast, lung, and colon cancers. Other miRNAs, such as miR-15-a and miR-16-1, are downregulated in chronic lymphocytic leukemia (CLL), prostate tumors, and pituitary adenomas. Current speculation suggests upregulated miRNAs act as oncogenes and lost miRNAs as tumor suppressors. However, much remains unknown about these tiny molecules.[151]

HALLMARK: EVASION OF IMMUNITY

Surveillance by the immune system is believed to be a real-time, vigilante mechanism that monitors for emergent cancer cells and destroys most of them before they are able to develop into a rapidly growing tumor. While this seems logical when contemplating the increased number of cancers in immunocompromised hosts, most of these cancers are associated with viral-initiated cancers, such as Kaposi's sarcoma.[152] More than 80% of malignancies have nonviral causes, and current research suggests the immune system represents a significant barrier to tumor formation and progression, especially for those cancers associated with viruses. Among individuals who have deficiencies in the development or function of the cells that direct the presentation, processing, or destruction of the foreign tissue/antigen (CD4+ Th1 helper cells, CD8+ cytotoxic T lymphocytes, or natural killer cells), there is an increased incidence of tumors.[153,154]

Lectins are types of glycoproteins that enable carbohydrate binding and other biological activities such as immunomodulatory activities and antitumor effects.[155] Upon recognition of a foreign agent, binding proteins on the cell membrane initiate the lectin pathway of complement activation by attaching proteases to the invaders. The resulting direct opsonization (binding to a phagocyte), neutralization, and agglutination limit the attack and concurrently direct a follow-up adaptive immune response.[156] Any alterations in lectin binding, however, enable leukocytes to adhere to and cover malignant cells. This change allows the malignant cell to escape surveillance and travel to distant sites in the body under the guise of a bolus of normal and abnormal cells.[1]

HALLMARK: DISRUPTION OF EPITHELIAL ADHESION AND POLARITY

DRIVER: ACTIVATING INVASION AND METASTASIS

Normal epithelial cells are held in place within the tissues through tight junctions and adhesion molecules. The adhesion of cells causes tight binding of cells to each other, such

that ions are unable to pass through a space between the cells. Transmembrane proteins, such as integrin and cadherin, support these tight junctions.[157]

Integrin has two types of functional subunits (α and β) and selectively binds to the *extracellular* membrane to the cell by identifying three specific amino acids. These subunits are present in multiple forms to promote functionality, including transferring information into the cell, with diverse tissues and their adhering companions. The *intercellular* adhesion molecule, cadherin, binds the same type of cells to each other with the additional function of information transference. These normal cells will not respond to mitogens as long as they are restrained by the cadherin-associated contact inhibition and continue to support the cellular matrix with integrins. Malignant cells, in contrast, no longer respond to the controls of the cadherin and integrin molecules as they come in contact with mitogens.[157]

The integrins are activated by Src family kinases (SFK), sometimes in combination with receptor tyrosine kinase (RTK) signaling molecules such as ErbB2 and Met; they energize the cytoplasmic tail of E-cadherin molecules to tag them for degradation. The machinery of the SFKs regulates essential processes of the cell such as cell growth, differentiation, migration, and survival; cell shape; and specialized cell signals.[158] While performing their specialized function, the differentiated epithelial cells are flat. As the cells go through cell division, however, they become more rounded and detach from the surrounding cells, although the mechanisms responsible for this change in shape remain unclear. Recently a protocadherin, PCDH7, has been implicated in the typical "rounding" of the cell at the beginning of mitosis. Research suggests that this biomarker may have potential as an anti-mitotic chemotherapy.[159] Because most cancer cells are constantly in some phase of cell division, it is possible that the loss of adhesion and rounding of the cells might enhance their metastatic capability. Mutated *CDH1*, which codes for E-cadherin, is associated with gastric and lobular breast cancers. Other genes associated with contact inhibition include Merlin, *LATS2*, and *YAP*. Merlin is a tumor suppressor gene located in the nucleus; when mutated, it is associated with development of neurofibromatosis 2 (*NF2*). The mutated forms of *LATS2* and *YAP* are associated with mesotheliomas and hepatocellular carcinomas, respectively.[160–163]

ALTERED SURFACE CHARGE DENSITY

The normal cell condition can be assessed through the electrical properties of the cell membrane. As the malignant cell becomes transformed, the membrane gains more phospholipids, especially anions, than are found in normal cells, causing the plasma membrane to have a more negative charge. A variety of changes affect the phospholipid content during membrane synthesis of the neoplastic cell as it moves through the ongoing progression of the cell cycle.[164] Most changes of the phospholipid content occur based on cell type, pH of the membrane, and the growth phase and transformation status of the malignancy. Amino acid components (which have their own electrical charges) of the phospholipid membrane affect the membrane's charge such that it is increased at both low pH (acidic, negative charges) and high pH (basic, positive charges).[18] Once cells detach from their neighborhood of cells and other anchorage-dependent cells, they can evade apoptosis and are capable of tumorigenesis.[141] Changes in the charge of the cell membrane inhibit apoptosis, contribute to the longevity of the malignant cell, and increase the loss of contact inhibition, thereby enhancing the ability to of the cell to spread, invade, and metastasize.

HALLMARK: MATRIX DEGRADATION AND GAIN IN MOTILITY

Like normal cells, the malignant cell has proteins, glycoproteins, and glycolipids with altered mobility on the surface membrane. However, the cancer cell membrane gains these molecules due to ectopic hormone production and/or malignant changes specific to the tissue type. Outcomes of this change enable the cancer to avoid immune surveillance, promote invasion, and metastasize.[1]

Cells have a skeleton with interior and exterior functions dependent upon the makeup of the protein filaments. The shape and mechanical strength of the cell; its ability to grow, divide, direct, and support the signaling network; and its ability to be mobile are all supported by the functions of the cytoskeleton. The internal function of the cytoskeleton permits substances to move within the cell. On the exterior membrane, microtubules evoke a rigidity to add strength to the membrane surface. Internally, they promote movement of organelles within the cytoplasm.

When malignancy is manifested, the cell loses external cytoskeleton control. Consequently, the cell may lose rigidity, round up, and be more amenable to continued cellular division. Multiple protein types participate in these changes associated with malignant transformation and locomotion.[165]

SIGNALING NETWORKS AND CIRCUITRY

Though not a hallmark themselves, a variety of signaling pathways can be correlated with one or more of the major characteristics of cancer. Much like the "motherboard" that contains the circuitry of a computer and directs the multiple pathways and branching trails, the signaling

networks of the cell direct the mechanisms that regulate the biology of the normal cell. If the normal circuitry is known, then the molecular networks that are altered due to malignancy could be inhibited, causing an "error" in the circuitry. Because therapeutic agents are directed at one target in the pathway, the side effects and nonspecific toxicities are less frequent than the generalized toxicities seen with chemotherapeutic or radiation results.[1] As seen in **Figure 1-3** and **Figure 1-4**, the hallmark capabilities have redundant and overlapping signaling pathways; thus, even if a targeting agent inhibits one circuit, it may not block the entire pathway, allowing the cancer cell to survive with remaining function(s) awaiting adaptation of the progeny.[1] As noted previously, this adaptation can be accomplished by "mutation, epigenetic reprogramming, or remodeling of the stromal microenvironment."[1(p667)] By targeting angiogenesis, some of the angiogenetic inhibitors have been successful in suppressing this hallmark characteristic, causing the tumor to adapt and shift from dependence on angiogenesis to increased activity of some other characteristic such as invasiveness and metastasis. The fact that the antiangiogenesis treatments have had transitory effects demonstrates the innate ability of the malignant cell to utilize mechanisms that enable survival skills.[166] The hypoxic cancer cells induced by antiangiogenic inhibitors may invade the nearby tissues in their quest for access to vascularized tissue.[167] This mechanism has been validated in glioblastomas but not in other tumors.[168]

The reprogrammed signaling networks of the cancer cell enable its survival. Figure 1-3 depicts the four circuits—motility, cytostasis and differentiation, proliferation, and viability—involved in this process. Though the figure dramatically simplifies matters, note the crosstalk between circuits within one cell; this crosstalk also occurs with other cancer cells within the microenvironment of the tumor.[1]

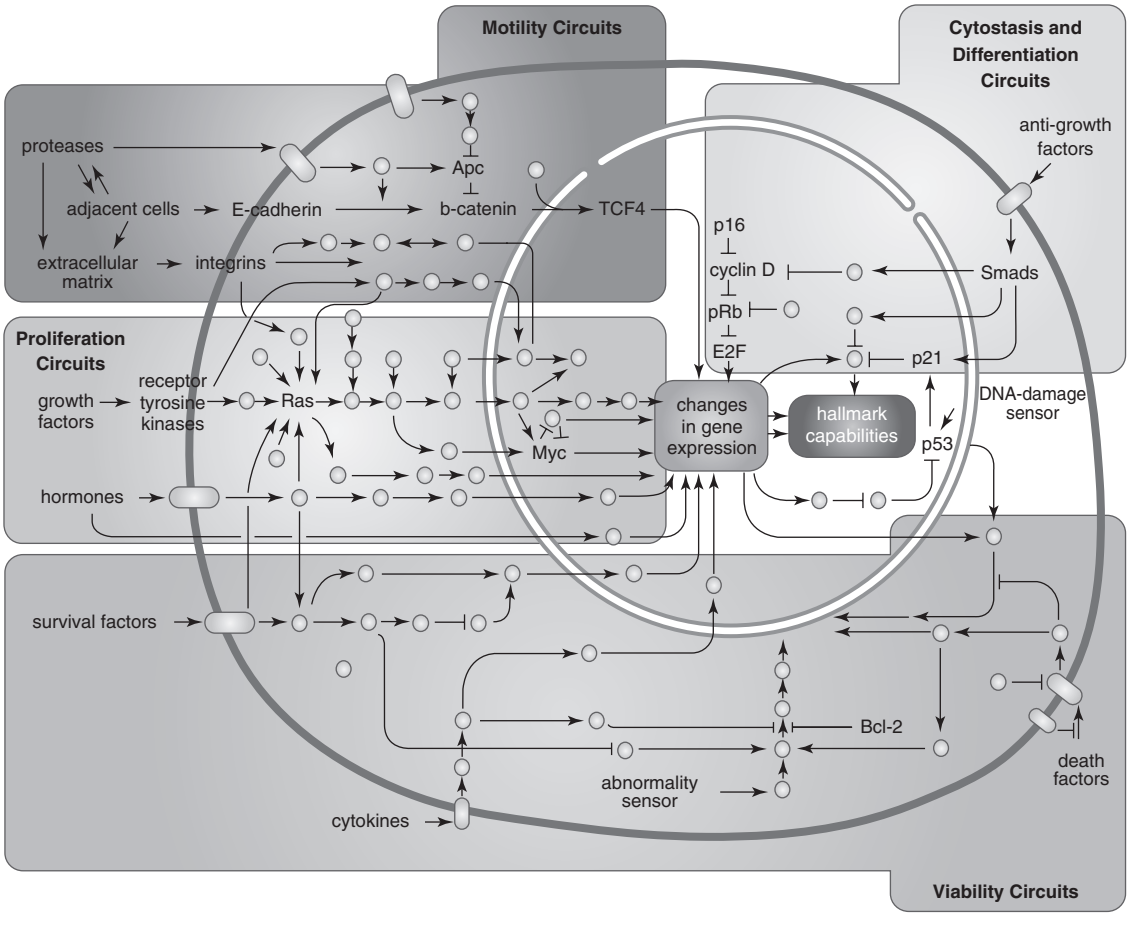

FIGURE 1-3

Intracellular signaling network for regulation of cancer cell operations.

Source: Reproduced from Hanahan and Weinberg.[1]

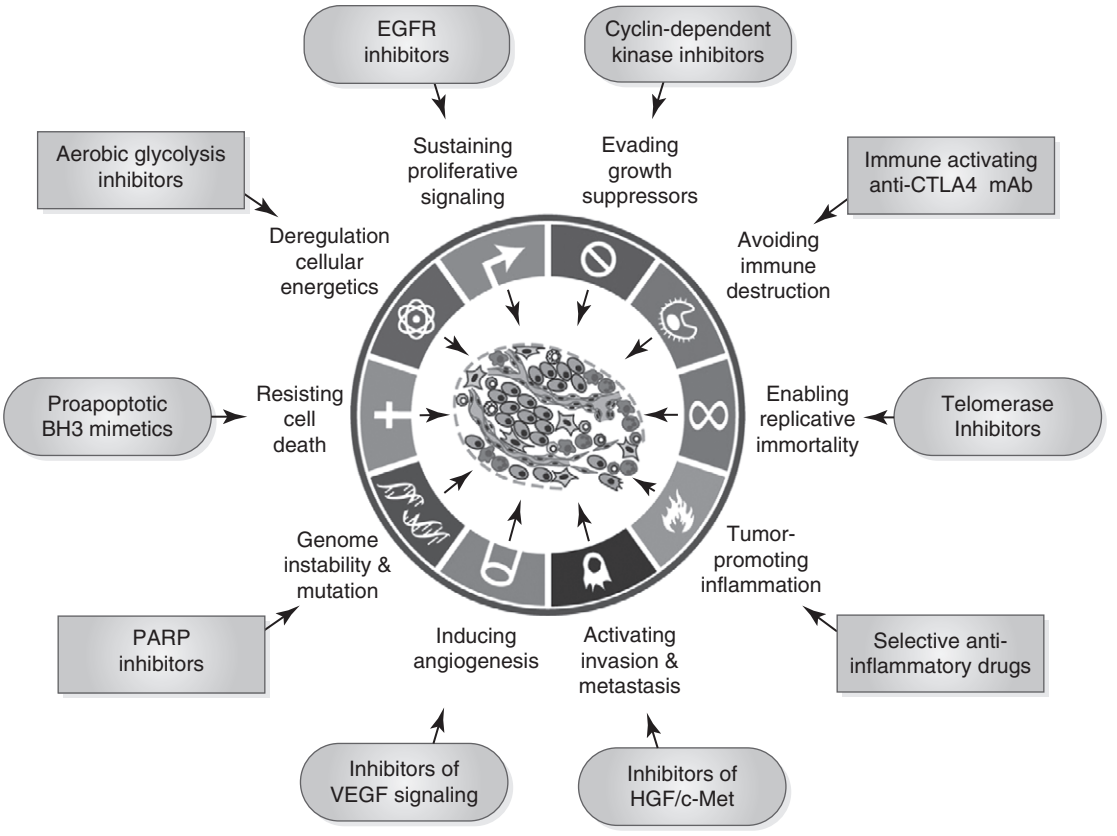

FIGURE 1-4

Targeted treatments directed at cancer hallmarks.

Source: Reproduced from Hanahan and Weinberg.[1]

MOTILITY CIRCUITS

Extracellular and transcellular signaling for the motility circuitry occurs via intramembrane receptors (proteases) and paracrine overproduction, as these enzymes and hormones are transported across the cellular membrane. The APC (activated protein C) receptor influences the innate immune response by decreasing the population of natural killer and Th17 (T-helper cells).[169] Other proteases affect E-cadherin and the extracellular–intracellular matrix, leading to stimulation of the intracellular integrins associated with breakdown of the matrix of the cellular infrastructure. Nuclear transcription factor 4 stimulates the tumor suppressor gene (*TCF4*), with direct signaling to other nuclear genes and indirect signaling to *cMyc* affecting changes in gene expression.[1,170]

CYTOSTASIS AND DIFFERENTIATION CIRCUITS

Transforming growth factor beta (TGF-β) has transmembrane receptors that signal an intracellular gene family, *Smad*, to produce proteins for regulation (or dysregulation, in this case) of cellular growth and proliferation.[171] The nuclear *p16* gene (*INK4a*) is a cyclin-dependent kinase inhibitor 2A (*CDKN2A*) tumor suppressor gene that normally monitors cellular division and proliferation.[62] pRB1 is a retinoblastoma gene protein with multiple actions, including (1) action as a tumor suppressor to regulate cell growth, (2) inhibition of DNA replication, (3) interaction with other proteins to promote apoptosis, and (4) promotion of cellular differentiation.[172] The E2F protein is a transcription factor that affects gene expression and ultimately changes in gene expression.[172,173] If any of these molecules are mutated, the results will be altered gene expression and the possibility of a transformed cell. Finally, the nuclear *p21* (also known as the *HRAS* oncogene) receives signals from the intracellular *Smad* genes to regulate cell division; these signals simply direct cell growth or division.[173]

PROLIFERATION CIRCUITS

The receptor tyrosine kinases promote proliferation through multiple signaling pathways and are also described in the

section "Hallmark: Deregulated Growth and Mitogenesis." When activated, the RTKs can activate the *Ras* family of oncogenes and ultimately *Myc* oncogenes. The *Myc* oncogene participates in cell-cycle progression as part of the proliferation circuitry. When this gene develops mutations, it acts to prevent apoptosis and promote cellular transformation. Overexpression of the mutated *Myc* gene is frequently associated with hematopoietic tumors.[58,60,173]

Importantly, all of the circuits merge to effect changes in gene expression, which then affect all of the hallmark capabilities. Integral to this collection of circuits is the DNA damage sensor, *TP53* (tumor protein p53) tumor suppressor gene, which is known as the "guardian of the genome." It is located in the nucleus of cells and binds directly to DNA when damage there needs to be repaired, or signals for apoptosis when unrepairable DNA damage is identified.[174]

VIABILITY CIRCUITS

The viability circuits mediate the death and survival factors associated with the cell mechanisms. Survival factors have receptors on the cell membrane. Once activated through phosphorylation, they have the ability to limit cell death, facilitate angiogenesis, resist apoptosis, deplete the immune response, and produce extracellular matrix–modifying enzymes to promote invasion and metastasis with wide dissemination.[1,175] One example, the *BCL2* gene product, is a mitochondrial membrane protein that blocks apoptosis in cells such as lymphocytes.[176] Death factors are also activated by transmembrane receptors and include HIF-α and other factors that signal core metabolic pathways driven by oncogenes or tumor suppressor genes to activate the expression of inflammation-related pathways.[177]

CONCLUSION

The biology of cancer is sometimes viewed as a multifaceted association of simultaneous processes "gone wrong" within the cell. Figure 1-4 suggests targeted therapies. This chapter has suggested additional hallmarks based on the literature and noted other changes as enablers or drivers.[14] Research is currently examining these factors to determine if a targeted therapy might be able to directly affect a specific malignant change in a tumor.

Since 2000, the complexity of the evolution of cancer has been visualized as an even more remarkably detailed process as a result of the generation of large data sets able to absorb multiple entities at a time—indeed, thousands of entities simultaneously.[1,17] By studying the elements of DNA, researchers have been able to describe not only the genome, but also many other "omes"—for example, exomes, transcriptomes, proteomes, and epigenomes, among others. The analysis of these data has made overwhelming quantities of "interactome" information available and led to better understanding of cancer biology and its treatment with innovative targeted therapies for sustained remissions or cure.[3]

The amount of data available to answer the questions of how cancer begins and transforms into an aggressive tumor with far-reaching metastatic processes suggests numerous possibilities for final answers. However, with the exploration of these enormous data sets, it has also become obvious that each tumor reveals only some of its personal genetics and secrets to the subpopulations of cells, thereby yielding varying phenotypes as the stages of the tumor evolve. The final answers to the questions about the biology of cancer will emerge only as understanding is created from the large data sets that are enabled to "chat among themselves" as new details are combined, condensed, and manipulated to yield new outcomes of knowledge to be anticipated in the future.[3]

This chapter has identified and described some of the new data that reveal complex relationships between genes, signaling pathways, and characteristics of malignant cells. Models for the development of cancer were also described. The "hallmarks of cancer" can serve as a guide through the complex mechanisms and interactions of the malignant cell as it attempts to survive.[1,17] Even specific characteristics of the malignant cell incorporate elements from other hallmarks, however. The ultimate goal is to clarify complex concepts into simple explanations so that cancer nurses can develop or update an understanding of the biology of cancer, including suggestions of rationale for the diagnosis and targeted treatment of various cancer types.

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