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obstacle. For example, when a tumor-suppressor gene is inactivated, some downstream component of the pathway is likely to be activated, thereby presenting a realistic target. Alternatively, the mutational inactivation of a DNA repair pathway might create a unique dependence on the repair pathways that remain intact. An example of this is provided by PARP-1 inhibitors, which have been successfully used to treat patients whose tumors have inactivating mutations of genes involved in DNA repair processes, such as BRCA1. Patterns of global gene expression can be used to help unravel such pathways and are already being used to predict drug sensitivities and provide prognostic information in addition to that provided by DNA sequence analysis. Evaluation of proteomic and metabolomic patterns may also prove useful for this purpose.

■ ■THE FUTURE A revolution in cancer genetics has occurred in the past 30 years. Most types of cancer are now understood at the DNA sequence level, and this accomplishment has led to an increasingly refined understanding of tumorigenesis. Cancer gene mutations have proven to be reliable biomarkers for cancer detection and monitoring as well as for informing therapeutics through precision medicine approaches. Gene-based tests are already standard of care for patients with certain tumor types, such as colorectal and lung cancers, and the utility of these tests will undoubtedly be expanded in the coming years as new therapies and ways of predicting responses to therapies are developed. While effective treatment of advanced cancers remains difficult, the early promise shown by immune-based therapies notwithstanding, it is expected that breakthroughs in these areas will continue to emerge and be applicable to an ever-increasing number of cancers. Moreover, with the hoped-for advances in diagnostics, particularly in the earlier detection of cancers, the new and old therapies for cancer can be expected to have a much greater impact on reducing cancer deaths. PART 4 Oncology and Hematology Acknowledgments The authors gratefully acknowledge the past contributions of Pat J. Morin, Jeff Trent, and Francis Collins to earlier versions of this chapter. ■ ■FURTHER READING Bunz F: Principles of Cancer Genetics, 3rd ed. Dordrecht, Springer, 2022. Le DT et al: PD-1 Blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 372:2509, 2015. Vogelstein B, Kinzler KW: The path to cancer—three strikes and you're out. *N Engl J Med* 373:1895, 2015. Vogelstein B et al: Cancer genome landscapes. *Science* 339:1546, 2013. Jeffrey W. Clark, Dan L. Longo

Cancer Cell Biology ■ ■CANCER CELL BIOLOGY Cancers are characterized by unregulated cell division, avoidance of cell death, tissue invasion, and the ability to spread to other areas of the body (metastasize). A neoplasm is benign when it grows in an unregulated fashion without tissue invasion or metastasizing. The presence of unregulated growth, tissue invasion, and the ability to metastasize is characteristic of malignant neoplasms. Cancers are named based on their tissue of origin: those derived from epithelial tissue are called carcinomas, those derived from

mesenchymal tissues are sarcomas, and

those derived from hematopoietic tissue are leukemias, lymphomas, and plasma cell dyscrasias (including multiple myeloma). Cancers arise as a consequence of genetic alterations, the vast majority of which begin in a single cell and therefore are monoclonal in origin. However, because a wide variety of genetic and epigenetic changes can occur in different cells within malignant tumors over time, as well as varied responses with their microenvironments and the biology of the patient, most cancers are characterized by plasticity and marked heterogeneity in the populations of cells and their composite behavior. In addition, extrinsic factors in the cancer environment (e.g., the stroma, infiltrating cells, various cell-to-cell interactions, spatial orientation, secreted factors, and availability of oxygen and nutrients) vary in different areas within the tumor or different metastases, compounding this heterogeneity. This heterogeneity significantly complicates the treatment of most cancers because it is likely that there are subsets of cells that will be resistant to therapy for a variety of reasons and will therefore survive and proliferate even if the majority of cells are killed. A few cancers appear to, at least initially, be primarily driven by an alteration in a dominant gene that produces uncontrolled cell proliferation. Examples include chronic myeloid leukemia (abl), about half of melanomas (braf), Burkitt's lymphoma (c-myc), and subsets of lung adenocarcinomas (egfr, alk, ros1, met, ret, braf, and ntrk). Genes that can promote cell growth when altered are often called oncogenes. They were first identified as critical elements of viruses that cause animal tumors; it was subsequently found that the viral genes had normal counterparts with important functions in the cell and had been captured and mutated by viruses as they passed from host to host. However, most human cancers are characterized by a multiple-step process involving many genetic abnormalities, each of which contributes to the loss of control of cell proliferation and differentiation and the acquisition of capabilities, such as tissue invasion, the ability to metastasize, angiogenesis (development of new blood vessels required for tumor growth), and alteration of the extracellular environment. These properties are not found in the normal adult cell from which the tumor is derived. Indeed, normal cells have a large number of safeguards against DNA damage (including multiple DNA repair and extensive DNA damage response mechanisms), uncontrolled proliferation, and invasion. Many cancers go through recognizable steps of progressively more abnormal phenotypes: hyperplasia, to adenoma, to dysplasia, to carcinoma in situ, to invasive cancer with the ability to metastasize (Table 77-1). For most cancers, these changes occur over a prolonged period of time, usually many years. In most organs, only primitive undifferentiated cells are capable of proliferating and cells lose the capacity to proliferate as they differentiate and acquire functional capabilities. The expansion of the primitive cells (stem cells) is linked to some functional need in the host, such as normal turnover of tissues or regeneration after acute injury, through receptors that receive signals from cells and other factors in the local tissue microenvironment or through hormonal and other influences delivered by the vascular supply. In the absence of such signals, the cells are at rest or quiescent (out of the cell cycle but capable of being activated to reenter the cell cycle). The signals that induce quiescence in primitive cells as well as those that keep the cells at rest are complex, including the process for quiescent entry, maintenance, and exit. Although much has been learned, including the importance of notch signaling, the STING pathway, other quiescent factors, and transcriptional, posttranscriptional, and epigenetic regulation in quiescent entry and maintenance, overall control of the process within the body remains incompletely understood. These signals must be, at least in part, environmental, based on the observations that a regenerating liver stops growing when it has replaced the portion that has been surgically removed after partial hepatectomy and regenerating

bone marrow stops growing when the peripheral blood counts return to normal. Cancer cells clearly have lost responsiveness to such controls and do not recognize when they have overgrown the niche normally occupied by the organ from which they are derived. A better understanding of these mechanisms of growth regulation in the context of organ homeostasis continues to evolve.

TABLE 77-1 Phenotypic Characteristics of Malignant Cells

Deregulated cell proliferation: Loss of function of negative growth regulators (tumor suppressor genes, i.e., Rb, p53) and increased action of positive growth regulators (oncogenes, i.e., Ras, Myc). Leads to aberrant cell cycle control and includes loss of normal checkpoint responses. Failure to differentiate: Arrest at a stage before terminal differentiation. May retain stem cell properties. (Frequently observed in leukemias due to transcriptional repression of developmental programs by the gene products of chromosomal translocations.) Loss of normal apoptosis pathways: Inactivation of p53, increases in Bcl-2 (antiapoptotic) family members. This defect enhances the survival of cells with oncogenic mutations and genetic instability and allows clonal expansion and diversification within the tumor without activation of physiologic cell death pathways. Genetic instability: Defects in DNA repair pathways leading to either single nucleotide or oligonucleotide mutations (as in microsatellite instability, MIN) or, more commonly, chromosomal instability (CIN) leading to aneuploidy (abnormal number of chromosomes in a cell). Caused by loss of function of a number of proteins including p53, BRCA1/2, mismatch repair genes, DNA repair enzymes, and the spindle checkpoint. Leads to accumulation of a variety of mutations in different cells within the tumor and heterogeneity. Loss of replicative senescence: Normal cells stop dividing in vitro after 25–50 population doublings. Arrest is mediated by the Rb, p16INK4a, and p53 pathways. While most cells remain arrested, genetic and epigenetic changes in a subset of cells allow further replication, leading to telomere loss, with crisis leading to death of many cells. Cells that survive often harbor gross chromosomal abnormalities and the ability to continue to proliferate. These cells express telomerase, which maintains telomeres and is important for ongoing growth of these cells. Relevance to human in vivo cancer remains uncertain. Many human cancers express telomerase. Nonresponsiveness to external growth-inhibiting signals: Cancer cells have lost responsiveness to signals normally present to stop proliferating when they have overgrown the niche normally occupied by the organ from which they are derived. Our understanding about this mechanism of growth regulation remains limited. Increased angiogenesis: Due to increased gene expression of proangiogenic factors (VEGF, FGF, IL-8, angiopoietin) by tumor or stromal cells, or loss of negative regulators (endostatin, tumstatin, thrombospondin). Invasion: Cell mobility and ability to move through extracellular matrix and into other tissues or organs. Loss of cell-cell contacts (gap junctions, cadherins) and increased production of matrix metalloproteinases (MMPs). Can take the form of epithelial-to-mesenchymal transition (EMT), with anchored epithelial cells becoming more like motile fibroblasts. Metastasis: Spread of tumor cells to lymph nodes or distant tissue sites. Limited by the ability of tumor cells to migrate out of initial site and to survive in a foreign environment, including evading the immune system (see below). Evasion of the immune system: Downregulation of MHC class I and II molecules; induction of T-cell tolerance; inhibition of normal dendritic cell and/or T-cell function; antigenic loss variants and clonal heterogeneity; increase in regulatory T cells. Shift in cell metabolism: Complex changes including alterations due to tumor stress such as hypoxia and energy generation shifts from oxidative phosphorylation to aerobic glycolysis generate building blocks for malignant cell production and proliferation. Complex interactions with the extracellular environment around the cancer cells: Induction of changes as well as complex interactions with the extracellular environment around cancer cells, including modifications to the extracellular matrix,

vasculature, chemokines, mesenchymal stromal cells, fibroblasts, immune cells, other hematopoietic cells, platelets, nerves, and potentially infectious agents impacting many of the above processes. Abbreviations: FGF, fibroblast growth factor; IL, interleukin; MHC, major histocompatibility complex; VEGF, vascular endothelial growth factor. ■ ■ DIFFERENCES BETWEEN PEDIATRIC AND ADULT CANCERS The underlying importance of genetic mutations and other molecular changes is similar for pediatric and adult cancers. However, some important differences exist. Childhood cancers have a different epidemiology (e.g., they do not have the same extent of environmental or lifestyle risk factors), are much less frequent, and have a different spectrum of frequency beginning primarily during embryogenesis in mesodermal (e.g., sarcomas or hematologic malignancies such as

acute lymphocytic leukemia [ALL] or lymphomas) or ectodermal (e.g., neuronal including brain and spinal) tissues. They generally have fewer genetic changes and lower mutational burdens than adult cancers. Likely due to a number of factors including the nature of the cancers (e.g., less genetic complexity than adult cancers) and the ability of children to tolerate more intense chemotherapy regimens, childhood cancers generally tend to be much more responsive to chemotherapy than adult cancers with significantly higher rates of cure.

■ ■ CANCER AS AN ORGAN THAT IGNORES ITS NICHE The fundamental cellular defects that create a malignant neoplasm act at the cellular level, and some of these are cell autonomous. However, that is not the entire story. Cancers consist of both malignant cells as well as other cells, blood vessels, extracellular matrix, and signaling and other molecules in the cancer microenvironment. They behave as organs that have lost their specialized function and stopped responding to signals that would limit their growth in tightly regulated normal tissue homeostasis. Most human cancers usually become clinically detectable when a primary mass is approximately 1 cm in diameter—such a mass consists of about 10^9 cells. Often, patients present with tumors that are approximately 10^{10} cells. Although it varies by type of cancer and where the primary tumor and metastases are located, a lethal tumor burden is usually about 10^{12} – 10^{13} cells. If all malignant cells were dividing without any cell death at the time of diagnosis, most patients would reach a lethal tumor burden in a very short time. However, human tumors grow by Gompertzian kinetics—this means that not every daughter cell produced by a cell division is actively dividing. In addition, the overall growth rate of a tumor depends on differences between growth rates of different cells within the tumor and rate of cell loss. The growth fraction of a tumor declines with time, largely due to factors in the microenvironment and accumulation of genetic damage over time. The growth fraction of the first malignant cell is 100%, and by the time a patient presents for medical care, the growth fraction is estimated to be <5%, although the fraction varies between different types of cancers and even different cancers of the same type in different individuals. This fraction is often similar to the growth fraction of normal bone marrow and normal intestinal epithelium, the most highly proliferative normal tissues in the human body, a fact that may explain the dose-limiting toxicities to these tissues of agents that target dividing cells. CHAPTER 77 Cancer Cell Biology The implication of these data is that the tumor is slowing its own growth over time. How does it do this? The tumor cells have multiple genetic lesions that tend to promote proliferation, yet by the time the tumor is clinically detectable, its capacity for proliferation has declined. Better understanding of how a tumor slows its own growth would provide important clues for better cancer treatment. A number of factors, including those in the tumor microenvironment, are known to contribute to the decreased proliferation of tumor cells over time in the patient. For

example, normal cells and other factors in the micro environment can contribute to slowing down the growth of cancer cells. Some cancer cells are hypoxemic and have inadequate supply of nutrients and energy. Some have sustained too much genetic damage to complete the cell cycle but have lost the capacity to undergo apoptosis and therefore survive but do not proliferate. However, an important subset is not actively dividing but retains the capacity to divide and can start dividing again under certain conditions such as when the tumor mass is reduced by treatments leading to improved conditions in the tumor microenvironment favorable for cell proliferation. Just as the bone marrow increases its rate of proliferation in response to bone marrow-damaging agents, the tumor also seems to sense when tumor cell numbers have been reduced and can respond by increasing growth rate. However, the critical difference is that the marrow stops growing when it has reached its production goals, whereas tumors do not. The ultimate structure and organization of an organ are based on a number of factors including growth, migration, elimination, and death of various cells; communication between cells to establish the correct architecture; competition between cells; and the composition of the extracellular matrix that is produced. In addition to normal cells

stopping proliferation in an organ when that is appropriate, normal tissues have various mechanisms for eliminating cells in both the process of development as well as ongoing homeostasis of an organ. These include mechanical processes based on a number of factors including cell size, cell shape, and topology between cells that can determine cell fate as well as an active process of cell extrusion, which plays a major role in the elimination of both cells that are no longer needed by the organ and cells that are damaged and potentially dangerous (such as those with mutations that might be precursors for malignancy). The process of cell extrusion may depend on cell cycle arrest in the S phase; aberrations in this process may contribute to the metastatic process. A variety of processes, including major alterations in cell cycle control, apoptosis and other mechanisms of cell death, and uncontrolled cell signaling, all contribute to defects in appropriate cell extrusion contributing to the development of cancer.

Additional tumor cell vulnerabilities are likely to be detected when we learn more about how normal cells respond to “stop” signals from their environment, and why and how tumor cells and tissues fail to heed such signals. ■ ■ CELL CYCLE CHECKPOINTS The cell division cycle consists of four phases—G1 (growth and preparation for DNA synthesis), S (DNA synthesis), G2 (preparation to divide), and M (mitosis, cell division). Cells can also exit the cell cycle and be quiescent (G0). Progression of a cell through the cell cycle is tightly regulated at a number of checkpoints (especially at the G1/S boundary, the G2/M boundary, and during M [spindle checkpoint]) by an array of genes that are targeted by specific genetic alterations in cancer. These checkpoints are quality-control features; at G1, the check point does not allow cells to proceed that are not ready for genome replication; at G2/M, the cell assesses whether the genome has been appropriately duplicated and is ready to divide. Critical proteins in these control processes that are frequently mutated or otherwise inactivated in cancers are called tumor-suppressor genes because when they function normally, they inhibit the development or growth of cancer cells. Examples include p53 and Rb (discussed below). PART 4 Oncology and Hematology In the first phase, G1, preparations are made to replicate the genetic material. The cell stops before entering the DNA synthesis phase, or S phase, to take inventory. Are we ready to replicate our DNA? Is the DNA repair machinery in place to fix any mutations that are detected? Are the DNA replicating enzymes available? Is there an adequate supply of nucleotides? Is there sufficient energy to proceed? The

retinoblastoma protein, Rb, plays a central role in placing a brake on the process until the cell is ready. When the cell determines that it is prepared to move ahead, sequential activation of cyclin-dependent kinases (CDKs) results in the inactivation of the brake, Rb, by phosphorylation. Phosphorylated Rb releases the S phase-regulating transcription factor, E2F/DP1, and genes required for S-phase progression are expressed. If the cell determines that it is unready to move ahead with DNA replication, a number of inhibitors are capable of blocking the action of the CDKs, including p21Cip2/Waf1, p16Ink4a, and p27Kip1. Nearly every cancer has one or more defects in the G1 checkpoint that permit progression to S phase despite abnormalities in DNA repair machinery or other deficiencies that would affect normal DNA synthesis. At the end of the G2 phase and before the M phase, after the cell has exactly duplicated its DNA content, a second inventory is taken at the G2 checkpoint. Have all of the chromosomes been fully duplicated? Were all segments of DNA copied only once? Has all damaged DNA been repaired? Do we have the right number of chromosomes and the right amount of DNA? If so, the cell proceeds to G2, in which the cell prepares for division by synthesizing mitotic spindle and other proteins needed to produce two daughter cells. If DNA damage is detected, the p53 pathway is normally activated. Called the guardian of the genome, p53 is a transcription factor that is normally present in the cell in very low levels. This level is generally regulated through its rapid turnover. Normally, p53 is bound to mdm2, a ubiquitin ligase that both inhibits p53 transcriptional activation and also targets p53 for degradation in the proteasome. When DNA damage is sensed, the ATM (ataxia telangiectasia mutated) pathway is activated; ATM phosphorylates

1. DNA DAMAGE CHECKPOINT

2. ONCOGENE CHECKPOINT myc, E2F, EIA ATM/ATR p53 mdm2 chk1/chk2 Induction of P14ARF P mdm2 P14ARF mdm2 P P Transcriptional activation of p53responsive genes P P p53 Tetramer FIGURE 77-1 Induction of p53 by the DNA damage and oncogene checkpoints. In response to noxious stimuli, p53 and mdm2 are phosphorylated by the ataxia telangiectasia mutated (ATM) and related ATR serine/threonine kinases, as well as the immediate downstream checkpoint kinases, Chk1 and Chk2. This causes dissociation of p53 from mdm2, leading to increased p53 protein levels and transcription of genes leading to cell cycle arrest (p21Cip1/Waf1) or apoptosis (e.g., the proapoptotic Bcl-2 family members Noxa and Puma). Inducers of p53 include hypoxemia, DNA damage (caused by ultraviolet radiation, gamma irradiation, or chemotherapy), ribonucleotide depletion, and telomere shortening. A second mechanism of p53 induction is activated by oncogenes such as Myc, which promote aberrant G1/S transition. This pathway is regulated by a second product of the Ink4a locus, p14ARF (p19 in mice), which is encoded by an alternative reading frame (ARF) of the same stretch of DNA that codes for p16Ink4a. Levels of ARF are upregulated by Myc and E2F, and ARF binds to mdm2 and rescues p53 from its inhibitory effect. This oncogene checkpoint leads to the death or senescence (an irreversible arrest in G1 of the cell cycle) of renegade cells that attempt to enter S phase without appropriate physiologic signals. Senescent cells have been identified in patients whose premalignant lesions harbor activated oncogenes, for instance, dysplastic nevi that encode an activated form of BRAF (see below), demonstrating that induction of senescence is a protective mechanism that operates in humans to prevent the outgrowth of neoplastic cells. mdm2, releasing it from its inhibitory bond to p53. p53 then stops cell cycle progression, directs the synthesis of repair enzymes, or if the damage is too great, initiates apoptosis (programmed cell death) of the cell to prevent the propagation of a

damaged cell (Fig. 77-1). A second method of activating p53 involves the induction of p14ARF by hyperproliferative signals from oncogenes. p14ARF competes with p53 for binding to mdm2, allowing p53 to escape the effects of mdm2 and accumulate in the cell. p53 then stops cell cycle progression by activating CDK inhibitors such as p21 and/or initiating the apoptosis pathway. Not surprisingly given its critical role in controlling cell cycle progression, mutations in the gene for p53 on chromosome 17p are among the most frequent mutations in human cancers, although percentages vary between different cancers. Most commonly these mutations are acquired in the malignant tissue in one allele and the second allele is inactivated (such as by deletion or epigenetic silencing), leaving the cell unprotected from DNA-damaging agents or activated oncogenes. Some environmental exposures produce signature mutations in p53; for example, aflatoxin exposure leads to mutation of arginine to serine at codon 249 and leads to hepatocellular carcinoma. In rare instances, p53 mutations are in the germline (Li-Fraumeni syndrome) and produce a familial cancer syndrome. Another mechanism for inactivation of p53 in malignant cells is due to alterations in regulators such as overexpression of the inhibitory mdm2 protein. Whether inactivated by mutation or inhibited by regulatory factors, absence of normal p53 function leads to chromosomal instability and accumulation of DNA damage including acquisition of properties that give the abnormal cell a proliferative and survival advantage. Like Rb dysfunction, most cancers have mechanisms that disable the p53 pathway. Indeed, the importance of p53 and Rb in the development of cancer is underscored by the neoplastic transformation mechanism of human papillomavirus. This virus has two main oncogenes, E6 and E7. E6 acts to increase the rapid

turnover of p53, and E7 acts to inhibit Rb function; inhibition of these two targets is required for transformation of epithelial cells by the virus. Another cell cycle checkpoint exists when the cell is undergoing division (M phase); this is the spindle checkpoint, which acts to ensure that there is proper attachment of chromosomes to the mitotic spindle before progression through the cell cycle can occur. If the spindle apparatus does not properly align the chromosomes for division, if the chromosome number is abnormal (i.e., greater or less than $4n$), or if the centromeres are not properly paired with their duplicated partners, then the cell initiates a cell death pathway to prevent the production of aneuploid progeny (having an altered number of chromosomes). Abnormalities in the spindle checkpoint facilitate the development of aneuploidy, which is frequently found in cancers. In some tumors, aneuploidy is a predominant genetic feature. In other tumors, a defect in the cells' ability to repair errors in the DNA, such as due to mutations in genes coding for the proteins critical for mismatched DNA repair, is the primary genetic lesion. Cancer cells can have defects in any of several DNA repair pathways in addition to mismatch repair, including deficient interstrand cross-link, double-strand breaks (homologous recombination or nonhomologous end joining repair), single-strand breaks, base excision, nucleotide excision, and translesional synthesis. In general, tumors have either defects in chromosome number or defective DNA repair pathways but not both. Defects that lead to cancer include abnormal cell cycle checkpoints, inadequate DNA repair, and failure to preserve genome integrity leading to DNA damage. These defects and the stress of the resultant increased DNA damage make cancer cells more vulnerable to additional DNA damage, which can be exploited by chemotherapy, radiation therapy, targeted therapy, and immunotherapy—the major systemic therapeutic approaches effective against cancer. Alternatively, research is ongoing in an attempt to therapeutically restore the defects in cell cycle regulation and DNA repair that characterize cancer, although this remains

a challenging problem because it is much more difficult to restore normal biologic function than to inhibit abnormal function of proteins driving cell proliferation, such as occurs with activated oncogenes. Newer approaches to gene editing (e.g., clustered regularly interspaced short palindromic repeats [CRISPR]) and subsequent modifications to this approach should eventually make gene editing more clinically feasible. ■ ■ CELLULAR SENESENCE The irreversible cessation of growth of normal cells while the cells remain viable has been termed cellular senescence. Senescence is important for several processes involved in normal development and homeostasis including embryogenesis and wound healing. It is also an important component of host mechanisms to prevent tumorigenesis by preventing replication of abnormal cells as well as other mechanisms including secreted substances that can stimulate an immune response against the abnormal senescent cell. However, paradoxically, senescent cells in tumors can also stimulate tumorigenesis and malignant progression, in part by other secreted substances that stimulate a harmful inflammatory response. It was initially identified by the fact that when normal cells are placed in culture in vitro, most are not capable of sustained growth. They quickly reach a point where they either undergo cell death due to excessive DNA damage or other factors or they become senescent. Fibroblasts are an exception to this rule. When they are cultured, fibroblasts may divide 30–50 times and then they undergo what has been termed a “crisis” during which the majority of cells stop dividing (usually due to an increase in p21 expression, a CDK inhibitor). This form of senescence is termed replicative senescence. Many other cells die, and a small fraction emerge that have acquired genetic and epigenetic changes that permit their uncontrolled growth. Among the cellular changes during in vitro propagation is telomere shortening. DNA polymerase is unable to replicate the tips of chromosomes, resulting in the loss of DNA at the specialized ends of chromosomes (called telomeres) with each replication cycle. At birth, human telomeres are 15- to 20-kb pairs long and are composed of tandem repeats of a six-nucleotide sequence (TTAGGG) that associate with specialized

telomere-binding proteins to form a T-loop structure that protects the ends of chromosomes from being mistakenly recognized as damaged. The loss of telomeric repeats with each cell division cycle causes gradual telomere shortening, leading to growth arrest when one or more critically short telomeres trigger a p53-regulated DNA-damage checkpoint response. Cell death usually ensues when the unprotected ends of chromosomes lead to chromosome fusions or other catastrophic DNA rearrangements. Cells with certain abnormalities, such as those with nonfunctional pRb and p53, can bypass this growth arrest. The ability to bypass telomere-based growth limitations is thought to be a critical step in the evolution of most malignancies. This occurs by reactivation of telomerase expression in cancer cells. Telomerase is an enzyme that adds TTAGGG repeats onto the 3' ends of chromosomes. It contains a catalytic subunit with reverse transcriptase activity (hTERT) and an RNA component that provides the template for telomere extension. Most normal somatic cells do not express sufficient telomerase to prevent telomere attrition with each cell division. Exceptions include stem cells (such as those found in hematopoietic tissues, gut and skin epithelium, and germ cells) that require extensive cell division to maintain tissue homeostasis. More than 90% of human cancers express high levels of telomerase that prevent telomere shortening to critical levels and allow indefinite cell proliferation. In vitro experiments indicate that inhibition of telomerase activity leads to tumor cell apoptosis. Major efforts are underway to develop methods to inhibit telomerase activity in cancer cells. For example, the protein component of telomerase (hTERT) may act as one of the most widely expressed tumor-associated antigens and can be targeted by vaccine approaches. However, a

caveat to targeting telomerase for anticancer treatment is the potential for inhibiting its activity in certain normal cells (such as stem cells) required for maintaining the normal physiologic state.

CHAPTER 77 Cancer Cell Biology Although most of the functions of telomerase relate to cell division, it also has several other effects including interfering with the differentiated functions of at least certain stem cells. However, the impact on differentiated function of normal nonstem cells is less clear. The picture is further complicated by the fact that rare genetic defects in the telomerase enzyme seem to cause dyskeratosis congenita (characterized by abnormalities in various rapidly dividing cells in the body including skin, nails, oral mucosa, hair, and bone marrow with increased risk for leukemia and certain other cancers). This can be associated with a number of other abnormalities including pulmonary fibrosis, bone marrow failure (aplastic anemia), or liver fibrosis. However, paradoxically, defects in nutrient absorption in the gastrointestinal tract, a site that should be highly sensitive to defective cell proliferation, are not seen. Much remains to be learned about how telomere shortening and telomere maintenance are related to human illness in general and cancer in particular. A variety of other stresses on cells (both environmental and intrinsic including radiation, chemotherapy, reactive oxygen species, and oncogenic mutations) can also lead to senescence, primarily those that induce DNA damage similar to that seen in cells with shortened telomeres. This is termed replicative senescence. ■ ■ **SIGNAL TRANSDUCTION**

PATHWAYS IN CANCER CELLS Signals that affect cell behavior come from adjacent cells, the stroma in which the cells are located, hormonal signals that originate remotely, and the cells themselves (autocrine signaling). These signals generally exert their influence on the receiving cell through activation of signal transduction pathways that have as their end result the induction of activated transcription factors that mediate a change in cell behavior or function or the acquisition of effector machinery to accomplish a new task. Although signal transduction pathways can lead to a wide variety of outcomes, many such pathways rely on cascades of signals that sequentially activate different proteins or glycoproteins and lipids or glycolipids, and the activation steps often involve the addition or removal of one or more phosphate groups on a downstream target. Other chemical changes can result from signal transduction pathways, but reversible phosphorylation and dephosphorylation play a major role. Proteins that add phosphate groups to other molecules

(proteins, lipids, or nucleic acids) are called kinases. Two major classes of kinases involved in signal transduction pathways important for cancer cells are tyrosine kinases that phosphorylate tyrosine and serine/threonine kinases that phosphorylate serine/threonine either directly or indirectly. However, some kinases can phosphorylate both, such as the MEK kinases that can phosphorylate both threonine and tyrosine. Phosphatases (protein tyrosine phosphatases and protein serine/threonine phosphatases) remove the phosphate groups to reverse the kinase activity.

Various kinases play critical roles in signal transduction pathways important for malignant cells. These include a number of receptor tyrosine kinases (RTKs) as well as various protein kinases (both tyrosine and serine/threonine kinases) downstream of receptors that transmit the signals within the cell (Fig. 77-2). Two important signaling pathways are the RAS-RAF-MEK-ERK pathway and the phosphatidylinositol-3-kinase (PI3K) pathway (Fig. 77-2). Although pathways are depicted as distinct, complex interactions between pathways occur within cells. Normally, kinase activity is short-lived and reversed by protein phosphatases. However, in many human cancers, RTKs or components of their downstream pathways are activated by mutation, gene **PART 4 Oncology and Hematology** PI3K inhibitors PIP2 RAS Grb2/mSOS PI3K PIP3 PDK1 AKT Multiple targets Everolimus

mTOR Protein synthesis p70S6k ERK1/2 Activated transcription factors ECM Integrin receptor Cytoskeleton FAK c-Src Activated kinases STAT Midostaurin JAK inhibitors JAK PKC Multiple targets PLC- γ Ca²⁺ Tamoxifen SERMS PIP2 DAG

FIGURE 77-2 Therapeutic targeting of signal transduction pathways in cancer cells. Three major signal transduction pathways are activated by receptor tyrosine kinases (RTKs). 1. The protooncogene Ras is activated by the Grb2/mSOS guanine nucleotide exchange factor, which induces an association with Raf and activation of downstream kinases (MEK and ERK1/2). 2. Activated PI3K phosphorylates the membrane lipid PIP2 to generate PIP3, which acts as a membrane-docking site for a number of cellular proteins including the serine/threonine kinases PDK1 and Akt. PDK1 has numerous cellular targets, including Akt and mTOR. Akt phosphorylates target proteins that promote resistance to apoptosis and enhance cell cycle progression, while mTOR and its target p70S6K upregulate protein synthesis to potentiate cell growth. 3. Activation of PLC- γ leads the formation of diacylglycerol (DAG) and increased intracellular calcium, with activation of multiple isoforms of PKC and other enzymes regulated by the calcium/ calmodulin system. Other important signaling pathways involve non-RTKs that are activated by cytokine or integrin receptors. Janus kinases (JAK) phosphorylate STAT (signal transducer and activator of transcription) transcription factors, which translocate to the nucleus and activate target genes. Integrin receptors mediate cellular interactions with the extracellular matrix (ECM), inducing activation of FAK (focal adhesion kinase) and c-Src, which activate multiple downstream pathways, including modulation of the cell cytoskeleton. Many activated kinases and transcription factors migrate into the nucleus, where they regulate gene transcription, thus completing the path from extracellular signals, such as growth factors, to a change in cell phenotype, such as induction of differentiation or cell proliferation. The nuclear targets of these processes include transcription factors (e.g., Myc, AP-1, and serum response factor) and the cell cycle machinery (cyclin-dependent kinases [CDKs] and cyclins). Inhibitors of many of these pathways have been developed for the treatment of human cancers. Examples of inhibitors that are either approved or are currently being evaluated in clinical trials are shown in purple type.

amplification, or chromosomal translocations to have enhanced and/ or prolonged activity. Because these pathways are important in regulating proliferation, survival, migration, and angiogenesis, they have been identified as important targets for cancer therapeutics. Inhibition of kinase activity is effective in the treatment of a number of neoplasms. Lung cancers with mutations in the epidermal growth factor receptor are highly responsive to osimertinib as well as other inhibitors (Table 77-2). Inhibitors have been developed to treat lung cancers with other tyrosine kinase-activating mutations (including anaplastic lymphoma kinase [ALK], ROS1, NTRK, MET, HER2, and RET). BRAF (a serine/threonine kinase) inhibitors are highly effective in melanomas and thyroid cancers and are also used in combination with other agents for lung and colon cancers as well as other solid tumors with BRAF V600E mutations. Targeting the MEK protein (which phosphorylates both threonine and tyrosine residues) downstream of BRAF also has activity against BRAF mutant melanomas, and combined inhibition of BRAF and MEK is more effective than either alone with activity that extends to BRAF-mutant lung cancer. Janus kinase (JAK) inhibitors are active in myeloproliferative syndromes in which JAK2 activation is a pathogenetic event. Imatinib (which targets a number of tyrosine kinases) is an effective agent in Ligand RTK Monoclonal antibody Under investigation Tyrosine kinase inhibitors Raf kinase inhibitors RAS inhibitors Raf GAP MEK inhibitors MEK ERK inhibitors Multiple cytoplasmic targets AP-1 (Jun/Fos) Serum response factor MYC Cyclin D1 CDK/cyclin complexes CDK H/b inhibitors Cell cycle regulation Nucleus Estrogen receptor

TABLE 77-2 Some FDA-Approved Molecularly Targeted Agents for the Treatment of Cancer

DRUG	MOLECULAR TARGET	DISEASE	MECHANISM OF ACTION
All-trans retinoic acid	PML-RAR α oncogene	Acute promyelocytic leukemia	M3 AML, t(15;17)
Imatinib, dasatinib, nilotinib, ponatinib, bosutinib	Bcr-Abl, c-Abl, c-Kit, PDGFR- α/β	Chronic myeloid leukemia, GIST	Blocks ATP binding to tyrosine kinase active site
Ripretinib	c-Kit, PDGFR- α	GIST	Inhibits tyrosine kinase activity
Asciminib	Bcr-Abl	Chronic myeloid leukemia	Allosteric inhibitor of BCR-ABL
Sunitinib	c-Kit, VEGFR-2, PDGFR- β , Flt-3	GIST, RCC, PNET	Inhibits activated c-Kit and PDGFR in GIST; inhibits VEGFR in RCC and probably in PNET
Sorafenib	RAF, VEGFR-2, PDGFR- α/β , Flt-3, c-Kit	RCC, hepatocellular carcinoma (HCC), differentiated thyroid cancer, desmoid	
Regorafenib	VEGFR1-3, TIE-2, FGFR1, KIT, RET, PDGFR	Colorectal cancer, GIST, HCC	
Larotrectinib, entrectinib	NTRK	Cancers with NTRK mutation	Competitive inhibitor ATP binding site of tyrosine kinase domain multiple kinases including VEGFR
Axitinib	VEGFR1-3	RCC	Competitive inhibitor ATP binding site of tyrosine kinase domain of VEGF receptors
Erlotinib	EGFR	NSCLC, pancreatic cancer	Competitive inhibitor of the ATP-binding site of the EGFR
Afinitinib	EGFR (and other HER family)	NSCLC	Irreversible inhibitor of ATP-binding site of HER family members
Osimertinib	EGFR (T790M)	NSCLC	Inhibits EGFR mutations including T790M mutant
Dacomitinib	EGFR	NSCLC (exon19 deletion/exon 21 L858R)	Inhibits EGFR mutant lung cancer
Mobocertinib	EGFR/NSCLC/Tumors with Exon20 insertion mutations		
Erdafitinib, pemigatinib, futibatinib, infigratinib	FGFR2, FGFR3	Urothelial (erdafitinib), myeloid/ lymphoid neoplasms (pemigatinib) cholangiocarcinoma (pemigatinib, futibatinib)	
Lapatinib, tucatinib, niratinib	HER2/neu	Breast cancer, CRC (tucatinib + trastuzumab)	
Crizotinib, ceritinib, alectinib, brigatinib, lorlatinib	ALK	NSCLC ALK+ large cell lymphoma, inflammatory myofibroblastic tumors (crizotinib)	
Crizotinib, entrectinib, repotrectinib	ROS1	NSCLC	Inhibitor of ROS1 tyrosine kinase
Palbociclib, ribociclib, abemaciclib	CDK4/6	Breast	Inhibitor of CDK4/6
Bortezomib, carfilzomib, ixazomib	Proteasome	Multiple myeloma	Inhibits proteolytic degradation of multiple cellular proteins
Vemurafenib, dabrafenib	BRAF V600E	Melanoma lung cancer, CRC (combined with Cetuximab)	
Trametinib, Cobimetinib, binimetinib	MEK	Melanoma	Inhibitor of serine-threonine kinase domain of MEK
Cabozantinib	RET, MET, VEGFR	MTC, RCC	Competitive inhibitor of ATP-binding site of tyrosine kinase domain of multiple kinases, including VEGFR2 and RET
Capmatinib, tepotinib	MET	NSCLC with MET exon14 deletions	
Selpercatinib, vandetinib, pralsetinib	RET	NSCLC, MTC, RET fusion thyroid cancer, RET fusion positive solid tumors	
Temsirolimus	mTOR	RCC	Competitive inhibitor of mTOR serine-threonine kinase
Everolimus	mTOR	RCC, PNET	Binds to immunophilin FK binding protein-12, which forms a complex that inhibits mTOR kinase
Vorinostat, romidepsin, belinostat	HDAC	CTCL/PTL	HDAC inhibitor, epigenetic modulation
Panobinostat	HDAC	MM	HDAC inhibitor, epigenetic modulation
Ruxolitinib	JAK-1, 2	Myelofibrosis	Competitive inhibitor of tyrosine kinase
Vismodegib	Hedgehog pathway	Basal cell cancer (skin)	Inhibits smoothened in hedgehog pathway
Lenvatinib	Multikinase inhibitor (VEGFR, FGFR, PGFR- α , others)	RCC, thyroid cancer, HCC	
Olaparib, rucaparib, niraparib, talazoparib	PARP	BRCA mutant ovarian, breast, prostate, pancreas cancers; not all agents approved for all cancers	
Venetoclax	BCL-2	CLL (with 17p deletion)	Inhibits BCL-2 and enhances apoptosis
Ibrutinib, acalabrutinib, pirtobrutinib, zanubrutinib	Bruton tyrosine kinase (BTK)	CLL, MCL, MZL, SLL, WM	Inhibitor of BTK

Inhibits transcriptional repression by PML-RAR α Adagrasib, Solorasib/KRAS12C/NSCLC/Inhibits KRAS12C Targets VEGFR pathways in RCC and HCC. Possible activity against BRAF in thyroid cancer CHAPTER 77 Inhibits tyrosine kinase of FGFR Cancer Cell Biology Competitive inhibitor of

the ATP-binding site of HER2 Inhibitor of ALK tyrosine kinase Inhibitor of serine-threonine kinase domain of V600E mutant of BRAF Inhibitor of RET, VEGFR1, VEGFR2 tyrosine kinases Inhibits PARP and DNA repair (Continued)

TABLE 77-2 Some FDA-Approved Molecularly Targeted Agents for the Treatment of Cancer DRUG MOLECULAR TARGET DISEASE MECHANISM OF ACTION Ivosidenib, olutasidenib IDH1 AML, MDS, cholangiocarcinoma IDH1 inhibitor Gilteritinib, quizartinib FLT3 AML FLT3 inhibitor Idelalisib PI3K-delta CLL Inhibits PI3k-delta, preventing proliferation and inducing apoptosis Alpelisib PIK3CA Breast cancer with a PIK3CA mutation Inhibits PIK3CA Belzutifan Hif-2 α HIF-1 α -associated RCC, pancreatic neuroendocrine, CNS hemangioblastoma Capivasertib AKT Breast cancer Inhibits AKT Umbralisib PI3K-delta, CK1-epsilon MZL, FL Inhibits PI3K-delta and CK1-epsilon Selinexor Exportin-1 MM, DLBCL Induces apoptosis of cells Tazemetostat EZH2 FL, epithelioid sarcoma Inhibits EZH2 Monoclonal Antibodies Trastuzumab, margetuximab HER2/neu (ERBB2) Breast cancer, gastric or GEJ cancer Binds HER2 on tumor cell surface and induces receptor internalization Pertuzumab HER2/neu (ERBB2) Breast cancer Binds HER2 on tumor cell surface at distinct site from trastuzumab and prevents binding to other receptors Cetuximab EGFR Colon cancer, squamous cell carcinoma of the head and neck PART 4 Oncology and Hematology Panitumumab EGFR Colon cancer Similar to cetuximab but fully humanized rather than chimeric Necitumumab EGFR Squamous NSCLC Binds EGFR Rituximab CD20 B-cell lymphomas and leukemias that express CD20 Alemtuzumab CD52 Chronic lymphocytic leukemia and CD52-expressing lymphoid tumors Bevacizumab VEGF Colorectal, lung cancers, RCC, glioblastoma Inhibits angiogenesis by high-affinity binding to VEGF Ziv-aflibercept VEGFA, VEGFB, PLGF Colorectal cancers Inhibits angiogenesis by high-affinity binding to VEGFA, VEGFB, and PLGF Ramucirumab VEGFR Gastric, colorectal, lung cancers Inhibits angiogenesis by binding to VEGFR Ipilimumab CTLA-4 Melanoma, HCC, MSI-high colorectal cancer Blocks CTLA-4, preventing interaction with CD80/86 and T-cell inhibition Nivolumab, pembrolizumab, dostarlimab-gxly, toripalimab, retifanlimab-dlwr, cemiplimab-rwlc PD-1 Melanoma, head and neck cancer, NSCLC, SCLC, Hodgkin's disease, urothelial cancer, RCC, HCC, gastric cancer, esophageal cancer, cholangiocarcinoma, MSI-high cancers, endometrial cancer, cervical cancer, cutaneous squamous cell carcinoma, basal cell carcinoma, breast cancer, nasopharyngeal cancer, Merkel cell tumor Atezolizumab, durvalumab, avelumab PD-L1 NSCLC, urothelial cancer, SCLC (durvalumab), HCC (atezolizumab), Merkel cell cancer (avelumab) Relatlimab LAG3 Melanoma (combined with nivolumab) Blocks LAG3 interaction with MHCII and other ligands inhibiting immune activation Denosumab Rank ligand Breast, prostate Inhibits Rank ligand, primary signal for bone removal Dinutuximab Glycolipid GD2 Neuroblastoma (pediatric) Immune-mediated attack on GD2-expressing cells Daratumumab, Isatuximab CD38 MM Binds to CD38 on MM cells causing apoptosis by antibody-dependent or complement-mediated cytotoxicity Elotuzumab SLAMF7 MM Activating NK cells to kill MM cells Olaratumab PDGFR α Soft tissue sarcomas Blocks PDGFR α activity Naxitamab GD2 Neuroblastoma Immune-mediated antitumor effect Bispecific Antibodies Blinatumomab CD19 and CD3 Ph-relapsed precursor B-cell ALL Binds CD19 on ALL cells and CD3 on T cells; immune attack on CD19-expressing cells Glofitamab-gxbl, epcoritamab-bysp, mosunetuzumab-axgb CD20 and CD3 DLBCL, FL Binds CD20 on DLBCL or FL and CD3 on T cells, immune attack on CD20-expressing cells Teclistamab-cqyv, elranatamab-bcmm B-cell maturation antigen (BCMA) and CD3 MM Binds BCMA on MM cells and CD3 on T cells Talquetamab CD3 and GPRC5D MM Binds CD3 T cells and GPRC5D-expressing MM cells

(Continued) Inhibits Hif-2 α Binds extracellular domain of EGFR and blocks binding of EGF and TGF- α ; induces receptor internalization. Potentiates the efficacy of chemotherapy and radiotherapy Multiple potential mechanisms, including direct induction of tumor cell apoptosis and immune mechanisms Immune mechanisms Blocks PD-1, preventing interaction with PD-L1 and T-cell inhibition Blocks PD-L1, preventing interaction with PD-1 and T-cell inhibition (Continued)

TABLE 77-2 Some FDA-Approved Molecularly Targeted Agents for the Treatment of Cancer
 DRUG MOLECULAR TARGET DISEASE MECHANISM OF ACTION
 Amivantamab-vmjw EGFR and MET NSCLC Targets EGFR exon 20 insertion mutations by also inhibiting MET
 Tebentafusp-tebn GP100 and CD3 Uveal melanoma Binds GP100 on melanoma cells and CD3 on T cells
 Antibody-Chemotherapy Conjugates Brentuximab vedotin CD30 Hodgkin's disease, anaplastic lymphoma
 Delivers chemotherapeutic agent MMAE to CD30-expressing Ado-trastuzumab emtansine HER2 Breast cancer
 Delivers chemotherapeutic agent emtansine to

HER2-expressing breast cancer cells Fam-trastuzumab HER2 Breast, NSCLC, and gastric cancers
 Delivers chemotherapeutic agent deruxtecan to

HER2-expressing breast cancer cells Sacituzumab govitecan Trop2 Breast, urothelial cancers
 Delivers chemotherapy to Trop2-expressing cells Enfortumab-vedotin Nectin-4 Urothelial cancers
 Delivers chemotherapeutic agent MMAE to

Nectin-4-expressing cells Polatuzumab-vedotin CD79b DLBCL or high-grade BCL Delivers MMAE chemotherapy to B-cell lymphomas
 Loncastuximab tesirine-lpyl CD19 DLBCL Delivers chemotherapy to CD19 expressing cells
 Mirvetuximab soravtansine-gynx Folate receptor alpha Ovarian, fallopian, peritoneal cancers
 Delivers chemotherapy to folate receptor alpha tumors Tisotumab vedotin-tftv Tissue factor (TF) Cervical cancer
 Delivers chemotherapy to TF-positive cells Gemtuzumab ozogamicin CD33 Pediatric CD33+ AML
 Delivery of chemotherapy to CD33+ cells CAR-T Cells and Tumor-Infiltrating Lymphocyte (TIL) Tisagenlecleucel, axicabtagene ciloleucel, brexucabtagene autoleucel, lisocabtagene maraleucel CD19 ALL (tisagenlecleucel), DLBCL/highgrade BCL (axicabtagene ciloleucel), B-cell precursor ALL (brexucabtagene), large BCL (lisocabtagene maraleucel) Ciltacabtagene autoleucel Idecabtagene vicleucel BCMA MM Targets T cells to protein on surface of MM cells
 Lifileucel Melanoma antigens Melanoma Tumor-infiltrating lymphocyte therapy Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BCL, B-cell lymphoma; CAR-T, chimeric antigen receptor T cells; CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; CTCL, cutaneous T cell lymphoma; DLBCL, diffuse large B-cell lymphoma; EGFR, epidermal growth factor receptor; FDA, U.S. Food and Drug Administration; FGFR, fibroblast growth factor receptor; FL, follicular lymphoma; Flt-3, fms-like tyrosine kinase-3; GEJ, gastroesophageal junction; GIST, gastrointestinal stromal tumor; HDAC, histone deacetylases; MCL, mantle cell lymphoma; MM, multiple myeloma; MSI, microsatellite instability; MMAE, monomethyl auristatin E; MTC, medullary thyroid cancer; mTOR, mammalian target of rapamycin; MZL, mantle zone lymphoma; NK, natural killer; NSCLC, non-small-cell lung cancer; PARP, poly-ADP ribose polymerase; PDGFR, platelet-derived growth factor receptor; PLGF, placenta growth factor; PML-RAR α , promyelocytic leukemia-retinoic acid receptor-alpha; PNET, pancreatic neuroendocrine tumors; PTL, peripheral T-cell lymphoma; RCC, renal cell cancer; t(15;17), translocation between chromosomes 15 and 17; SCLC, small-cell lung cancer; SLL, small lymphocytic lymphoma; TGF- α , transforming growth factor-alpha; VEGFR, vascular endothelial growth factor receptor; WM,

Waldenström's macroglobulinemia. Note: The pace of drug discovery is rapid and this list is not comprehensive. Tumors that have translocations of the c-Abl and BCR gene (such as chronic myeloid leukemia), mutant c-Kit (gastrointestinal stromal cell tumors), or mutant platelet-derived growth factor receptor (PDGFR α ; gastrointestinal stromal tumors). Second-generation inhibitors of BCR-Abl, dasatinib and nilotinib, are even more effective, and the third-generation agent bosutinib has activity in some patients who have progressed on other inhibitors, while the third-generation agent ponatinib has activity against the T315I mutation, which is resistant to the other agents. Although almost all tyrosine kinase inhibitors are not entirely selective for one protein, certain inhibitors have significant activity against a broad number of proteins. These include sorafenib, regorafenib, cabozantinib, sunitinib, and lenvatinib. These have shown antitumor activity in various malignancies, including renal cell cancer (RCC) (sorafenib, sunitinib, cabozantinib, lenvatinib), hepatocellular carcinoma (sorafenib, regorafenib, lenvatinib), gastrointestinal stromal tumor (GIST) (sunitinib, regorafenib), thyroid cancer (sorafenib, cabozantinib, lenvatinib), colorectal cancer (regorafenib), and pancreatic neuroendocrine tumors (sunitinib). Inhibitors of the PI3K pathway also have been approved for cancer therapy. The PI3K family includes three classes and several isoforms within each class. Inhibitors against different isoforms have proved effective against different types of malignancies, with inhibitors of the delta isoform (either specifically or also with inhibition of other isoforms; e.g., idelalisib) having activity against lymphoid malignancies (CLL), whereas the specific inhibitor of a mutation in the alpha isoform (alpelisib) has activity against breast cancers with this mutation. Inhibitors of mammalian target of rapamycin (mTOR; which is downstream of PI3K; e.g., everolimus, temsirolimus) are active in RCC; well-differentiated nonfunctional neuroendocrine tumors of pancreatic,

(Continued) tumor cells CHAPTER 77 Targeted T cells to protein on surface of malignant cells Cancer Cell Biology gastrointestinal, or lung origin; and breast cancer. Additional inhibitors of the PI3K pathway and other phospholipid signaling pathways such as the phospholipase C-gamma pathway, which are involved in a large number of cellular processes important in cancer development and progression, are being evaluated. The list of active agents and treatment indications is growing rapidly (Table 77-2). These agents have ushered in a new era of personalized therapy. For many cancers, it is now standard for tumor biopsies to be assessed for specific molecular changes that predict response and to have clinical decision-making guided by those results. This is now an important component of choosing therapy for metastatic lung, gastroesophageal, melanoma, breast, and colorectal cancers as well as in adjuvant therapy for breast cancer. This list will continue to evolve as both new agents against existing targets are developed and new targets are discovered. An alternative approach to testing samples directly from tumors is to test blood for the presence of mutations or amplification in circulating tumor DNA, which has the significant advantage of being noninvasive. As cancers grow, some of the cells die, break apart, and release cellular contents, including DNA, into the circulation. Sensitive methods have been developed to detect this DNA and to identify mutations and other DNA changes in the malignant cells. This has the potential advantage over tumor biopsies of sampling all of the tumor and not being limited to one site that may not be representative of the overall tumor heterogeneity. Distinct metastatic lesions may have different genetic abnormalities that will not be detected in a biopsy of a single site. In addition to identifying potential changes that can be targeted for therapy, there is also the potential for monitoring a patient's response to therapy, identifying resistance mechanisms to therapy earlier, detecting

disease recurrence before it can be detected by tumor markers or scans, monitoring bodily fluids in addition to blood, and possibly providing a means of earlier initial detection of cancer if sufficiently sensitive and specific detection methods can be developed. Optimizing the sensitivity and specificity of these tests is essential for their potential utility in patient care. Research is ongoing to determine if other cellular components specific to cancer cells (e.g., mRNA, proteins from mutant genes, or other protein modifications found in cancer cells) might also be useful for diagnosis or monitoring therapeutic response.

However, none of these targeted therapies has yet been curative by themselves for any malignancy, although prolonged periods of disease control lasting many years frequently occur in chronic myeloid leukemia (CML), including a >80% survival rate at 10 years, and antibodies to HER2 have increased survival for breast cancer patients in combination with chemotherapy. The reasons for the failure of targeted agents to cure are not completely defined, although resistance to the treatment ultimately develops in most patients. In some tumors, resistance to kinase inhibitors is related to proliferation of cells with a mutation in the target kinase that inhibits drug binding. Many of these kinase inhibitors act as competitive inhibitors of the ATP-binding pocket. ATP is the phosphate donor in these phosphorylation reactions. For example, mutation in the critical BCR-ABL kinase in the ATP-binding pocket (such as the threonine to isoleucine change at codon 315 [T315I]) can prevent imatinib binding. Other resistance mechanisms include alterations in other signal transduction pathways to bypass the inhibited pathway. As resistance mechanisms continue to be better defined, rational strategies to overcome resistance are emerging. In addition, many kinase inhibitors are less specific for an oncogenic target than was hoped, and toxicities related to off-target inhibition of kinases limit the use of the agent at a dose that would optimally inhibit the cancer-relevant kinase.

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Antibodies against protein targets more highly expressed on malignant than normal cells can also be used to deliver highly toxic compounds relatively specifically to cancer cells. Examples of protein targets for currently approved antibody-drug conjugates include CD30 for Hodgkin's and anaplastic lymphomas; HER2 on breast cancer; CD33 on acute myeloid leukemias; CD22 on B-cell acute lymphocytic and hairy cell leukemias; and CD79b on diffuse large B-cell lymphomas. Another strategy to enhance the antitumor effects of targeted agents is to use them in rational combinations with each other as well as with chemotherapy or immunotherapy agents that kill cells in ways distinct from agents targeting specific mutant or overexpressed proteins. Combinations of trastuzumab (a monoclonal antibody that targets the HER2 receptor [member of the EGFR family]) with chemotherapy have significant activity against breast, gastric, and esophageal cancers that have high levels of expression of the HER2 protein. The activity of trastuzumab and chemotherapy can be enhanced further by combinations with another targeted monoclonal antibody (pertuzumab), which prevents dimerization of the HER2 receptor with other HER family members including HER3, or in some cases with immunotherapy, such as combinations of chemotherapy, trastuzumab, and the immunotherapeutic agent pembrolizumab against HER2-positive gastric and esophageal cancers. Although targeted therapies have not yet resulted in cures when used alone, their use in the adjuvant setting and when combined with other effective treatments has substantially increased the fraction of patients cured. For example, the addition of rituximab, an anti-CD20 antibody, to combination chemotherapy in patients with diffuse large B-cell lymphoma improves cure rates by ~15%. The addition of trastuzumab, an antibody to HER2, to combination chemotherapy in the adjuvant treatment of HER2-positive breast cancer significantly improves overall survival. A major effort continues to develop targeted therapies for mutations in the ras

family of genes, which play a critical role in transmitting signals through a number of downstream signaling pathways including the MAP (mitogen-activated protein) kinase and PI3K pathways. Mutations in ras are the most common mutations in oncogenes in cancers (especially kras) but have proved to be very difficult targets for a number of reasons related to the structure of RAS proteins as well

as mechanisms of activation and inactivation (active when bound to guanosine triphosphate [GTP] and inactive when bound to guanosine diphosphate [GDP]). RAS proteins are not kinases but bind directly to the BRAF serine/threonine kinase with preferential binding when RAS is in the active GTP bound state. Agents that target one of the mutant forms of KRAS (12C), which is the most common RAS mutation in lung cancer and is also found in a subset of other cancers, have sufficient antitumor activity to now be approved for the treatment of these lung cancers. They are under active study (often in combination with other agents) for the treatment of other cancers that have the KRAS12C mutation. Agents targeting other mutations in the RAS genes (especially other KRAS-mutant proteins) are also being evaluated in clinical trials. One strategy for new drug development is to take advantage of so-called oncogene addiction. This situation (Fig. 77-3) is created when a tumor cell develops an activating mutation in an oncogene that becomes a dominant pathway for survival and growth with reduced contributions from other pathways, even when there may be abnormalities in those pathways. This dependency on a single pathway creates a cell that is vulnerable to inhibitors of that oncogene pathway. For example, cells harboring mutations in BRAF are sensitive to MEK inhibitors that inhibit signaling via the BRAF pathway. Proteins critical for transcription of other proteins essential for malignant cell survival or proliferation provide another potential target for treating cancers. The transcription factor nuclear factor (NF)- κ B is a heterodimer composed of p65 and p50 subunits that associate with an inhibitor, I κ B, in the cell cytoplasm. In response to growth factor or cytokine signaling, a multisubunit kinase called IKK (I κ B-kinase) phosphorylates I κ B and directs its degradation by the ubiquitin/ proteasome system. NF- κ B, free of its inhibitor, translocates to the nucleus and activates target genes, many of which promote the survival of tumor cells. One of the mechanisms by which novel drugs called proteasome inhibitors are thought to produce an anticancer effect is by blocking the proteolysis of I κ B, thereby preventing NF- κ B activation. For reasons that have not been fully elucidated, this has a differential toxicity effect on tumor, as compared to normal cells. Although this mechanism appears to be an important aspect of the antitumor effects of proteasome inhibitors, other effects involving the inhibition of the degradation of multiple cellular proteins important in malignant cell survival or proliferation also play a role. Proteasome inhibitors (e.g., bortezomib, carfilzomib, ixazomib) have activity in patients with multiple myeloma, including partial and complete remissions. Inhibitors of IKK are also in development, with the hope of more selectively blocking the degradation of I κ B, thus “locking” NF- κ B in an inhibitory complex and rendering the cancer cell more susceptible to apoptosis-inducing agents. Many other transcription factors are activated by phosphorylation, which can be prevented by tyrosine or serine/threonine kinase inhibitors, a number of which are currently in clinical trials. Estrogen receptors (ERs) and androgen receptors (ARs), members of the steroid hormone family of nuclear receptors, are targets of inhibition by drugs used to treat breast and prostate cancers, respectively. Selective estrogen receptor modulators (SERMs) have been developed as a treatment approach for ER-positive breast cancer. Tamoxifen, a partial agonist and antagonist of ER function, is frequently used in breast cancer, can mediate tumor regression in metastatic breast cancer, and can prevent disease recurrence in the adjuvant setting. Tamoxifen binds to the ER and modulates its transcriptional activity, inhibiting activity in the breast but

promoting activity in bone but unfortunately also in uterine epithelium, leading to a small increased risk of uterine cancer. Attempts have been made to develop SERMs that would have antiestrogenic effects in both breast and uterus while maintaining protective effects on bone. However, none of these to date has been an improvement over tamoxifen. Aromatase inhibitors, which block the conversion of androgens to estrogens in breast and subcutaneous fat tissues, have demonstrated improved clinical efficacy compared with tamoxifen in postmenopausal women and are often used as first-line therapy in postmenopausal patients with ER-positive disease. They are occasionally used in premenopausal patients with ER-positive disease

Normal cell Base excision repair Tumor cell BRCA1, 2 nonmutated Normal cell Base excision repair Tumor cell BRCA1, 2 mutated

FIGURE 77-3 Synthetic lethality. Genes are said to have a synthetic lethal relationship when mutation of either gene alone is tolerated by the cell, but mutation of both genes leads to lethality, as originally noted by Bridges and later named by Dobzhansky. Thus, mutant gene a and gene b have a synthetic lethal relationship, implying that the loss of one gene makes the cell dependent on the function of the other gene. In cancer cells, loss of function of a DNA repair gene like BRCA1, which repairs double-strand breaks, makes the cell dependent on base excision repair mediated in part by PARP. If the PARP gene product is inhibited, the cell attempts to repair the break using the error-prone nonhomologous end-joining method, which results in tumor cell death. High-throughput screens can now be performed using isogenic cell line pairs in which one cell line has a defined defect in a DNA repair pathway. Compounds can be identified that selectively kill the mutant cell line; targets of these compounds have a synthetic lethal relationship to the repair pathway and are potentially important targets for future therapeutics. In combination with ovarian suppression approaches such as luteinizing hormone-releasing hormone (LHRH) agonists. A number of approaches have been developed for blocking androgen stimulation of prostate cancer, including decreasing production by the testicles (e.g., orchiectomy, LHRH agonists or antagonists), directly blocking actions of androgen (a number of agents have been developed to do this), or blocking production by inhibiting the enzyme CYP17, which is central in production of androgens from cholesterol.

■ ■ CANCER-SPECIFIC GENETIC CHANGES AND SYNTHETIC LETHALITY The concepts of oncogene addiction and synthetic lethality have spurred new drug development targeting oncogene- and tumor-suppressor pathways. As discussed earlier in this chapter and outlined in Fig. 77-3, cancer cells can become dependent upon signaling pathways containing activated oncogenes; this can effect proliferation (i.e., mutated KRAS, BRAF, overexpressed MYC, or activated tyrosine kinases). Additional genetic changes in malignant cells or unique features of tumors including defects in DNA repair (e.g., loss of BRCA1 or BRCA2 gene function), modifications in cell cycle control (e.g., changes in protein levels or mutations in cyclins and CDKs), enhanced survival mechanisms (overexpression of Bcl-2 or NF- κ B), altered cell metabolism (such as occurs when mutant KRAS enhances glucose uptake and aerobic glycolysis), tumor-stromal interactions, and angiogenesis (e.g., production of vascular endothelial growth factor [VEGF] in response to HIF-2 α in RCC) can also be successfully exploited to relatively specifically target cancers. However, resistance to inhibition of specific oncogenic pathways almost always eventually develops. In addition, targeting defects in tumor-suppressor genes has been much more difficult, both because the target of mutation is

- PARP inhibition PARP PARP PARP PARP PARP PARP PARP PARP Homologous double strand break repair No cell killing CHAPTER 77 Homologous double strand break repair Selective tumor cell killing – Cancer Cell Biology often deleted and because it is much more difficult

to restore normal function than to inhibit abnormal function of a protein. Synthetic lethality occurs when loss of function in either of two or more genes individually has limited effects on cell survival but loss of function in both (or more) genes leads to cell death. In the case of oncogene-addicted pathways, identifying genes that have a synthetic lethal relationship with the activated pathway may allow enhanced cell killing and decreased resistance by targeting those genes or their proteins. In the case of mutant tumor-suppressor genes, identifying genes that have a synthetic lethal relationship to those mutated pathways may allow targeting by inhibiting proteins required uniquely by those cells for survival or proliferation (Fig. 77-3). This is a much more tractable approach than attempting to repair normal function of the mutant suppressor gene itself. Examples of synthetic lethality with clinical impact have been identified. For instance, cells with mutations in the BRCA1 or BRCA2 tumor-suppressor genes (e.g., a subset of breast and ovarian cancers) are unable to repair DNA damage by homologous recombination. Poly-ADP ribose polymerase (PARP) is a family of proteins important for single-strand break (SSB) DNA repair. PARP inhibition results in selective killing of cancer cells that have lost BRCA1 or BRCA2 function. A number of PARP inhibitors have been approved for treatment of ovarian, breast, and pancreatic cancers with BRCA mutations and are likely to have activity in other tumors with defective DNA repair mechanisms. The concept of synthetic lethality provides a framework for genetic screens to identify other synthetic lethal combinations involving known tumor-suppressor genes and development of novel therapeutic agents to target dependent pathways. Other unique aspects of malignant tumors, including those outlined elsewhere in the chapter, may also be vulnerable to synthetic lethal interactions.

■ ■ EPIGENETIC INFLUENCES ON CANCER GENE TRANSCRIPTION Chromatin structure regulates the hierarchical order of sequential gene transcription that governs differentiation and tissue homeostasis. Disruption of chromatin remodeling (the process of modifying chromatin structure to control exposure of specific genes to transcriptional proteins, thereby controlling the expression of those genes) leads to aberrant gene expression that can significantly alter the biology of cells including inducing proliferation or migration of cells. Epigenetic changes are those that alter the pattern of gene expression that persist across at least one cell division but are not caused by changes in the DNA code. These include alterations of chromatin structure mediated by methylation of cytosine residues of DNA (primarily in context of CpG dinucleotides in somatic cells), modification of histones by altering acetylation or methylation, or changes in higher-order chromosome structure (Fig. 77-4). Appropriate control of DNA methylation is essential for normal cell function and development, and both altered methylation and hypomethylation of histones occur in cancers. Hypermethylation of DNA promoter regions is a common mechanism by which tumor-suppressor loci are epigenetically silenced in cancer cells. Thus, one allele of a tumor-suppressor gene may be inactivated by mutation or deletion, while expression of the other allele is epigenetically silenced, usually by methylation, leading to loss of gene function. Aberrant hypomethylation is also frequently found in a number of cancers consistent with the dysregulated pattern of gene transcription that is a hallmark of cancer cells, with some genes being inappropriately turned off while others are inappropriately turned on. Specific changes in DNA methylation in cancer cells provide a potentially more sensitive and specific approach to utilizing circulating tumor DNA to identify the presence of cancer than utilizing only DNA mutational analysis.

PART 4 Oncology and Hematology Acetylation of the amino terminus of the core histones H3 and H4 induces an open chromatin conformation that promotes transcription HDAC MeCP Nucleosomes CpG Island in promoter region HAT: histone acetyl transferase HDAC: histone deacetylase :unmethylated CpG :methylated CpG DNMT: DNA methyltransferase MeCP: methylcytosine binding protein Co-activator complex HAT HAT Tc factor Tc factor Tc factor “Open” chromatin configuration permits binding of multiple sequence-specific transcription factors that cooperatively promote gene expression. Nucleosomes Nucleosomes FIGURE 77-4 Epigenetic regulation of gene expression in cancer cells. Tumor-suppressor genes are often epigenetically silenced in cancer cells. In the upper portion, a CpG island within the promoter and enhancer regions of the gene has been methylated, resulting in the recruitment of methyl-cytosine binding proteins (MeCP) and complexes with histone deacetylase (HDAC) activity. Chromatin is in a condensed, nonpermissive conformation that inhibits transcription. Clinical trials are under way utilizing the combination of demethylating agents such as 5-aza-2'-deoxycytidine plus HDAC inhibitors, which together confer an open, permissive chromatin structure (lower portion). Transcription factors bind to specific DNA sequences in promoter regions and, through protein-protein interactions, recruit coactivator complexes containing histone acetyl transferase (HAT) activity. This enhances transcription initiation by RNA polymerase II and associated general transcription factors. The expression of the tumor-suppressor gene commences, with phenotypic changes that may include growth arrest, differentiation, or apoptosis.

initiation. Histone acetylases are components of coactivator complexes recruited to promoter/enhancer regions by sequence-specific transcription factors during the activation of genes (Fig. 77-4). Histone deacetylases (HDACs; multiple HDACs are encoded in the human genome) are recruited to genes by transcriptional repressors and prevent the initiation of gene transcription. Methylated cytosine residues in promoter regions become associated with methyl cytosine-binding proteins that recruit protein complexes with HDAC activity. The balance between permissive and inhibitory chromatin structure is therefore largely determined by the activity of transcription factors in modulating the “histone code” and the methylation status of the genetic regulatory elements of genes. The pattern of gene transcription is aberrant in all human cancers, and in many cases, epigenetic events are responsible. Epigenetic events play a critical role in carcinogenesis (e.g., long-lasting changes in methylation induced by smoking) and are found in premalignant lesions. Unlike genetic events that alter DNA primary structure (e.g., deletions), epigenetic changes are potentially reversible and appear amenable to therapeutic intervention. In certain human cancers, including a subset of pancreatic cancers and multiple myeloma, the p16Ink4a promoter is inactivated by methylation, thus permitting the unchecked activity of CDK4/cyclin D and rendering pRb nonfunctional. In sporadic forms of renal, breast, and colon cancer, the von Hippel-Lindau (VHL), breast cancer 1 (BRCA1), and serine/threonine kinase 11 (STK11) genes, respectively, can be epigenetically silenced. Other targeted genes include the p15Ink4b CDK inhibitor, glutathione S-transferase (which detoxifies reactive oxygen species [ROS]), and the E-cadherin molecule (important for junction formation between epithelial cells). Epigenetic silencing can affect genes involved in DNA repair, thus predisposing to further genetic damage. Examples include MLH1 (mutL homologue in sporadic colon cancers that have micro satellite instability) and MSH2 in a subset of hereditary nonpolyposis colon cancer patients who have a mutation in the 3' end of epithelial cell adhesion molecule (EPCAM). These are critical genes involved in No transcription Differentiation arrested Deregulated proliferation DNMT HDAC MeCP Nucleosomes Treatment: 5-aza-2'-deoxycytidine HDAC inhibitors Active transcription of tumor

suppressor genes RNA polymerase II and general transcription machinery

repair of mismatched bases that occur during DNA synthesis, and their silencing can lead to mutations in the DNA. Human leukemias often have chromosomal translocations that code for novel fusion proteins with activities that alter chromatin structure by interacting with HDACs or histone acetyl transferases (HATs). For example, the promyelocytic leukemia-retinoic acid receptor α (PML-RAR α) fusion protein, generated by the t(15;17) translocation observed in most cases of acute promyelocytic leukemia (APL), binds to promoters containing retinoic acid response elements and recruits HDACs to these promoters, effectively inhibiting gene expression. This arrests differentiation at the promyelocyte stage and promotes tumor cell proliferation and survival. Treatment with pharmacologic doses of all-trans retinoic acid (ATRA), the ligand for RAR α , results in the release of HDAC activity and the recruitment of coactivators, which overcome the differentiation block. This induced differentiation of APL cells has improved treatment of these patients but also has led to a novel treatment toxicity when newly differentiated tumor cells infiltrate the lungs. ATRA represents a treatment paradigm for the reversal of epigenetic changes in cancer. Other leukemia-associated fusion proteins, such as Tel-acute myeloid leukemia (AML1), AML1eight-twenty-one (ETO), and the MLL fusion proteins seen in acute myeloid leukemia (AML) and acute lymphocytic leukemia, also lead to repression through the HDAC complex. Therefore, efforts are ongoing to determine the structural basis for interactions between translocation fusion proteins and chromatin-remodeling proteins and to use this information to rationally design small molecules that will disrupt specific protein-protein associations, although this has proven to be technically difficult. Several drugs that block the enzymatic activity of HDACs (HDAC inhibitors [HDACis]) are approved for cancer treatment, and others are being tested. HDACis have demonstrated sufficient antitumor activity against cutaneous T-cell lymphoma (vorinostat, romidepsin), peripheral T-cell lymphoma (romidepsin, belinostat), and multiple myeloma (panobinostat) to be approved by the U.S. Food and Drug Administration (FDA). HDACis have also demonstrated antitumor activity in clinical studies against some solid tumors, and additional studies are ongoing. HDACis may target cancer cells via a number of mechanisms including both epigenetic modulation via histone acetylation and effects on other proteins that are acetylated. The pleiotropic effects of some HDACis include enhancement of apoptosis by upregulation of a number of proteins that enhance apoptosis including death receptors (DR4/5, FAS, and their ligands) and downregulation of proteins that inhibit apoptosis (e.g., X-linked inhibitor of apoptosis [XIAP]); upregulation of proteins that inhibit cell cycle progression (e.g., p21Cip1/Waf1); inhibition of DNA repair and generation of ROS leading to increased DNA damage; and disruption of the chaperone protein HSP90. Efforts are also under way to modulate other epigenetic processes such as reversing the hypermethylation of CpG islands that characterizes many malignancies. Drugs that induce DNA demethylation, such as 5-aza-2-deoxycytidine, can lead to reexpression of silenced genes in cancer cells with restoration of function, and 5-aza-2-deoxycytidine is approved for use in myelodysplastic syndrome. However, 5-aza-2-deoxycytidine has limited aqueous solubility and is myelosuppressive, limiting its usefulness. Other inhibitors of DNA methyltransferases are in development. In ongoing clinical trials, inhibitors of DNA methylation are being combined with HDACis, with the idea that reversing coexisting epigenetic changes will reverse the deregulated patterns of gene transcription in cancer cells. Epigenetic gene regulation can also occur via microRNAs or long noncoding RNAs (lncRNA). MicroRNAs (miRNA) are short (average 22 nucleotides in length) single strand RNA molecules that regulate gene expression after transcription by specifically binding to and inhibiting the translation or promoting the degradation of mRNA transcripts. It is estimated

that >1000 miRNAs are encoded in the human genome. Each tissue has a distinctive repertoire of miRNA expression, and this pattern is altered in specific ways in cancers. miRNAs are involved in controlling multiple aspects of cell biology through modulating protein expression (primarily by down regulation) and thus are also involved in multiple aspects of cancer biology.

Specific correlations between expression of different miRNA molecules and tumor biology and clinical behavior are continuing to emerge. Therapies targeting miRNAs are not currently at hand but represent an ongoing area of treatment development. LncRNAs are longer than 200 nucleotides and comprise the largest group of noncoding RNAs. Some of them have been shown to play important roles in gene regulation. The potential for altering these RNAs for therapeutic benefit is an area of active investigation. In addition to epigenetic changes, mutations in genes (such as enhancer and promoter regions) involved in controlling expression of other genes important in cancer cell biology can also lead to enhanced or decreased expression of the protein products of these genes.

APOPTOSIS AND OTHER MECHANISMS OF CELL DEATH Tissue homeostasis requires a balance between the death of aged, terminally differentiated cells or severely damaged cells and their renewal by proliferation of committed progenitors. Genetic damage to growth-regulating genes of stem cells could lead to catastrophic results for the host as a whole. Thus, in normal cells, the genetic events causing activation of oncogenes or loss of tumor suppressors, which would be predicted to lead to unregulated cell proliferation unless corrected, also usually activate signal transduction pathways that block aberrant cell proliferation. These pathways can lead to forms of programmed cell death including apoptosis or autophagy (degradation of proteins and organelles by lysosomal proteases) or irreversible growth arrest (senescence). A number of other regulated cell death processes have been identified, including: pyroptosis, a caspase-1-dependent process leading to cleavage of gasdermins with subsequent formation of pores in the plasma membrane; ferroptosis (iron and reactive oxygen species dependent); and necroptosis (caspase-independent regulated cell death involving breakdown of cellular components and cell rupture, leading to inflammation and damage to surrounding tissues), which also play roles in tissue homeostasis and cell death. However, the exact roles they play in the fate of cancer cells and tissues are still being elucidated. Much as a panoply of intra- and extracellular signals impinge upon the core cell cycle machinery to regulate cell division, so too these signals are transmitted to a core enzymatic machinery that regulates cell death and survival. Cancer cells have developed mechanisms that either inhibit these processes to prevent cell death or utilize them to enhance survival. **CHAPTER 77 Cancer Cell Biology** Apoptosis is a tightly regulated process induced by two main pathways (Fig. 77-5). The extrinsic pathway of apoptosis is activated by cross-linking members of the tumor necrosis factor (TNF) receptor superfamily, such as CD95 (Fas) and death receptors DR4 and DR5, by their ligands, Fas ligand or TRAIL (TNF-related apoptosis-inducing ligand), respectively. This induces the association of FADD (Fas-

associated death domain) and procaspase-8 to death domain motifs of the receptors. Caspase-8 is activated and then cleaves and activates effector caspases-3 and -7, which then target cellular constituents (including caspase-activated DNase, cytoskeletal proteins, and a number of regulatory proteins), inducing the morphologic appearance characteristic of apoptosis, which pathologists term karyorrhexis (literally “nucleus bursting”). The intrinsic pathway of apoptosis is initiated by the release of cytochrome c and SMAC (second mitochondrial activator of caspases)

from the mitochondrial intermembrane space in response to a variety of noxious stimuli, including DNA damage, loss of adherence to the extracellular matrix (ECM), oncogene-induced proliferation, and growth factor deprivation. Upon release into the cytoplasm, cytochrome c associates with dATP, procaspase-9, and the adaptor protein APAF-1, leading to the sequential activation of caspase-9 and effector caspases. SMAC binds to and blocks the function of inhibitor of apoptosis proteins (IAP), negative regulators of caspase activation. The release of apoptosis-inducing proteins from the mitochondria is regulated by pro- and antiapoptotic members of the Bcl-2 family. Antiapoptotic members (e.g., Bcl-2, Bcl-XL, and Mcl-1) associate with the mitochondrial outer membrane via their carboxyl termini, exposing to the cytoplasm a hydrophobic binding pocket composed of Bcl-2

Trail

DR4 or DR5 FADD Caspase 8 Pro-caspase 9 Cyt c APAF-1 dATP

SMAC IAP BH3-only proteins Intermembrane space Bak

Bcl2 Matrix Bax PART 4 Oncology and Hematology Outer membrane Mitochondrion FIGURE 77-5

Therapeutic strategies to overcome aberrant survival pathways in cancer cells. 1. The extrinsic pathway of apoptosis can be selectively induced in cancer cells by TRAIL (the ligand for death receptors 4 and 5) or by agonistic monoclonal antibodies. 2. Inhibition of antiapoptotic Bcl-2 family members with antisense oligonucleotides or inhibitors of the BH3-binding pocket will promote formation of Bak- or Bax-induced pores in the mitochondrial outer membrane. 3. Epigenetic silencing of APAF-1, caspase-8, and other proteins can be overcome using demethylating agents and inhibitors of histone deacetylases. 4. Inhibitor of apoptosis proteins (IAP) blocks activation of caspases; small-molecule inhibitors of IAP function (mimicking SMAC action) should lower the threshold for apoptosis. 5. Signal transduction pathways originating with activation of receptor tyrosine kinase receptors (RTKs) or cytokine receptors promote survival of cancer cells by a number of mechanisms. Inhibiting receptor function with monoclonal antibodies, such as trastuzumab or cetuximab, or inhibiting kinase activity with small-molecule inhibitors can block the pathway. 6. The Akt kinase phosphorylates many regulators of apoptosis to promote cell survival; inhibitors of Akt may render tumor cells more sensitive to apoptosis-inducing signals; however, the possibility of toxicity to normal cells may limit the therapeutic value of these agents. 7 and 8. Activation of the transcription factor NF- κ B (composed of p65 and p50 subunits) occurs when its inhibitor, I κ B, is phosphorylated by I κ B-kinase (IKK), with subsequent degradation of I κ B by the proteasome. Inhibition of IKK activity should selectively block the activation of NF- κ B target genes, many of which promote cell survival. Inhibitors of proteasome function are U.S. Food and Drug Administration approved and may work in part by preventing destruction of I κ B, thus blocking NF- κ B nuclear localization. NF- κ B is unlikely to be the only target for proteasome inhibitors. homology (BH) domains 1, 2, and 3 that is crucial for their activity. Perturbations of normal physiologic processes in specific cellular compartments lead to the activation of BH3-only proapoptotic family members (e.g., Bad, Bim, Bid, Puma, Noxa, and others) that can alter the conformation of the outer-membrane proteins Bax and Bak, which then oligomerize to form pores in the mitochondrial outer membrane resulting in cytochrome c release. If proteins composed only by BH3 domains are sequestered by Bcl-2, Bcl-XL, or Mcl-1, pores do not form and apoptosis-inducing proteins are not released from the mitochondria. The ratio of levels of antiapoptotic Bcl-2 family members and the

levels of proapoptotic BH3-only proteins at the mitochondrial membrane determines the activation state of the intrinsic pathway. The mitochondrion must therefore be recognized not only as an organelle with vital roles in intermediary metabolism and oxidative phosphorylation but also as a central regulatory structure of the apoptotic process. The evolution of tumor cells to a more malignant phenotype requires the acquisition of genetic changes that subvert apoptosis pathways and promote cancer cell survival and resistance to anticancer therapies.

GF RTK

PI3K

Mdm2 AKT Cytokine receptor Effector caspases BAD Caspase

FKHR

Substrate cleavage

IKK IκB p65 p50 Cytoskeletal disruption

Proteasome NF-κB genes activated DNA degradation Chromatin condensation Lamin cleavage Nucleus Death-inducing signals • DNA damage • Oncogene-induced proliferation • Loss of attachment to ECM • Chemotherapy, radiation therapy However, this means that cancer cells may be more vulnerable than normal cells to therapeutic interventions that target the apoptosis pathways that cancer cells depend upon. For instance, overexpression of Bcl-2 as a result of the t(14;18) translocation contributes to follicular lymphoma, and it is highly expressed in many lymphoid malignancies including chronic lymphocytic leukemia (CLL). Upregulation of Bcl-2 expression is also observed in other cancers including prostate, breast and lung cancers, and melanoma. Targeting of antiapoptotic Bcl-2 family members has been accomplished by the identification of several low-molecular-weight compounds that bind to the hydrophobic pockets of either Bcl-2 or Bcl-XL and block their ability to associate with death-inducing BH3-only proteins. An oral BH3 mimetic inhibitor of BCL-2, venetoclax, is approved for use in patients with refractory CLL with 17p deletion, and is active in AML. Preclinical studies targeting death receptors DR4 and -5 have demonstrated that recombinant, soluble, human TRAIL or humanized monoclonal antibodies with agonist activity against DR4 or -5 can induce apoptosis of tumor cells while sparing normal cells. The mechanisms for this selectivity may

include expression of decoy receptors or elevated levels of intracellular inhibitors (such as FLIP, which competes with caspase-8 for FADD) by normal cells but not tumor cells. Synergy has been shown between TRAIL-induced apoptosis and chemotherapeutic agents in some preclinical studies. However, studies have not yet shown significant clinical activity of approaches targeting the TRAIL pathway. Many of the signal transduction pathways perturbed in cancer promote tumor cell survival (Fig. 77-5). These include activation of the PI3K/Akt pathway, increased levels of the NF-κB transcription factor, and epigenetic silencing of genes such as APAF-1 (apoptosis protease activating factor-1 involved in activating caspase-9 and essential for apoptosis) and caspase-8. Each of these pathways is a target for therapeutic agents that, in addition to affecting cancer cell proliferation or gene expression, may render cancer cells more susceptible to apoptosis, thus promoting synergy when combined with other chemotherapeutic agents. Some tumor cells resist

drug-induced apoptosis indirectly by eliminating the noxious stimulus-inducing apoptosis through expression of one or more members of the ABC (ATP-binding cassette proteins) family of ATP-dependent efflux pumps that mediate the multidrug resistance (MDR) phenotype. The prototype member of this family, P-glycoprotein (PGP), spans the plasma membrane 12 times and has two ATP-binding sites. Hydrophobic drugs (e.g., anthracyclines and vinca alkaloids) are recognized by PGP as they enter the cell and are pumped out. Numerous clinical studies have failed to demonstrate that drug resistance can be overcome using inhibitors of PGP. However, ABC transporters have different substrate specificities, and inhibition of a single family member may not be sufficient to overcome the MDR phenotype. Efforts to reverse PGP-mediated drug resistance continue. In addition to its role in cell death, autophagy can also serve as a homeostatic mechanism to promote cell survival by recycling cellular components to provide necessary energy. The mechanisms that control the balance between enhancing survival versus leading to cell death are still not fully understood. Autophagy appears to play conflicting roles in the development and survival of cancer. Early in the carcinogenic process, it can act as a tumor suppressor by preventing the cell from accumulating abnormal proteins and organelles. However, in established tumors, it may serve as a mechanism of survival for cancer cells when they are stressed by damage such as from chemotherapy. Preclinical studies have indicated that inhibition of this process can enhance the sensitivity of cancer cells to chemotherapy or radiation therapy, and ongoing trials are evaluating inhibitors of autophagy in combination with chemotherapy and/or radiation therapy. Better understanding of the factors that control the survival-promoting versus death-inducing aspects of autophagy is required in order to know how to best manipulate it for therapeutic benefit. ■ ■

METASTASIS The metastatic process accounts for the vast majority of deaths from solid tumors, and therefore, an understanding of this process is critical for improvements in survival from cancer. The biology of metastasis is complex and requires multiple steps. The initial step involves cell migration and invasion through the ECM. The three major features of tissue invasion are cell adhesion to the basement membrane, local proteolysis of the membrane, and movement of the cell through the rent in the membrane and the ECM. Cells that lose contact with the ECM normally undergo programmed cell death (anoikis-apoptosis induced by the loss of contact), and this process has to be suppressed in cells that metastasize. Another process important for many, but not necessarily all, metastasizing epithelial cancer cells is epithelial mesenchymal transition (EMT). This is a process by which cells lose their epithelial properties and gain mesenchymal properties. This normally occurs during the developmental process in embryos, allowing cells to migrate to their appropriate destinations in the embryo. It also occurs in wound healing, tissue regeneration, and fibrotic reactions, but in all of these processes, cells stop proliferating when the process is complete. Malignant cells that metastasize often undergo EMT as an important step in that process but retain the capacity for unregulated proliferation. However, there is evidence that not all metastasizing cancer cells

require EMT, and the exact role of EMT in different metastasizing cancer cells continues to be elucidated. Malignant cells that gain access to the circulation must then repeat those steps at a remote site, find a hospitable niche in a foreign tissue, avoid detection and elimination by host defenses including the immune system, and induce the growth of new blood vessels. Some metastatic cells occur as oligoclonal clusters, which appear to be more potent in establishing metastasis than single cells, perhaps, in part, through differential and cooperative effects in evading host defenses. The rate-limiting step for metastasis is the ability for tumor cells to survive and expand in the novel microenvironment of the metastatic site, and multiple host-tumor

interactions determine the ultimate outcome (Fig. 77-6).

As is true for cells in primary cancers, there is significant heterogeneity as well as plasticity in metastatic cancer cells. In addition to actively dividing cells, a population of quiescent cells are present that can evade the immune system as well as chemotherapy targeting dividing cells. The processes that keep metastatic cancer cells quiescent as well as lead them to divide are complex, as discussed in the introductory section. Efforts to inhibit growth of metastatic cells by modulating these pathways are being explored. Few drugs have been developed to attempt to directly target the process of metastasis, in part because the specifics of the critical steps in the process that would be potentially good targets for drugs are still being identified. However, a number of potential targets are known. HER2 can enhance the metastatic potential of breast cancer cells, and as discussed above, the monoclonal antibody trastuzumab, which targets HER2, improves survival in the adjuvant setting for HER2-positive breast cancer patients. A number of other potential targets that increase metastatic potential of cells in preclinical studies include HIF-1 and -2, transcription factors induced by hypoxia within tumors, growth factors (e.g., cMET and VEGFR), oncogenes (e.g., SRC), adhesion molecules (e.g., focal adhesion kinase [FAK]), ECM proteins (e.g., matrix metalloproteinases 1 and 2), and inflammatory molecules (e.g., COX-2).

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The metastatic phenotype is likely restricted to a fraction of tumor cells (Fig. 77-6). A number of genetic and epigenetic changes are required for tumor cells to be able to metastasize, including activation of metastatic-promoting genes and inhibition of genes that suppress the metastatic ability. Given the role of microRNAs in controlling gene expression (see epigenetic section) including those critical to the metastatic process, efforts are under way to modulate these to try to inhibit metastasis. Cells with metastatic capability frequently express chemokine receptors that are likely important in the metastatic process. A number of candidate metastasis-suppressor genes have been identified, including genes coding for proteins that enhance apoptosis, suppress cell division, are involved in the interactions of cells with each other or the ECM, or suppress cell migration. The loss of function of these genes enhances metastasis. Gene expression profiling is being used to study the metastatic process and other properties of tumor cells that may predict susceptibilities. An example of the ability of malignant cells to survive and grow in a novel microenvironment is bone metastases. Bone metastases can be extremely painful, cause fractures of weight-bearing bones, can lead to hypercalcemia, and are a major cause of morbidity for cancer patients. Osteoclasts and their monocyte-derived precursors express the surface receptor RANK (receptor activator of NF- κ B), which is required for terminal differentiation and activation of osteoclasts. Osteoblasts and other stromal cells express RANK ligand (RANKL), as both a membrane-bound and soluble cytokine. Osteoprotegerin (OPG), a soluble receptor for RANKL produced by stromal cells, acts as a decoy receptor to inhibit RANK activation. The relative balance of RANKL and OPG determines the activation state of RANK on osteoclasts. Bone modulation and resorption by osteoclasts is an important component of the establishment and progression of metastases in bone. Many tumors increase osteoclast activity by secretion of substances such as parathyroid hormone (PTH), PTH-related peptide, interleukin (IL) 1, or Mip1 that perturb the homeostatic balance of bone remodeling by increasing RANK signaling. One example is multiple myeloma, where tumor cell-stromal cell interactions activate osteoclasts and inhibit osteoblasts, leading to the development of multiple lytic bone lesions.

Basement membrane Normal epithelial cells Cytokeratin Adherens junction E-cadherin Tumor cell TGF- β receptor TGF- β N-Cadherin Snail Twist HGF New integrin expression N-Cadherin C-Met PART

4 Oncology and Hematology **FIGURE 77-6** Oncogene signaling pathways are activated during tumor progression and promote metastatic potential. This figure shows a cancer cell that has undergone epithelial to mesenchymal transition (EMT) under the influence of several environmental signals. Critical components include activated transforming growth factor beta (TGF- β) and the hepatocyte growth factor (HGF)/c-Met pathways, as well as changes in the expression of adhesion molecules that mediate cell-cell and cell-extracellular matrix interactions. Important changes in gene expression are mediated by the Snail and Twist family of transcriptional repressors (whose expression is induced by the oncogenic pathways), leading to reduced expression of E-cadherin, a key component of adherens junctions between epithelial cells. This, in conjunction with upregulation of N-cadherin, a change in the pattern of expression of integrins (which mediate cell-extracellular matrix associations that are important for cell motility), and a switch in intermediate filament expression from cytokeratin to vimentin, results in the phenotypic change from adherent highly organized epithelial cells to motile and invasive cells with a fibroblast or mesenchymal morphology. EMT is thought to be an important step leading to metastasis in some human cancers. Host stromal cells, including tumor-associated fibroblasts and macrophages, play an important role in modulating tumor cell behavior through secretion of growth factors and proangiogenic cytokines, and matrix metalloproteinases that degrade the basement membrane. VEGF-A, -C, and -D are produced by tumor cells and stromal cells in response to hypoxemia or oncogenic signals and induce production of new blood vessels and lymphatic channels through which tumor cells metastasize to lymph nodes or tissues. Inhibition of RANKL by an antibody (denosumab) can prevent further bone destruction. Bisphosphonates are also effective inhibitors of osteoclast function that are used in the treatment of cancer patients with bone metastases. ■

■ **CANCER STEM CELLS** Normal tissues have stem cells capable of self-renewal and repairing damaged tissue, whereas the majority of cells in normal tissues do not have this capacity. Similarly, only a small proportion of the cells within a tumor are capable of initiating colonies in vitro or forming tumors at high efficiency when injected into immunocompromised NOD/ SCID mice. For example, AML and CML have a small population of cells (estimated to be <1%) that have properties of stem cells, such as unlimited self-renewal and the capacity to cause leukemia when serially transplanted in mice. These cells have an undifferentiated phenotype (Thy1-CD34+CD38- and do not express other differentiation markers) and resemble normal stem cells in many ways but are no longer under homeostatic control (Fig. 77-7). Solid tumors may also contain a population of stem cells. It is not yet known how often cancers may originate within a stem cell population, although a body of evidence argues that stem cells are likely involved in the development of the majority of cancers. Cancer stem cells, like their normal counterparts, have unlimited proliferative capacity and paradoxically traverse the cell cycle at a slow rate; cancer growth occurs largely due to expansion of the stem cell pool, the unregulated proliferation of an

Lamina propria Tumor-associated fibroblast New lymph vessel MMP Cytokines growth factors
 Tumor-associated macrophage Invasion New blood vessel VEGF-A **HOST STROMAL CELLS**
 amplifying population, and failure of apoptosis pathways (Fig. 77-7). Slow cell cycle progression and high levels of expression of antiapoptotic Bcl-2 family members and drug efflux pumps of the MDR family render cancer stem cells less vulnerable to cancer chemotherapy or radiation therapy. Implicit in the cancer stem cell hypothesis is the idea that failure to cure most human cancers is due to the fact that current therapeutic agents are not very effective in killing stem cells. Efforts are ongoing to identify and isolate cancer stem cells from different types of malignancies, which should allow determination of the aberrant signaling pathways that distinguish these cells from

normal tissue stem cells. These would serve as potential therapeutic targets. Evidence that cells with stem cell properties can arise from other epithelial cells within the cancer by processes such as epithelial mesenchymal transition also implies that it is essential to treat all of the cancer cells, and not just those with current stem cell-like properties, in order to eliminate the self-renewing cancer cell population. The exact nature of cancer stem cells remains an area of investigation. One of the unanswered questions is the exact origin of cancer stem cells for the different cancers.

PLASTICITY AND RESISTANCE Cancer cells, and especially stem cells, have the capacity for significant plasticity, allowing them to alter multiple aspects of cell biology in response to external factors (e.g., chemotherapy, radiation therapy, inflammation, immune response). In addition, heterogeneity between

NORMAL TISSUE **CANCER Stem Cells** Stem cell niche Paracrine signals Polarized division Daughter cell Stem cell Transit-amplifying cells Exponential growth Regulated activation of differentiation program Loss of self-renewal capacity Multilineage differentiation Growth arrest Maintenance of tissue architecture and homeostasis

FIGURE 77-7 Cancer stem cells play a critical role in the initiation, progression, and resistance to therapy of malignant neoplasms. In normal tissues (left), homeostasis is maintained by asymmetric division of stem cells, leading to one progeny cell that will differentiate and one cell that will maintain the stem cell pool. This occurs within highly specific niches unique to each tissue, such as in close apposition to osteoblasts in bone marrow, or at the base of crypts in the colon. Here, paracrine signals from stromal cells, such as sonic hedgehog or Notch ligands, as well as upregulation of β -catenin and telomerase, help to maintain stem cell features of unlimited self-renewal while preventing differentiation or cell death. This occurs in part through upregulation of the transcriptional repressor Bmi-1 and inhibition of the p16Ink4a/Arf and p53 pathways. Daughter cells leave the stem cell niche and enter a proliferative phase (referred to as transit-amplifying) for a specified number of cell divisions, during which time a developmental program is activated, eventually giving rise to fully differentiated cells that have lost proliferative potential. Cell renewal equals cell death, and homeostasis is maintained. In this hierarchical system, only stem cells are long-lived. The hypothesis is that cancers harbor stem cells that make up a small fraction (i.e., 0.001–1%) of all cancer cells. These cells share several features with normal stem cells, including an undifferentiated phenotype, unlimited self-renewal potential, and a capacity for some degree of differentiation; however, due to initiating mutations (mutations are indicated by lightning bolts), they are no longer regulated by environmental cues. The cancer stem cell pool is expanded, and rapidly proliferating progeny, through additional mutations, may attain stem cell properties, although most of this population is thought to have a limited proliferative capacity. Differentiation programs are dysfunctional due to reprogramming of the pattern of gene transcription by oncogenic signaling pathways. Within the cancer transit-amplifying population, genomic instability generates aneuploidy and clonal heterogeneity as cells attain a fully malignant phenotype with metastatic potential. The cancer stem cell hypothesis has led to the idea that current cancer therapies may be effective at killing the bulk of tumor cells but do not kill tumor stem cells, leading to a regrowth of tumors that is manifested as tumor recurrence or disease progression. Research is in progress to identify unique molecular features of cancer stem cells that can lead to their direct targeting by novel therapeutic agents. the different clones of cells within the tumor population and their interactions with each other and the tumor microenvironment provides the tumor with the capacity for significant plasticity in dealing with both internal and external stresses. Thus, a major problem in cancer therapy is that malignancies have a wide spectrum of mechanisms for both initial and adaptive resistance to treatments. These include inhibiting drug

delivery to the cancer cells, blocking drug uptake and retention, increasing drug metabolism, altering levels of target proteins making them less sensitive to drugs, acquiring mutations in target proteins making them no longer sensitive to the drug, modifying metabolism and cell signaling pathways, using alternate signaling pathways, adjusting the cell replication process including mechanisms by which the cell deals with DNA damage, inhibiting apoptosis, and evading the immune system. Thus, most metastatic cancers (except those curable with chemotherapy such as germ cell tumors) eventually become resistant to the therapy being utilized. Overcoming resistance is a major area of research. ■ ■ **CANCER METABOLISM** One of the distinguishing characteristics of cancer cells is that they have altered metabolism as compared with normal cells in supporting survival, their high rates of proliferation, and ability to metastasize. Complicating studies evaluating metabolic differences between normal and malignant cells is that there is heterogeneity in metabolism between different cells within a cancer. Malignant cells must focus a significant fraction of their energy resources into synthesis of proteins and other molecules (building blocks required for the production of new cells) while still maintaining sufficient ATP production to survive and grow.

Differentiation Cancer Stem Cells Altered or expanded stem cell niche Initiating mutations Transiently amplifying cells Exponential growth Altered transcription program Differentiation arrest Genetic instability Secondary mutations Limited self-renewal capacity Partial differentiation No growth arrest

CHAPTER 77 Loss of tissue architecture and homeostasis control Cancer Cell Biology

Although normal proliferating cells also have similar needs, there are differences in how cancer cells metabolize glucose and a number of other compounds including the amino acid glutamine as compared to normal cells in part because of genetic and epigenetic changes within cancer cells but also likely due to differences in the environments of cancer and normal cells. Many cancer cells utilize aerobic glycolysis (the Warburg effect) (Fig. 77-8) to metabolize glucose, leading to increased lactic acid production, whereas normal cells utilize oxidative phosphorylation in mitochondria under aerobic conditions, a much more efficient process for generating ATP for energy utilization but one that does not produce the same level of building blocks needed for new cells. One consequence is increased glucose uptake and utilization by cancer cells, a fact utilized in fluorodeoxyglucose (FDG)-positron emission tomography (PET) scanning to detect tumors. A number of proteins in cancer cells, including cMYC, HIF1, RAS, p53, pRB, and AKT, are involved in modulating glycolytic processes and controlling the Warburg effect. Although these pathways overall remain difficult to target therapeutically, some progress has been made in targeting HIF1 and the RAS pathways with inhibitors approved to treat cancers with mutations in HIF1 α or KRAS12C. In addition, both the PI3K pathway with signaling through mTOR and the AMP-activated kinase (AMPK) pathway that inhibits mTORC1 (a protein complex that includes mTOR) are important in controlling the glycolytic process and thus provide potential targets for inhibiting this process. An inhibitor of mTOR is approved for use against RCC (temsirolimus), and another inhibitor (everolimus) has activity against breast and neuroendocrine cancer and RCC. Other mTOR inhibitors are in trials, and modulators of AMPK are being investigated. The inefficient utilization of glucose

Differentiated tissue Tumor Proliferative tissue or +O₂ -O₂ +/-O₂ Glucose Glucose Glucose
 Pyruvate Pyruvate Pyruvate O₂ O₂ Lactate Lactate Lactate CO₂ Anaerobic glycolysis 2 mol ATP/
 mol glucose Oxidative phosphorylation -36 mol ATP/ mol glucose

FIGURE 77-8 Warburg versus oxidative phosphorylation. In most normal tissues, the vast majority of cells are differentiated and

dedicated to a particular function within the organ in which they reside. The metabolic needs are mainly for energy and not for building blocks for new cells. In these tissues, ATP is generated by oxidative phosphorylation that efficiently generates about 36 molecules of ATP for each molecule of glucose metabolized. By contrast, proliferative tumor tissues, especially in the setting of hypoxia, a typical condition within tumors, use aerobic glycolysis to generate energy for cell survival and generation of building blocks for new cells. PART 4 Oncology and Hematology by malignant cells also leads to a need for alternative metabolic pathways for other compounds as well, one of which is glutamine. Similar to glucose, this provides both a source for structural molecules as well as energy production. Similarly to glucose, glutamine is also inefficiently utilized by cancer cells. Cancer cells can also take up nutrients released by surrounding cells and tissues, increasing the complexity of successfully therapeutically inhibiting metabolism in cancer. Mutations in genes involved in the metabolic process occur in a number of cancers. Among the most frequently found to date are mutations in isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2). These have been most commonly seen in gliomas, AMLs, and intrahepatic cholangiocarcinomas. These mutations lead to the production of an oncometabolite (2-hydroxyglutarate [2HG]) instead of the normal product α -ketoglutarate. Although the exact mechanisms of oncogenesis by 2HG are still being elucidated, α -ketoglutarate is a key cofactor for a number of dioxygenases involved in controlling DNA methylation. 2HG can act as a competitive inhibitor for α -ketoglutarate, leading to alterations in methylation status (primarily hypermethylation) of genes (leading to epigenetic changes) that can have profound effects on a number of cellular processes including differentiation. Inhibitors of mutant IDH1 and/or IDH2 are approved for treating IDH mutant AML and cholangiocarcinoma; and a dual IDH1/2 inhibitor is approved for treatment of low-grade gliomas and astrocytomas with IDH mutations. Much needs to be learned about the specific differences in metabolism between cancer cells and normal cells in order to develop more effective approaches to using these differences therapeutically; however, even with the currently limited state of knowledge, modulators of metabolism are being tested clinically. One of these is the antidiabetic agent metformin, both alone and in combination with chemotherapeutic agents. Metformin inhibits gluconeogenesis and may have direct effects on tumor cells by activating AMPK, a serine/threonine protein kinase that is downstream of the LKB1 tumor suppressor, and thus inhibiting mTOR complex 1 (mTORC1). This leads to decreased protein synthesis and proliferation. Studies to date have not yet established metformin to have a clear role as an anticancer agent. ■

■ **TUMOR MICROENVIRONMENT, ANGIOGENESIS, AND IMMUNE EVASION** Tumors consist not only of malignant cells but also of a complex microenvironment including many other types of cells (including lymphocytes, macrophages, myeloid cells; other inflammatory cells;

5% 85% CO₂ Aerobic glycolysis (Warburg effect) –4 mol ATP/mol glucose vascular cells, lymphatic endothelial cells, nerve cells, fibroblasts, and fat cells), ECM, stroma, secreted factors (including growth factors and hormones), reactive oxygen and nitrogen species, mechanical factors, blood vessels, and lymphatics. There is extensive cross-talk between the cells with each other, the ECM, and the various secreted factors within the tumor microenvironment. This microenvironment is not static but rather is dynamic and continually evolving. Both the complexity and dynamic nature of the microenvironment enhance the difficulty of treating tumors. The microenvironment is involved in altered tumor metabolism, tumor maintenance, growth, phenotypic plasticity, metastasis, and immune escape, and can contribute to resistance to anticancer therapies through a number of mechanisms. These include immune evasion by a variety of mechanisms including suppression of effector T cells, increase in regulatory T cells, induction of an inflammatory environment, and

altered vasculature that inhibits effector T-cell access to malignant cells. Similarly, it contributes to drug resistance through multiple mechanisms, alteration in metabolic pathways including creating a hypoxic and acidic environment, vascular and mechanical factors that limit drug access to malignant cells, various secreted factors that inhibit apoptosis or stimulate survival pathways, and generation of ROS that enhance drug resistance. Multiple additional mechanisms are also involved in enhancing resistance to immune-mediated anticancer effects and anticancer drug therapy. ■

■ **OBESITY AND CANCER** Significant evidence links obesity and the increased risk of developing certain cancers including postmenopausal breast, colorectal, ovarian, endometrial, esophageal, gallbladder, thyroid, and kidney cancers, among others. Less certain are the mechanisms responsible for this risk. As outlined above, cancers arise in an environment with multiple factors, many of which can stimulate cell proliferation. Obesity impacts a variety of factors including hormonal factors, altered metabolism (especially adipose metabolism), and mediators of inflammatory response that all can impact the development of malignancy. Obesity is associated with a number of hormonal changes including high insulin, glucagon, and leptin levels that can stimulate growth of cells. It also leads to insulin resistance, which may contribute to cancer cell development, in part by increasing insulin-like growth factor-1 (IGF-1) levels. Obesity also leads to alterations in adipose, including fatty acid, metabolism with production of compounds important for energy metabolism as well as for membrane function within cells that

may contribute to carcinogenic process. Obesity contributes to an inflammatory environment in a variety of ways including increased levels of inflammatory proteins such as IL-6 and TNF- α . In terms of impact on survival with cancer, data primarily from breast cancer suggest that obesity is associated with decreased survival likely due, at least in part, to the impact of obesity on hormonal factors in development of certain breast cancers, although this may be limited to subsets of breast cancer patients. Some studies have suggested, paradoxically, that obesity may be associated with improved survival in some patients such as those with advanced-stage colorectal cancer. Furthermore, immune checkpoint inhibitor therapy has appeared to be more effective in obese patients.

Clearly, the biology of the association between obesity and cancer and its impact on disease outcome is complex, and additional studies are necessary to better define the mechanisms involved. **Vascular mimicry—tumor cells as part of vessel wall** Tumor ■ ■ **MECHANISMS OF TUMOR VESSEL FORMATION** One of the critical elements of tumor cell proliferation is delivery of oxygen, nutrients, and circulating factors important for growth and survival. Thus, a critical element in growth of primary tumors and formation of metastatic sites is the angiogenic switch: the ability of the tumor to promote the formation of new blood vessels, including the recruitment of vascular endothelial cells (ECs). The angiogenic switch is a phase in tumor development when the dynamic balance of pro- and antiangiogenic factors is tipped in favor of vessel formation by the effects of the tumor on its immediate environment. Stimuli for tumor angiogenesis include hypoxemia, inflammation, and genetic lesions in oncogenes or tumor suppressors that alter tumor cell gene expression. Angiogenesis consists of several steps, including the stimulation of ECs by growth factors, degradation of the ECM by proteases, proliferation and migration of ECs into the tumor, and the eventual formation of new capillary tubes. Tumors use a number of mechanisms to promote vascularization, subverting normal angiogenic processes for this purpose (Fig. 77-9). Primary or metastatic tumor cells sometimes arise in proximity to host blood vessels and grow around these vessels, parasitizing nutrients by co-opting the local blood supply. However, most tumor blood vessels arise by the process of sprouting, in which tumors secrete trophic angiogenic molecules, the most potent being vascular endothelial growth factors (VEGFs), that induce the

proliferation and migration of host ECs into the tumor. Sprouting in normal and pathologic angiogenesis is regulated by three families of transmembrane RTKs expressed on ECs and their ligands (VEGFs, angiopoietins, and ephrins; Fig. 77-10), which are produced by tumor cells, inflammatory cells, or stromal cells in the tumor microenvironment. FIGURE 77-9 Tumor angiogenesis is a complex process involving many different cell types that must proliferate, migrate, invade, and differentiate in response to signals from the tumor microenvironment. Endothelial cells (ECs) sprout from host vessels in response to VEGF, bFGF, Ang2, and other proangiogenic stimuli. Sprouting is stimulated by VEGF/VEGFR2, Ang2/Tie-2, and integrin/extracellular matrix (ECM) interactions. Bone marrow-derived circulating endothelial precursors (CEPs) migrate to the tumor in response to VEGF and differentiate into ECs, while hematopoietic stem cells differentiate into leukocytes, including tumor-associated macrophages that secrete angiogenic growth factors and produce matrix metalloproteinases (MMPs) that remodel the ECM and release bound growth factors. Tumor cells themselves may directly form parts of vascular channels within tumors. The pattern of vessel formation is haphazard: vessels are tortuous, dilated, leaky, and branch in random ways. This leads to uneven blood flow within the tumor, with areas of acidosis and hypoxemia (which stimulate release of angiogenic factors) and high intratumoral pressures that inhibit delivery of therapeutic agents. Central to the angiogenic response are hypoxia-inducible factors (HIFs; especially 1 and 2), which are transcription factors that normally, in response to hypoxia, stimulate the transcription of a large number of genes responsive to hypoxia, including genes involved in metabolism as well as angiogenesis. HIF1 has a bigger role in stimulating metabolism (glycogenesis), whereas HIF2 plays a bigger role in angiogenesis. HIF protein function can also be enhanced in a number of ways in cancer not involving hypoxia, including mutations in the von Hippel-Lindau tumor suppressor gene (an E3 ubiquitin ligase that controls HIF levels by targeting it for degradation), such as occurs in some RCCs. Among

CEP contributes newly differentiated EC HSC-derived macrophage Tumor Leaky vessels Tumor Region of hypoxemia 100 μ m High intratumoral pressure Dilated leaky tumor vessel VEGF VEGF VEGFR2 VEGFR2 Destabilization Tie 2 Tie 2 Ang2 α v β 2 α v β 3 α v β 5 α 5 β 1 α 5 β 1 ECM Tie 2 New sprout Follows VEGF gradient to tumor CHAPTER 77 Migrate to tumor Host blood vessel VEGFR2 CEP CD133 Cancer Cell Biology Bone marrow-derived cells (from hemangioblast) VEGFR1 HSC c-kit Tumor cells Host EC Tumor EC Circulating endothelial precursors (CEP) Hematopoietic cell-derived leukocytes (HSC) the genes stimulated by HIF are VEGF and VEGF receptors. VEGFs and their receptors are required for embryonic vasculogenesis (development of new blood vessels when none preexist) and normal (wound healing, corpus luteum formation) and pathologic angiogenesis (tumor angiogenesis, inflammatory conditions such as rheumatoid arthritis). VEGF-A is a heparin-binding glycoprotein with at least four isoforms (splice variants) that regulates blood vessel formation by binding to the RTKs VEGFR1 and VEGFR2, which are expressed on all ECs in addition to a subset of hematopoietic cells (Fig. 77-9). VEGFR2 plays a more direct role in regulating EC proliferation, migration, and survival, whereas VEGFR1 appears to have more nuanced functions with a less direct role in stimulating EC processes in the normal adult (even acting as a decoy protein for VEGFA to decrease binding to VEGFR2) but with important effects during embryogenesis and on tumor angiogenesis. Tumor vessels may be more dependent on VEGFR signaling for growth and survival than normal ECs.

Endothelial cell–“specific” ligand/receptor complexes PIGF VEGF-A VEGF-B VEGF-C Ang-1 Ang-2 Ephrins Extracellular matrix bFGF PDGF Kinase domain $\alpha\text{v}\beta 3$ Matrix (attachment) EPHB4 (Artery/vein differentiation, vessel remodeling) VEGFR1 VEGFR2 VEGFR3 (Endothelial cells) (Lymphatics) Tie-2 (Blood vessel stabilization and remodeling) Downstream pathways Ras/MAPK PI3K/AKT Rho/Rac/cdc42 NF κ B PART 4 Oncology and Hematology Endothelial cell proliferation, migration, survival FIGURE 77-10 Critical molecular determinants of endothelial cell biology.

Angiogenic endothelium expresses a number of receptors not found on resting endothelium. These include receptor tyrosine kinases (RTKs) and integrins that bind to the extracellular matrix and mediate endothelial cell (EC) adhesion, migration, and invasion. ECs also express RTKs (i.e., the fibroblast growth factor [FGF] and platelet-derived growth factor [PDGF] receptors) that are found on many other cell types. Critical functions mediated by activated RTK include proliferation, migration, and enhanced survival of endothelial cells, as well as regulation of the recruitment of perivascular cells and bloodborne circulating endothelial precursors and hematopoietic stem cells to the tumor. Intracellular signaling via EC-specific RTK utilizes molecular pathways that may be targets for future antiangiogenic therapies. While VEGF signaling is a critical initiator of angiogenesis, this is a complex process regulated by additional signaling pathways (Fig. 77-10). The angiopoietin, Ang1, produced by stromal cells, binds to the EC RTK Tie-2 and promotes the interaction of ECs with the ECM and perivascular cells, such as pericytes and smooth-muscle cells, to form tight, nonleaky vessels. PDGF and basic fibroblast growth factor (bFGF) help to recruit these perivascular cells. Ang1 is required for maintaining the quiescence and stability of mature blood vessels and prevents the vascular permeability normally induced by VEGF and inflammatory cytokines. For tumor cell-derived VEGF to initiate sprouting from host vessels, the stability conferred by the Ang1/Tie2 pathway must be perturbed; this occurs by the secretion of Ang2 by ECs that are undergoing active remodeling. Ang2 binds to Tie2 and is a competitive inhibitor of Ang1 action: under the influence of Ang2, preexisting blood vessels become more responsive to remodeling signals, with less adherence of ECs to stroma and associated perivascular cells and more responsiveness to VEGF. Therefore, Ang2 is required at early stages of tumor angiogenesis for destabilizing the vasculature by making host ECs more sensitive to angiogenic signals. In the presence of Ang2, there is no stabilization by the Ang1/Tie2 interaction, and tumor blood vessels are leaky, hemorrhagic, and have poor association of ECs with underlying stroma. Sprouting tumor ECs express high levels of the transmembrane protein ephrin-B2 and its receptor, the RTK EPH, whose signaling appears to work with the angiopoietins during vessel remodeling. During embryogenesis, EPH receptors are expressed on the endothelium of primordial venous vessels while the transmembrane ligand ephrin-B2 is expressed by cells of primordial arteries; the reciprocal expression may regulate differentiation and patterning of the vasculature. A number of additional ubiquitously expressed host molecules play critical roles in normal and pathologic angiogenesis. Proangiogenic

cytokines, chemokines, and growth factors secreted by stromal cells or inflammatory cells make important contributions to neovascularization, including bFGF, transforming growth factor- β (TGF- β), TNF- α , and IL-8. In contrast to normal endothelium, angiogenic endothelium overexpresses specific members of the integrin family of ECM-binding proteins that mediate EC adhesion, migration, and survival. Specifically, expression of integrins $\alpha\text{v}\beta 3$, $\alpha\text{v}\beta 5$, and $\alpha 5\beta 1$ mediates spreading and migration of ECs and is required for angiogenesis induced by VEGF and bFGF, which in turn can upregulate EC integrin expression. The $\alpha\text{v}\beta 3$ integrin physically associates with VEGFR2 in the plasma membrane and promotes signal transduction from each receptor to promote EC proliferation

tion (via focal adhesion kinase, src, PI3K, and other pathways) and survival (by inhibition of p53 and increasing the Bcl-2/Bax expression ratio). In addition, $\alpha v\beta 3$ forms cell-surface complexes with matrix metalloproteinases (MMPs), zinc-requiring proteases that cleave ECM proteins, leading to enhanced EC migration and the release of heparin-binding growth factors, including VEGF and bFGF. EC adhesion molecules can be upregulated (i.e., by VEGF, TNF- α) or downregulated (by TGF- β); this, together with chaotic blood flow, explains poor leukocyte-endothelial interactions in tumor blood vessels and may help tumor cells avoid immune surveillance. Generalized growth factor receptors FGF receptor PDGF receptor (Recruitment of smoothmuscle cells and pericytes) Tumor blood vessels are not normal; they have chaotic architecture and blood flow. Due to an imbalance of angiogenic regulators such as VEGFs and angiopoietins (see below), tumor vessels are tortuous and dilated with an uneven diameter, excessive branching, and shunting. Tumor blood flow is variable, with areas of hypoxemia and acidosis leading to the selection of cancer cell variants that are resistant to hypoxemia-induced apoptosis (often involving the loss of p53 expression). Tumor vessel walls have numerous openings, widened interendothelial junctions, and discontinuous or absent basement membrane. This contributes to the high permeability of these vessels and, together with lack of functional intratumoral lymphatics, causes increased interstitial pressure within the tumor (which also interferes with the delivery of therapeutics to the tumor; Figs. 77-9, 77-10, and 77-11). Tumor blood vessels have a deficit of perivascular cells such as pericytes and smooth-muscle cells that normally regulate flow in response to tissue metabolic needs. Unlike normal blood vessels, the vascular lining of tumor vessels is not a homogeneous layer of ECs but often consists of a mosaic of ECs and tumor cells, which, because of their plasticity, can upregulate expression of genes normally only seen in ECs under hypoxic conditions. These cancer cell-derived vascular channels, which may be lined by ECM secreted by the tumor cells, are referred to as vascular mimicry. During tumor angiogenesis, ECs are highly proliferative and express a number of plasma membrane proteins that are characteristic of activated endothelium, including growth factor receptors and adhesion molecules such as integrins. These abnormalities in tumor vasculature provide potential differential sensitivities from normal vessels to approaches inhibiting the process, allowing for the use of antiangiogenic agents in cancer treatment. Lymphatic vessels also exist within tumors. Development of tumor lymphatics is associated with expression of VEGFR3 and its ligands VEGF-C and VEGF-D. The role of these vessels in tumor cell metastasis to regional lymph nodes remains to be determined. However, VEGF-C levels correlate significantly with metastasis to regional lymph nodes in lung, prostate, and colorectal cancers.

A. Normal blood vessel Low IP Normoxic Physiologic pH Hierarchical branching Even blood distribution Lumen EC BM BM Pericytes Tight junctions between EC Well-formed BM Pericyte coverage Normal permeability C. Treatment with bevacizumab (Early) D. Treatment with bevacizumab (Late) Low IP Less hypoxemia Less acidosis Normalization of vessels Improved blood flow Lumen EC BM Pericytes More efficient delivery of chemotherapy and oxygen Reduced permeability Death of EC due to loss of VEGF survival signals (plus chemotherapy or radiotherapy) Apoptosis of tumor due to starvation and/or effects of chemotherapy FIGURE 77-11 Normalization of tumor blood vessels due to inhibition of VEGF signaling. A. Blood vessels in normal tissues exhibit a regular hierarchical branching pattern that delivers blood to tissues in a spatially and temporally efficient manner to meet the metabolic needs of the tissue (top). At the microscopic level, tight junctions are maintained between endothelial cells (ECs), which are adherent to a thick and evenly distributed basement membrane (BM). Pericytes form a surrounding layer that provides trophic signals to the EC and helps maintain proper vessel tone. Vascular permeability is regulated,

interstitial fluid pressure (IP) is low, and oxygen tension and pH are physiologic. B. Tumors have abnormal vessels with tortuous branching and dilated, irregular interconnecting branches, causing uneven blood flow with areas of hypoxemia and acidosis. This harsh environment selects genetic events that result in resistant tumor variants, such as the loss of p53. High levels of VEGF (secreted by tumor cells) disrupt gap junction communication, tight junctions, and adherens junctions between EC via src-mediated phosphorylation of proteins such as connexin 43, zonula occludens-1, VE-cadherin, and α/β -catenins. Tumor vessels have thin, irregular BM, and pericytes are sparse or absent. Together, these molecular abnormalities result in a vasculature that is permeable to serum macromolecules, leading to high tumor interstitial pressure, which can prevent the delivery of drugs to the tumor cells. This is made worse by the binding and activation of platelets at sites of exposed BM, with release of stored VEGF and microvessel clot formation, creating more abnormal blood flow and regions of hypoxemia. C. In experimental systems, treatment with bevacizumab or blocking antibodies to VEGFR2 leads to changes in the tumor vasculature that have been termed vessel normalization. During the first week of treatment, abnormal vessels are eliminated or pruned (dotted lines), leaving a more normal branching pattern. ECs partially regain features such as cell-cell junctions, adherence to a more normal BM, and pericyte coverage. These changes lead to a decrease in vascular permeability, reduced interstitial pressure, and a transient increase in blood flow within the tumor. Note that in murine models, this normalization period lasts only for ~5–6 days. D. After continued anti-VEGF/VEGFR therapy (which is often combined with chemo- or radiotherapy), ECs die, leading to tumor cell death (either due to direct effects of the chemotherapy or lack of blood flow). ■ ■ ANTIANGIOGENIC THERAPY Angiogenesis inhibitors function by targeting the critical molecular pathways involved in EC proliferation, migration, and/or survival, many of which are highly expressed in the activated endothelium in tumors. Inhibition of growth factor and adhesion-dependent signaling

B. Tumor blood vessel High IP High VEGF Hypoxemia Acidosis Tortuous vessels Haphazard blood flow Lumen EC Tumor cells Loss of EC junction complexes Irregular or no BM Absent (or few) pericyte Increased permeability CHAPTER 77 Collapse of tumor vasculature Cancer Cell Biology Lumen EC BM Tumor cells pathways can induce EC apoptosis with concomitant inhibition of tumor growth. Different types of tumors can use distinct combinations of molecular mechanisms to activate the angiogenic switch. Therefore, it is doubtful that a single antiangiogenic strategy will suffice for all human cancers; rather, a number of agents or combinations of agents

Ang 1 Ang 2 Novel inhibitors Anti-VEGF MoAb VEGF VEGFR2 Kinase domain Tie2 receptor Enhanced binding to ECM, vessel stabilization Specific kinase inhibitors Proliferation survival migration Anti-integrin MoAb, RGD peptides $\alpha\beta3$ $\alpha\beta5$ $\alpha5\beta1$ Nucleus Microtubules Extracellular matrix (ECM) 2-Methoxy estradiol MMPs (invasion, growth factor release) MMP inhibitors PART 4 Oncology and Hematology FIGURE 77-12 Knowledge of the molecular events governing tumor angiogenesis has led to a number of therapeutic strategies to block tumor blood vessel formation. The successful therapeutic targeting of VEGF and its receptors VEGFR is described in the text. Other endothelial cell (EC)-specific receptor tyrosine kinase pathways (e.g., angiopoietin/Tie2 and ephrin/EPH) are likely targets for the future. Ligation of the $\alpha\beta3$ integrin is required for EC survival. Integrins are also required for EC migration and are important regulators of matrix metalloproteinase (MMP) activity, which modulates EC movement through the ECM as well as release of bound growth factors. Targeting of integrins includes development of blocking antibodies, small peptide inhibitors of integrin signaling, and arg-gly-asp-containing peptides that prevent integrin:ECM binding.

Peptides derived from normal proteins by proteolytic cleavage, including endostatin and tumstatin, inhibit angiogenesis by mechanisms that include interfering with integrin function. Signal transduction pathways that are dysregulated in tumor cells indirectly regulate EC function. Inhibition of EGF-family receptors, whose signaling activity is upregulated in a number of human cancers (e.g., breast, colon, and lung cancers), results in downregulation of VEGF and IL-8, while increasing expression of the antiangiogenic protein thrombospondin-1. The Ras/MAPK, PI3K/Akt, and Src kinase pathways constitute important antitumor targets that also regulate the proliferation and survival of tumor-derived EC. The discovery that ECs from normal tissues express tissue-specific “vascular addressins” on their cell surface suggests that targeting specific EC subsets may be possible. will be needed, depending on distinct programs of angiogenesis used by different human cancers. Despite this, experimental data indicate that for some tumor types, blockade of a single growth factor (e.g., VEGF) may inhibit tumor-induced vascular growth. Bevacizumab, an antibody that binds circulating VEGF, modestly potentiates the effects of a number of different types of active chemo therapeutic regimens used to treat a variety of different tumor types including colon, lung, ovarian, and cervical cancers. It also has some activity in combination with immunotherapy against RCCs and alone for glioblastomas. Other protein inhibitors of the VEGF signaling path way approved for anticancer therapy include ramucirumab (a mono clonal antibody directed against VEGFR2, approved for use against gastric/gastroesophageal, colon, and lung cancers) and ziv-aflibercept (a recombinant protein inhibitor of VEGF, approved for colorectal cancer). Hypertension is the most common side effect of inhibitors of VEGF (or its receptors) but can be treated with antihypertensive agents and uncommonly requires discontinuation of therapy. Rare but seri ous potential risks include arterial thromboembolic events, including stroke and myocardial infarction, hemorrhage, bowel perforation, and inhibition of wound healing. Several small-molecule inhibitors (SMIs) that target VEGF RTK activity but are also inhibitory to other kinases have also been approved to treat certain cancers. Sunitinib (see above and Table 77-2) has activ ity directed against mutant c-Kit receptors (approved for GIST), but also targets VEGFR and PDGFR, and has antitumor activity against pancreatic neuroendocrine and metastatic RCCs, presumably on the basis of its antiangiogenic activity. Similarly, sorafenib, originally developed as a Raf kinase inhibitor but with potent activity against VEGFR and PDGFR, has activity against RCC, differentiated thyroid and hepatocellular cancers, and desmoid tumors. A closely related mol ecule to sorafenib, regorafenib, has activity against colorectal cancer,

Stromal cell Novel inhibitors EPH receptor Ephrin-B2 Endothelial cell GIST, gastric, and hepatocellular cancers. Other inhibitors of the VEGF pathway approved for the treatment of various cancers include axitinib, pazopanib, lenvatinib, and cabozantinib. Antiangiogenic agents have been particularly effective against RCC for which angiogenic factors are important for its development and growth. The modest success in targeting tumor angiogenesis against most other cancers has led to enhanced enthusiasm for the develop ment of drugs that target other aspects of the angiogenic process; some of these therapeutic approaches are outlined in Fig. 77-12. An inhibitor of HIF2- α has sufficient antitumor activity against RCC, pancreatic neuroendocrine tumors, and hemangioblastomas develop ing in patients with germline VHL mutations to be approved for these indications. Evidence of enhanced activity has been seen when anti-VEGF agents are used in combination with immunomodulators including immune checkpoint inhibitors. Examples of approved combinations include durvalumab plus bevacizumab for HCC and lenvatinib plus pembrolizumab for endometrial cancer and RCC. ■ ■EVASION OF THE IMMUNE SYSTEM BY CANCERS The immune system plays a critical role in maintaining organismal integrity including by

defending against pathogens as well as preventing and limiting the growth of cancers. There is a complex interaction between cancer and the host from the development of the first malignant cell to the establishment of a clinical cancer and its subsequent growth, invasion, and metastasis. The immune system plays a critical role in the prevention of cancer development. This is exemplified by the increased risk for cancer development in individuals who are significantly immunosuppressed, such as by inherited defects in mechanisms important for immune function, the immunosuppression necessary to maintain allogeneic organ transplants, and immunosuppression seen

from certain infections such as human immunodeficiency virus. It also plays a critical role in inhibiting the process of cells metastasizing as well as growth of metastatic cells at the sites of metastasis. There are two components of the immune system. The first is innate immunity (present in the organism and not dependent on prior exposure to a specific antigen, such as those present in a pathogen or malignant cell), which tends to be general and not specific and a stimulus is not remembered if encountered again. The second is the adaptive immune component, which depends on the innate immune process for activation and provides the specificity to the response with significant expansion of cells to target the specific antigens present on the pathogen or malignant cell and memory of the encounter such that exposure to the same stimulus elicits an even more rapid and vigorous response. Thus, while the innate process provides the first line of defense, the adaptive process is necessary for the specificity of response and providing memory to more rapidly attack cells should the pathogen infection recur or the malignant cells grow. The immune system has to be tightly regulated to allow for clearance of unwanted antigens while preventing an immune-mediated attack on the self. (See Chap. 360 for details on the function of the immune system.) Not surprisingly, since cancers arise from normal cells within the body that have a variety of processes to prevent harm or destruction by the immune system, they have a variety of mechanisms that allow them to evade detection and elimination by the immune system. These include downregulation of cell surface proteins involved in immune recognition (including MHC proteins and tumor-specific antigens), expression of other cell surface proteins that inhibit immune function (including members of the B7 family of proteins such as PD-L1), secretion of proteins and other molecules that are immunosuppressive such as TGF- β , recruitment and expansion of immunosuppressive cells such as regulatory T cells (which are important for maintaining tolerance against self-antigens), induction of T-cell tolerance, and downregulation of death receptors. Due to the marked heterogeneity of cells within a cancer, as well as the complexity and dynamic changes in the tumor microenvironment, a variety of immune-suppressive mechanisms are continuously occurring and changing. In addition, the inflammatory effects of some of the immune mediator cells in the tumor microenvironment (including tissue-associated macrophages and myeloid-derived suppressor cells) can suppress effector T-cell responses against the tumor as well as stimulate inflammation that can enhance tumor growth. There are marked differences in the way different malignancies respond to current immunotherapeutic approaches. For example, melanomas, RCC, Merkel cell carcinomas, cancers with defects in DNA repair associated with microsatellite instability with accumulation of gene mutations, and lymphomas (including Hodgkin's) respond well to current immunotherapeutic approaches, whereas microsatellite-stable pancreatic and colon cancers do not. While there is not a complete understanding of why these differences exist and many factors both within the cancer cells and in the microenvironment may play a role, several factors have been identified that appear to be important. These include the number of mutations present in the tumor (tumor mutational burden), presence of increased

neoantigens, expression of immune checkpoint proteins (e.g., PD-L1 for anti-PD-1 or anti-PD-L1 therapy), density of tumor-infiltrating lymphocytes, and host genetic factors. One of these (PD-L1 expression by the tumor) has sufficient predictive value for certain tumors (e.g., non-small-cell lung cancer or gastroesophageal cancers) to be used in making treatment decisions regarding the use of antibodies targeting PD-1 or PD-L1. However, neither PD-L1 expression nor any other marker can predict responsiveness of most tumors to immunotherapy. Better biomarkers that define potential responsiveness of specific cancers to immunotherapy are badly needed. A major area of research is to try to identify approaches that would convert cancers that are not responsive to immunotherapy to being responsive. Immunotherapy approaches to treat cancer can be divided into those aimed at activating the immune response and those designed to release the brakes that prevent an effective immune response against tumors. Releasing the brakes is also important for maintaining the

effectiveness of approaches that activate the immune response since, given the normally tight regulation of immune function, activation induces changes in the braking system to prevent the immune system from damaging normal tissues. Approaches at activating the immune response against cancer including using immunostimulatory molecules such as interferons, IL-2, and especially monoclonal antibodies have had success in treating a number of different cancers. For example, antibodies that target molecules highly expressed on certain cancers, such as CD20 on malignant B cells or HER2 on a variety of cancers including breast and gastroesophageal cancers, which activate the immune response locally against those malignancies, have proven highly effective.

A more direct approach to enhance the activity of T cells directed against specific tumors involves isolating T cells from patients and reengineering the cells to express chimeric antigen receptors (CAR-T) that recognize antigens present on the cells of that individual's tumor. The most commonly used approach to date has been to engineer the cells to express receptors targeting the CD19 antigen on ALL, diffuse large B-cell lymphoma (DLBCL) cells, follicular lymphoma, and mantle cell lymphoma. These have been shown to have significant antitumor activity in the treatment of patients with ALL and DLBCL, including durable remissions in patients refractory to standard therapies, and are approved for these malignancies. In addition, anti-B-cell maturation antigen (BCMA) CAR-T therapies have been approved for the treatment of multiple myeloma. CHAPTER 77

However, there have also been significant issues with toxicity including cytokine release syndrome, organ toxicity felt to be due to inadvertent targeting of antigens present in the organ, neurotoxicity, and potentially an increased risk for subsequent development of T-cell malignancies. These patients often require aggressive supportive care by individuals experienced in the delivery of CAR-T therapy. In addition, as is true for most anticancer therapies, mechanisms of resistance have developed, most commonly the outgrowth of tumor cells no longer expressing the antigen. Mechanisms for preventing the development of resistant cells are being explored, including combinations targeting different antigens. In addition to potentially preventing or overcoming resistance to the targeting of a single antigen, this could potentially increase efficacy and better reflects the normal immune response to pathogens or cancers in targeting multiple different antigens. CAR-T therapies are undergoing clinical investigation against other hematologic malignancies and solid tumors. Approaches to develop allogeneic CAR-T therapies are also being explored with the aim of having an off-the-shelf product that could be used in a number of patients rather than

generating each treatment specifically for one recipient. Cancer Cell Biology Another approach utilizing lymphocytes to treat cancer involves utilization of autologous tumor-derived T cells. Tumor-infiltrating lymphocyte therapy in combination with IL-2 is now approved for the treatment of melanoma. Given previously demonstrated efficacy against other cancers, such as RCC, tumor-infiltrating lymphocyte therapy may eventually be approved for other cancers as well. However, technical issues of getting adequate expansion of the cells may limit this approach. The immune response against cancers may also be able to be enhanced through targeting of proteins or cells (e.g., regulatory T cells) involved in normal homeostatic control to prevent autoimmune damage to the host but that malignant cells and their stroma can also utilize to inhibit the immune response directed against them. A component of this process includes a number of immune checkpoints that involve interaction of proteins on the surface of effector T cells with proteins on self-cells (or cancer cells that arise from normal cells) that inhibit activation of the T cells. By inhibiting the binding of the proteins involved in this process, the brake on the effector T cells are released and they can be activated. The presence of neoantigens (e.g., mutant proteins) enhances the activation of the T cells against cancer cells as compared to normal cells. Sufficient clinical antitumor activity has been seen for monoclonal antibodies targeting various proteins involved in this process, including CTLA-4, PD-1, PD-L1, and LAG3 (others continue to be explored), for them to be approved. These are

Tumor cells Elaboration of
immunosuppressive cytokines
TGF- β Interleukin-4 Interleukin-6
Interleukin-10 Immunosuppressive
immune cells PART 4 Oncology and
Hematology T regulatory cells
CD11+ granulocytes Macrophages
FIGURE 77-13 Tumor-host
interactions that suppress the

immune response to the tumor. co-inhibitory molecules that are expressed on the surface of cancer cells, and/or cells of the immune system, and/or stromal cells and are involved in inhibiting the immune response against both normal cells (their normal protective mechanism for the host) and also cancer cells that use this inhibitory process to evade immune-mediated cell death (Figs. 77-13 and 77-14). This approach has had clinical activity against a wide variety of cancers. A monoclonal antibody directed

against CTLA-4 is approved for the treatment of melanoma and several other malignancies, and antibodies targeting PD-1 or PD-L1 are approved for use against melanoma, RCC, lung cancer (both non-small-cell lung and small-cell lung), head and neck cancer, nasopharyngeal cancer, urothelial cancer, cervical cancer, endometrial cancer, hepatocellular carcinoma, gastric cancer, esophageal cancer, cutaneous squamous cell carcinoma, basal cell carcinoma, Merkel cell cancer, primary B-cell mediastinal

lymphoma, Hodgkin's lymphoma, and in a cancer-agnostic PD-L1 PD- L1 Cancer cells CD28 CD80/86 MHC

CD80/86 CTLA-4 Tumor antigens (TA) Antigen-presenting cell/dendritic cell FIGURE 77-14 Inhibition of T-cell activation against cancer cells by engagement of co-inhibitory molecules including PD-1, PD-L1, and CTLA-4 and reversal of this inhibition by antibodies against these proteins. The red ovals in the T cell indicate inhibitory signals, and the green oval indicates stimulatory signals.

T-cell inactivation Induction of CTLA-4 Induction of PD-1 Cell signaling disruption Class I MHC loss in tumor cells STAT-3 signaling loss in T cells Generation of indoleamine 2, 3-dioxygenase Degradation of T-cell receptor ζ chain approach, cancers with high microsatellite instability (MSI) or high tumor mutational burden (TMB). They continue to be evaluated against other malignancies as well. The combination of anti-CTLA-4 and anti-PD-1 antibodies has been approved for treatment of a number of malignancies, including mela noma, RCC, HCC, NSCLC, mesothelioma, and MSI-high metastatic colorectal cancers. Specific determinants of response to immune checkpoint inhibitors are still being defined, but in addition to high PD-L1 expression, the presence of increased neoantigens in the tumor, such as seen in patients with MSI-high and TMB-high cancers, may be one important determinant of better responses. A number of other proteins are involved in controlling the immune response (both ones that enhance activity [e.g., CD27 and CD40] as well as ones involved in inhibiting response [e.g., TIM-3, TIGIT]). Antibodies have been developed to modulate function of these Anti-PD-L1 antibodies Anti-PD-1 antibodies PD-1 + - PD-1 + + MHC T-cell receptor T cell receptor - + Cancer cell T cell Anti-CTLA-4 antibodies

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