Chapter 2: Hallmarks of Cancer: An Organizing Principle for Cancer Medicine

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Introduction

The hallmarks of cancer comprise eight biologic capabilities acquired by incipient cancer cells during the multistep development of human tumors. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming energy metabolism, and evading immune destruction. Facilitating the acquisition of these hallmark capabilities are genome instability, which enables mutational alteration of hallmark-enabling genes, and immune inflammation, which fosters the acquisition of multiple hallmark functions. In addition to cancer cells, tumors exhibit another dimension of complexity: They contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the *tumor microenvironment*. Recognition of the widespread applicability of these concepts will increasingly influence the development of new means to treat human cancer.

At the beginning of the new millennium, we proposed that six *hallmarks of cancer* embody an organizing principle that provides a logical framework for understanding the remarkable diversity of neoplastic diseases.[1] Implicit in our discussion was the notion that, as normal cells evolve progressively to a neoplastic state, they acquire a succession of these hallmark capabilities, and that the multistep process of human tumor pathogenesis can be rationalized by the need of incipient cancer cells to acquire the diverse traits that in aggregate enable them to become tumorigenic and, ultimately, malignant.

We noted as an ancillary proposition that tumors are more than insular masses of proliferating cancer cells. Instead, they are complex tissues composed of multiple distinct types of neoplastic and normal cells that participate in heterotypic interactions with one another. We depicted the recruited normal cells, which form tumor-associated stroma, as active participants in tumorigenesis rather than passive bystanders; as such, these stromal cells contribute to the development and expression of certain hallmark capabilities. This notion has been solidified and extended during the intervening period, and it is now clear that the biology of tumors can no longer be understood simply by enumerating the traits of the cancer cells, but instead must encompass the contributions of the *tumor microenvironment* to tumorigenesis. In 2011, we revisited the original hallmarks, adding two new ones to the roster, and expanded on the functional roles and contributions made by recruited stromal cells to tumor biology.[2] Herein we reiterate and further refine the hallmarks-of-cancer perspectives we presented in 2000 and 2011, with the goal of informing students of cancer medicine about the concept and its potential utility for understanding the pathogenesis of human cancer, and the potential relevance of this concept to the development of more effective treatments for this disease.

Hallmark Capabilities, in Essence

The eight hallmarks of cancer—distinct and complementary capabilities that enable tumor growth and

metastatic dissemination—continue to provide a solid foundation for understanding the biology of cancer (**Fig. 2.1**). The sections that follow summarize the essence of each hallmark, providing insights into their regulation and functional manifestations.

FIGURE 2.1

The hallmarks of cancer.

Eight functional capabilities—the hallmarks of cancer—are thought to be acquired by developing cancers in the course of the multistep carcinogenesis that leads to most forms of human cancer. The order in which these hallmark capabilities are acquired and the relative balance and importance of their contributions to malignant disease appears to vary across the spectrum of human cancers.

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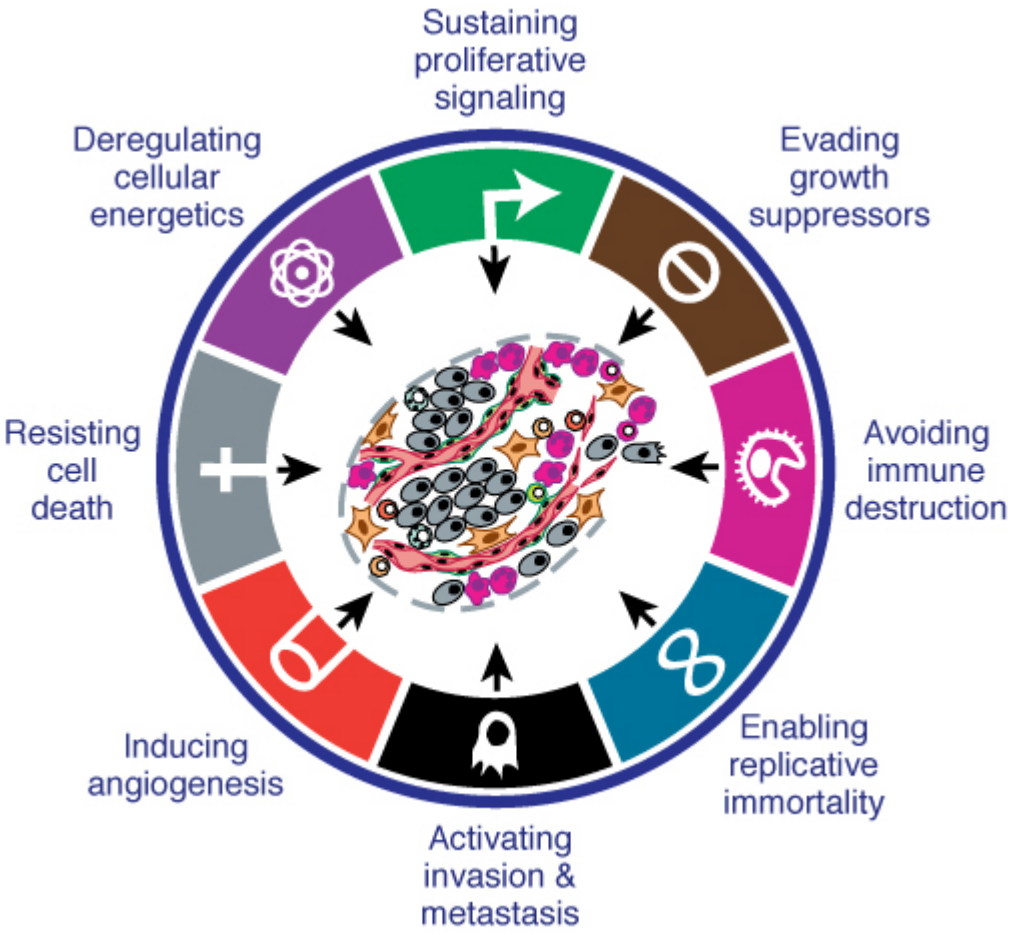


Sustaining Proliferative Signaling

Arguably, the most fundamental trait of cancer cells involves their ability to sustain chronic proliferation. Normal tissues carefully control the production and release of growth-promoting signals that instruct entry of cells into and progression through the growth-and-division cycle, thereby ensuring proper control of cell number and thus maintenance of normal tissue architecture and function. Cancer cells, by deregulating these signals, become masters of their own destinies. The enabling signals are conveyed in large part by growth factors that bind cell-surface receptors, typically containing intracellular tyrosine kinase domains. The latter proceed to emit signals via branched intracellular signaling pathways that regulate progression through the cell cycle as well as cell growth (that is, increase in cell size); often, these signals influence yet other cell-biologic properties, such as cell survival and energy metabolism.

Remarkably, the precise identities and sources of the proliferative signals operating within normal tissues remain poorly understood. Moreover, we still know relatively little about the mechanisms controlling the release of these mitogenic signals. In part, the study of these mechanisms is complicated by the fact that the growth factor signals controlling cell number and position within normal tissues are thought to be transmitted in a temporally and spatially regulated fashion from one cell to its neighbors; such paracrine signaling is difficult to access experimentally. In addition, the bioavailability of growth factors is regulated by their sequestration in the pericellular space and associated extracellular matrix. Moreover, the actions of these extracellular mitogenic proteins is further controlled by a complex network of proteases, sulfatases, and possibly other enzymes that liberate and activate these factors, apparently in a highly specific and localized fashion.

The mitogenic signaling operating in cancer cells is, in contrast, far better understood.[3],[4],[5],[6] Cancer cells can acquire the capability to sustain proliferative signaling in a number of alternative ways: They may produce growth factor ligands themselves, to which they can then respond via the coexpression of cognate receptors, resulting in autocrine proliferative stimulation. Alternatively, cancer cells may send signals to stimulate normal cells within the supporting tumor-associated stroma; the stromal cells then reciprocate by supplying the cancer cells with various growth factors.[7],[8] Mitogenic signaling can also be deregulated by elevating the levels of receptor proteins displayed at the cancer cell surface, rendering such cells hyperresponsive to otherwise limiting amounts of growth factor ligands; the same outcome can result from structural alterations in the receptor molecules that facilitate ligand-independent firing.

Independence from externally supplied growth factors may also derive from the constitutive activation of components of intracellular signaling cascades operating downstream of these receptors within cancer cells. These intracellular alterations obviate the need to stimulate cell proliferation pathways by ligand-mediated activation of cell-surface receptors. Of note, because a number of distinct downstream signaling pathways radiate from ligand-stimulated receptors, the activation of one or another of these downstream branches (e.g., the pathway responding to the Ras signal transducer) may only provide a subset of the regulatory instructions transmitted by a ligand-activated receptor.

Somatic Mutations Activate Additional Downstream Pathways

DNA sequencing analyses of cancer cell genomes have revealed somatic mutations in certain human tumors that predict constitutive activation of the signaling circuits, cited previously, that are normally triggered by activated growth factor receptors. The past 3 decades have witnessed the identification in tens of thousands of human tumors of mutant, oncogenic alleles of the *RAS* proto-oncogenes, most of which have sustained point mutations in the 12th codon, which results in RAS proteins that are constitutively active in downstream signaling. Thus, more than 90% of pancreatic adenocarcinomas carry mutant K-*RAS* alleles. More recently, the repertoire of frequently mutated genes has been expanded to include those encoding the downstream effectors of the RAS proteins. For example, we now know that ~40% of human melanomas contain activating mutations affecting the structure of the B-RAF protein, resulting in constitutive signaling through the RAF to the mitogen-activated protein (MAP)–kinase pathway.[9] Similarly, mutations in the catalytic subunit of phosphoinositide 3-kinase (PI3K) isoforms are being detected in an array of tumor types; these mutations typically serve to hyperactivate the PI3K signaling pathway, causing in turn, excess signaling through the crucial Akt/PKB signal transducer.[10],[11] The advantages to tumor cells of activating upstream (receptor) versus downstream (transducer) signaling remain obscure, as does the functional impact of cross-talk between the multiple branched pathways radiating from individual growth factor receptors.

Disruptions of Negative-Feedback Mechanisms that Attenuate Proliferative Signaling

Recent observations have also highlighted the importance of negative-feedback loops that normally operate to dampen various types of signaling and thereby ensure homeostatic regulation of the flux of signals coursing through the intracellular circuitry.[12],[13],[14],[15] Defects in these negative-feedback mechanisms are capable of enhancing proliferative signaling. The prototype of this type of regulation involves the RAS oncoprotein. The oncogenic effects of mutant RAS proteins do not result from a hyperactivation of its downstream signaling powers; instead, the oncogenic mutations affecting *RAS* genes impair the intrinsic GTPase activity of RAS that normally serves to turn its activity off, ensuring that active signal transmission (e.g., from upstream growth factor receptors) is transient; as such, oncogenic RAS mutations disrupt an autoregulatory negative-feedback mechanism, without which RAS generates chronic proliferative signals.

Analogous negative-feedback mechanisms operate at multiple nodes within the proliferative signaling circuitry. A prominent example involves phosphatase and tensin homolog (PTEN), which counteracts PI3K by degrading its product, phosphatidylinositol 3,4,5-phosphate (PIP_3). Loss-of-function mutations in PTEN amplify PI3K signaling and promote tumorigenesis in a variety of experimental models of cancer; in human tumors, PTEN expression is often lost by the methylation of DNA at specific sites associated with the promoter of the *PTEN* gene, resulting in the shutdown of its transcription.[10],[11]

Yet another example involves the mammalian target of rapamycin (mTOR) kinase, a key coordinator of cell growth and metabolism that lies both upstream and downstream of the PI3K pathway. In the circuitry of some cancer cells, mTOR activation results, via negative feedback, in the inhibition of PI3K signaling. Accordingly, when mTOR is pharmacologically inhibited in such cancer cells (e.g., by the drug rapamycin), the associated loss of negative feedback results in increased activity of PI3K and its effector, the Akt/PKB kinase, thereby blunting the antiproliferative effects of mTOR inhibition.[16],[17] It is likely that compromised negative feedback loops in this and other signaling pathways will prove to be widespread among human cancer cells, serving as important means by which cancer cells acquire the capability of signaling chronically through these pathways. Moreover, disruption of such normally self-attenuating signaling can contribute to the development of adaptive resistance toward therapeutic drugs targeting mitogenic signaling.

Excessive Proliferative Signaling can Trigger Cell Senescence

Early studies of oncogene action encouraged the notion that ever-increasing expression of such genes and the signals released by their protein products would result in proportionately increased cancer cell proliferation and, thus, tumor growth. More recent research has undermined this notion, in that it is now apparent that excessively elevated signaling by oncoproteins, such as RAS, MYC, and RAF, can provoke counteracting (protective) responses from cells, such as induction of cell death; alternatively, cancer cells expressing high levels of these oncoproteins may be forced to enter into the nonproliferative but viable state called senescence. These responses contrast with those seen in cells expressing lower levels of these proteins, which permit cells to avoid senescence or cell death and, thus, proliferate.[18],[19],[20],[21]

Cells with morphologic features of senescence, including enlarged cytoplasm, the absence of proliferation markers, and the expression of the senescence-induced β-galactosidase enzyme, are abundant in the tissues of mice whose genomes have been reengineered to cause overexpression of certain oncogenes[19],[20]; such senescent cells are also prevalent in some cases of human melanoma.[22]

These ostensibly paradoxical responses seem to reflect intrinsic cellular defense mechanisms designed to eliminate cells experiencing excessive levels of certain types of mitogenic signaling. Accordingly, the intensity of oncogenic signaling observed in naturally arising cancer cells may represent compromises between maximal mitogenic stimulation and avoidance of these anti-proliferative defenses. Alternatively, some cancer cells may adapt to high levels of oncogenic signaling by disabling their senescence- or apoptosis-inducing circuitry.

Evading Growth Suppressors

In addition to the hallmark capability of inducing and sustaining positively acting growth-stimulatory signals, cancer cells must also circumvent powerful programs that negatively regulate cell proliferation; many of these programs depend on the actions of tumor suppressor genes. Dozens of tumor suppressors that operate in various ways to limit cell proliferation or survival have been discovered through their inactivation in one or another form of animal or human cancer; many of these genes have been validated as bona fide tumor suppressors through gain- or loss-of-function experiments in mice. The two prototypical tumor suppressor genes encode the retinoblastoma (RB)-associated and TP53 proteins; they operate as central control nodes within two key, complementary cellular regulatory circuits that govern the decisions of cells to proliferate, or alternatively, to activate growth arrest, senescence, or the cell-suicide program known as apoptosis.

The RB protein integrates signals from diverse extracellular and intracellular sources and, in response, decides whether or not a cell should proceed through its growth-and-division cycle.[23],[24],[25] Cancer cells with defects in the RB pathway function are thus missing the services of a critical gatekeeper of cell-cycle progression whose absence permits persistent cell proliferation. Whereas RB transduces growth-inhibitory signals that largely originate outside of the cell, TP53 receives inputs from stress and abnormality sensors that function within the cell's intracellular operating systems. For example, if the degree of damage to a cell's genome is excessive, or if the levels of nucleotide pools, growth-promoting signals, glucose, or oxygenation are insufficient, TP53 can call a halt to further cell-cycle progression until these conditions have been normalized. Alternatively, in the face of alarm signals indicating overwhelming or irreparable damage to such cellular systems, TP53 can trigger apoptosis. Of note, the alternative effects of activated TP53 are complex and highly context dependent, varying by cell type as well as by the severity and persistence of conditions of cell-physiologic stress and genomic damage.

Although the two canonical suppressors of proliferation—TP53 and RB—have preeminent importance in regulating cell proliferation, various lines of evidence indicate that each operates as part of a larger network that is wired for functional redundancy. For example, chimeric mice populated throughout their bodies with individual cells lacking a functional *Rb* gene are surprisingly free of proliferative abnormalities, despite the expectation that a loss of RB function should result in unimpeded advance through the cell division cycle by these cells and their lineal descendants; some of the resulting clusters of *Rb*-null cells should, by all rights, progress to neoplasia. Instead, the *Rb*-null cells in such chimeric mice have been found to participate in relatively normal tissue morphogenesis throughout the body; the only neoplasia observed is of pituitary tumors developing late in life.[26] Similarly, *TP53*-null mice develop normally, show largely normal cell and tissue homeostasis, and again develop abnormalities only later in life in the form of leukemias and sarcomas.[27]

Mechanisms of Contact Inhibition and Its Evasion

Four decades of research have demonstrated that the cell-to-cell contacts formed by dense populations of normal cells growing in 2-dimensional culture operate to suppress further cell proliferation, yielding

confluent cell monolayers. Importantly, such *contact inhibition* is abolished in various types of cancer cells in culture, suggesting that contact inhibition is an in vitro surrogate of a mechanism that operates in vivo to ensure normal tissue homeostasis that is abrogated during the course of tumorigenesis. Until recently, the mechanistic basis for this mode of growth control remained obscure. Now, however, mechanisms of contact inhibition are beginning to emerge.[28]

One mechanism involves the product of the *NF2* gene, long implicated as a tumor suppressor because its loss triggers a form of human neurofibromatosis. Merlin, the cytoplasmic *NF2* gene product, orchestrates contact inhibition by coupling cell-surface adhesion molecules (e.g., E-cadherin) to transmembrane receptor tyrosine kinases (e.g., the EGF receptor). In so doing, Merlin strengthens the adhesiveness of cadherin-mediated cell-to-cell attachments. Additionally, by sequestering such growth factor receptors, Merlin limits their ability to efficiently emit mitogenic signals.[28],[29],[30],[31]

Corruption of the TGF-β Pathway Promotes Malignancy

Transforming growth factor beta (TGF-β is best known for its antiproliferative effects on epithelial cells. The responses of carcinoma cells to TGF-β's proliferation–suppressive effects is now appreciated to be far more elaborate than a simple shutdown of its signaling circuitry.[32],[33],[34],[35] In normal cells, exposure to TGF-β blocks their progression through the G1 phase of the cell cycle. In many late-stage tumors, however, TGF-β signaling is redirected away from suppressing cell proliferation and is found instead to activate a cellular program, termed the epithelial-to-mesenchymal transition (EMT), which confers on cancer cells multiple traits associated with high-grade malignancy, as will be discussed in further detail.

Resisting Cell Death

The ability to activate the normally latent apoptotic cell-death program appears to be associated with most types of normal cells throughout the body. Its actions in many if not all multicellular organisms seems to reflect the need to eliminate aberrant cells whose continued presence would otherwise threaten organismic integrity. This rationale explains why cancer cells often, if not invariably, inactivate or attenuate this program during their development.[21],[36],[37],[38]

Elucidation of the detailed design of the signaling circuitry governing the apoptotic program has revealed how apoptosis is triggered in response to various physiologic stresses that cancer cells experience either during the course of tumorigenesis or as a result of anticancer therapy. Notable among the apoptosis-inducing stresses are signaling imbalances resulting from elevated levels of oncogene signaling and from DNA damage. The regulators of the apoptotic response are divided into two major circuits, one receiving and processing extracellular death-inducing signals (the extrinsic apoptotic program, involving for example the Fas ligand/Fas receptor), and the other sensing and integrating a variety of signals of intracellular origin (the intrinsic program). Each of these circuits culminates in the activation of a normally latent protease (caspase 8 or 9, respectively), which proceeds to initiate a cascade of proteolysis involving effector caspases that are responsible for the execution phase of apoptosis. During this final phase, an apoptotic cell is progressively disassembled and then consumed, both by its neighbors and by professional phagocytic cells. Currently, the intrinsic apoptotic program is more widely implicated as a barrier to cancer pathogenesis.

The molecular machinery that conveys signals between the apoptotic regulators and effectors is controlled by counterbalancing pro- and antiapoptotic members of the Bcl-2 family of regulatory proteins.[36],[37] The archetype, Bcl-2, along with its closest relatives (Bcl-XL, Bcl-W, Mcl-1, A1) are inhibitors of

apoptosis, acting in large part by binding to and thereby suppressing two proapoptotic triggering proteins (Bax and Bak); the latter are embedded in the mitochondrial outer membrane. When relieved of inhibition by their antiapoptotic relatives, Bax and Bax disrupt the integrity of the outer mitochondrial membrane, causing the release into the cytosol of proapoptotic signaling proteins, the most important of which is cytochrome C. When the normally sequestered cytochrome C is released, it activates a cascade of cytosolic caspase proteases that proceed to fragment multiple cellular structures, thereby executing the apoptotic death program.[37],[39]

Several abnormality sensors have been identified that play key roles in triggering apoptosis.[21],[37] Most notable is a DNA damage sensor that acts through the TP53 tumor suppressor[40]; TP53 induces apoptosis by upregulating expression of the proapoptotic, Bcl-2-related Noxa and Puma proteins, doing so in response to substantial levels of DNA breaks and other chromosomal abnormalities. Alternatively, insufficient survival factor signaling (e.g., inadequate levels of interleukin (IL)-3 in lymphocytes or of insulinlike growth factors 1/2 [IGF1/2] in epithelial cells) can elicit apoptosis through another proapoptotic Bcl-2–related protein called Bim. Yet another condition triggering apoptosis involves hyperactive signaling by certain oncoproteins, such as Myc, which acts in part via Bim and other Bcl-2–related proteins.[18],[21],[40]

Tumor cells evolve a variety of strategies to limit or circumvent apoptosis. Most common is the loss of TP53 tumor suppressor function, which eliminates this critical damage sensor from the apoptosis-inducing circuitry. Alternatively, tumors may achieve similar ends by increasing the expression of antiapoptotic regulators (Bcl-2, Bcl-XL) or of survival signals (IGF1/2), by downregulating proapoptotic Bcl-2–related factors (Bax, Bim, Puma), or by short-circuiting the extrinsic ligand-induced death pathway. The multiplicity of apoptosis-avoiding mechanisms presumably reflects the diversity of apoptosis-inducing signals that cancer cell populations encounter during their evolution from the normal to the neoplastic state.

Autophagy Mediates Both Tumor Cell Survival and Death

Autophagy represents an important cell-physiologic response that, like apoptosis, normally operates at low, basal levels in cells but can be strongly induced in certain states of cellular stress, the most obvious of which is nutrient deficiency.[41],[42],[43] The autophagic program enables cells to break down cellular organelles, such as ribosomes and mitochondria, allowing the resulting catabolites to be recycled and thus used for biosynthesis and energy metabolism. As part of this program, intracellular vesicles (termed autophagosomes) envelope the cellular organelles destined for degradation; the resulting vesicles then fuse with lysosomes in which degradation occurs. In this fashion, low–molecular-weight metabolites are generated that support survival in the stressed, nutrient-limited environments experienced by many cancer cells. When acting in this fashion, autophagy favors cancer cell survival.

However, the autophagy program intersects in more complex ways with the life and death of cancer cells. Like apoptosis, the autophagy machinery has both regulatory and effector components.[41],[42],[43] Among the latter are proteins that mediate autophagosome formation and delivery to lysosomes. Of note, recent research has revealed intersections between the regulatory circuits governing autophagy, apoptosis, and cellular homeostasis. For example, the signaling pathway involving PI3K, AKT, and mTOR, which is stimulated by survival signals to block apoptosis, similarly inhibits autophagy; when survival signals are insufficient, the PI3K signaling pathway is downregulated, with the result that autophagy and/or apoptosis may be induced.[41],[42],[44],[45]

Another interconnection between these two programs resides in the Beclin-1 protein, which has been

shown by genetic studies to be necessary for the induction of autophagy.[41],[42],[43],[44] Beclin-1 is a member of the Bcl-2 family of apoptotic regulatory proteins, and its BH3 domain allows it to bind the Bcl-2/Bcl-XL proteins. Stress sensor–coupled BH3-containing proteins (e.g. Bim, Noxa) can displace Beclin-1 from its association with Bcl-2/Bcl-XL, enabling the liberated Beclin-1 to trigger autophagy, much as they can release proapoptotic Bax and Bak to trigger apoptosis. Hence, stress-transducing Bcl-2–related proteins can induce apoptosis and/or autophagy depending on the physiologic state of the cell.

Genetically altered mice bearing inactivated alleles of the *Beclin-1* gene or of certain other components of the autophagy machinery exhibit increased susceptibility to cancer.[42],[46] These results suggest that the induction of autophagy can serve as a barrier to tumorigenesis that may operate independently of or in concert with apoptosis. For example, excessive activation of the autophagy program may cause cells to devour too many of their own critical organelles, such that cell growth and division are crippled. Accordingly, autophagy may represent yet another barrier that needs to be circumvented by incipient cancer cells during multistep tumor development.[41],[46]

Perhaps paradoxically, nutrient starvation, radiotherapy, and certain cytotoxic drugs can induce elevated levels of autophagy that apparently protect cancer cells.[45],[46],[47],[48] Moreover, severely stressed cancer cells have been shown to shrink via autophagy to a state of reversible dormancy.[46],[49] This particular survival response may enable the persistence and eventual regrowth of some late-stage tumors following treatment with potent anticancer agents. Together, observations like these indicate that autophagy can have dichotomous effects on tumor cells and, thus, tumor progression.[46],[47] An important agenda for future research will involve clarifying the genetic and cell-physiologic conditions that determine when and how autophagy enables cancer cells to survive or, alternatively, causes them to die.

Necrosis has Proinflammatory and Tumor-Promoting Potential

In contrast to apoptosis, in which a dying cell contracts into an almost invisible corpse that is soon consumed by its neighbors, necrotic cells become bloated and explode, releasing their contents into the local tissue microenvironment. A body of evidence has shown that cell death by necrosis, like apoptosis, is an organized process under genetic control, rather than being a random and undirected process.[50],[51],[52]

Importantly, necrotic cell death releases proinflammatory signals into the surrounding tissue microenvironment, in contrast to apoptosis, which does not. As a consequence, necrotic cells can recruit inflammatory cells of the immune system,[51],[53],[54] whose dedicated function is to survey the extent of tissue damage and remove associated necrotic debris. In the context of neoplasia, however, multiple lines of evidence indicate that immune inflammatory cells can be actively tumor-promoting by fostering angiogenesis, cancer cell proliferation, and invasiveness (discussed in subsequent sections). Additionally, necrotic cells can release bioactive regulatory factors, such as IL1α, which can directly stimulate neighboring viable cells to proliferate, with the potential, once again, to facilitate neoplastic progression.[53] Consequently, necrotic cell death, while seemingly beneficial in counterbalancing cancer-associated hyperproliferation, may ultimately do more damage to the patient than good.

Enabling Replicative Immortality

Cancer cells require unlimited replicative potential in order to generate macroscopic tumors. This capability stands in marked contrast to the behavior of the cells in most normal cell lineages in the body,

which are only able to pass through a limited number of successive cell growth-and-division cycles. This limitation has been associated with two distinct barriers to proliferation: *replicative senescence*, a typically irreversible entrance into a nonproliferative but viable state, and *crisis*, which involves cell death. Accordingly, when cells are propagated in culture, repeated cycles of cell division lead first to induction of replicative senescence and then, for those cells that succeed in circumventing this barrier, to the crisis phase, in which the great majority of cells in the population die. On rare occasion, cells emerge from a population in crisis and exhibit unlimited replicative potential. This transition has been termed immortalization, a trait that most established cell lines possess by virtue of their ability to proliferate in culture without evidence of either senescence or crisis.

Multiple lines of evidence indicate that telomeres protecting the ends of chromosomes are centrally involved in the capability for unlimited proliferation.[55],[56],[57],[58] The telomere-associated DNA, composed of multiple tandem hexanucleotide repeats, shortens progressively in the chromosomes of nonimmortalized cells propagated in culture, eventually losing the ability to protect the ends of chromosomal DNA from end-to-end fusions; such aberrant fusions generate unstable dicentric chromosomes, whose resolution during the anaphase of mitosis results in a scrambling of karyotype and entrance into crisis that threatens cell viability. Accordingly, the length of telomeric DNA in a cell dictates how many successive cell generations its progeny can pass through before telomeres are largely eroded and have consequently lost their protective functions.

Telomerase, the specialized DNA polymerase that adds telomere repeat segments to the ends of telomeric DNA, is almost absent in nonimmortalized cells but is expressed at functionally significant levels in the great majority (~90%) of spontaneously immortalized cells, including human cancer cells. By extending telomeric DNA, telomerase is able to counter the progressive telomere erosion that would otherwise occur in its absence. The presence of telomerase activity, either in spontaneously immortalized cells or in the context of cells engineered to express the enzyme, is correlated with a resistance to induction of both senescence and crisis/apoptosis; conversely, the suppression of telomerase activity leads to telomere shortening and to activation of one or the other of these proliferative barriers.

The two barriers to proliferation—replicative senescence and crisis/apoptosis—have been rationalized as crucial anticancer defenses that are hardwired into our cells and are deployed to impede the outgrowth of clones of preneoplastic and, frankly, neoplastic cells. According to this thinking, most incipient neoplasias exhaust their endowment of replicative doublings and are stopped in their tracks by either of these barriers. The eventual immortalization of rare variant cells that proceed to form tumors has been attributed to their ability to maintain telomeric DNA at lengths sufficient to avoid triggering either senescence or apoptosis, which is achieved most commonly by upregulating the expression of telomerase or, less frequently, via an alternative recombination-based (ALT) telomere maintenance mechanism.[59] Hence, telomere shortening has come to be viewed as a clocking device that determines the limited replicative potential of normal cells and, thus, one that must be overcome by cancer cells.

Reassessing Replicative Senescence

The senescent state induced by oncogenes, as described previously, is remarkably similar to that induced when cells are explanted from living tissue and introduced into culture, the latter being the replicative senescence just discussed. Importantly, the concept of replication-induced senescence as a general barrier requires refinement and reformulation. Recent experiments have revealed that the induction of senescence in certain cultured cells can be delayed and possibly eliminated by the use of

improved cell culture conditions, suggesting that recently explanted primary cells may be intrinsically able to proliferate unimpeded in culture up the point of crisis and the associated induction of apoptosis triggered by critically shortened telomeres.[60],[61],[62],[63] This result indicates that telomere shortening does not necessarily induce senescence prior to crisis. Additional insight comes from experiments in mice engineered to lack telomerase; this work has revealed that shortening telomeres can shunt premalignant cells into a senescent state that contributes (along with apoptosis) to attenuated tumorigenesis in mice genetically destined to develop particular forms of cancer.[58] Such telomerase-null mice with highly eroded telomeres exhibit multiorgan dysfunction and abnormalities that provide evidence of both senescence and apoptosis, perhaps similar to the senescence and apoptosis observed in cell culture.[58],[64] Thus, depending on the cellular context, the proliferative barrier of telomere shortening can be manifested by the induction of senescence and/or apoptosis.

Delayed Activation of Telomerase May Both Limit and Foster Neoplastic Progression

There is now evidence that clones of incipient cancer cells in spontaneously arising tumors experience telomere loss-induced crisis relatively early during the course of multistep tumor progression due to their inability to express significant levels of telomerase. Thus, extensively eroded telomeres have been documented in premalignant growths through the use of fluorescence in situ hybridization (FISH), which has also revealed the end-to-end chromosomal fusions that signal telomere failure and crisis.[65],[66] These results suggest that such incipient cancer cells have passed through a substantial number of successive telomere-shortening cell divisions during their evolution from fully normal cells of origin. Accordingly, the development of some human neoplasias may be aborted by telomere-induced crisis long before they have progressed to become macroscopic, frankly neoplastic growths.

A quite different situation is observed in cells that have lost the TP53-mediated surveillance of genomic integrity and, thereafter, experience critically eroded telomeres. The loss of the TP53 DNA damage sensor can enable such cells to avoid apoptosis that would otherwise be triggered by the DNA damage resulting from dysfunctional telomeres. Instead, such cells lacking TP53 continue to divide, suffering repeated cycles of interchromosomal fusion and subsequent breakage at mitosis. Such breakage-fusion-bridge (BFB) cycles result in deletions and amplifications of chromosomal segments, evidently serving to mutagenize the genome, thereby facilitating the generation and subsequent clonal selection of cancer cells that have acquired mutant oncogenes and tumor suppressor genes.[58],[67] One infers, however, that the clones of cancer cells that survive this telomere collapse must eventually acquire the ability to stabilize and thus protect their telomeres via the activation of telomerase or the ALT mechanism noted previously.

These considerations present an interesting dichotomy: Although dysfunctional telomeres are an evident barrier to chronic proliferation, they can also facilitate the genomic instability that generates hallmark-enabling mutations, as will be discussed further. Both mechanisms may be at play in certain forms of carcinogenesis in the form of transitory telomere deficiency prior to telomere stabilization. Circumstantial support for this concept of transient telomere deficiency in facilitating malignant progression has come from comparative analyses of premalignant and malignant lesions in the human breast.[68],[69] The premalignant lesions did not express significant levels of telomerase and were marked by telomere shortening and chromosomal aberrations. In contrast, overt carcinomas exhibited telomerase expression concordantly with the reconstruction of longer telomeres and the fixation of the aberrant karyotypes that would seem to have been acquired after telomere failure but before the acquisition of telomerase activity. When portrayed in this way, the delayed acquisition of telomerase function serves to generate tumor-promoting mutations, whereas its subsequent expression stabilizes the mutant genome and confers the unlimited replicative capacity that cancer cells require in order to generate clinically apparent tumors.

Inducing Angiogenesis

Like normal tissues, tumors require sustenance in the form of nutrients and oxygen as well as an ability to evacuate metabolic wastes and carbon dioxide. The tumor-associated neovasculature, generated by the process of angiogenesis, addresses these needs. During embryogenesis, the development of the vasculature involves the birth of new endothelial cells and their assembly into tubes (vasculogenesis) in addition to the sprouting (angiogenesis) of new vessels from existing ones. Following this morphogenesis, the normal vasculature becomes largely quiescent. In the adult, as part of physiologic processes such as wound healing and female reproductive cycling, angiogenesis is turned on, but only transiently. In contrast, during tumor progression, an *angiogenic switch* is almost always activated and remains on, causing normally quiescent vasculature to continually sprout new vessels that help sustain expanding neoplastic growths.[70]

A compelling body of evidence indicates that the angiogenic switch is governed by countervailing factors that either induce or oppose angiogenesis.[71]'[72] Some of these angiogenic regulators are signaling proteins that bind to stimulatory or inhibitory cell-surface receptors displayed by vascular endothelial cells. The well-known prototypes of angiogenesis inducers and inhibitors are vascular endothelial growth factor-A (VEGF-A) and thrombospondin-1 (Tsp-1), respectively.

The VEGF-A gene encodes ligands that are involved in orchestrating new blood vessel growth during embryonic and postnatal development, in the survival of endothelial cells in already-formed vessels, and in certain physiologic and pathologic situations in the adult. VEGF signaling via three receptor tyrosine kinases (VEGFR1–3) is regulated at multiple levels, reflecting this complexity of purpose. VEGF gene expression can be upregulated both by hypoxia and by oncogene signaling.[73]'[74]'[75] Additionally, VEGF ligands can be sequestered in the extracellular matrix in latent forms that are subject to release and activation by extracellular matrix-degrading proteases (e.g., matrix metallopeptidase 9 [MMP-9]).[76] In addition, other proangiogenic proteins, such as members of the fibroblast growth factor (FGF) family, have been implicated in sustaining tumor angiogenesis.[71] TSP-1, a key counterbalance in the angiogenic switch, also binds transmembrane receptors displayed by endothelial cells and thereby triggers suppressive signals that can counteract proangiogenic stimuli.[77]

The blood vessels produced within tumors by an unbalanced mix of proangiogenic signals are typically aberrant: Tumor neovasculature is marked by precocious capillary sprouting, convoluted and excessive vessel branching, distorted and enlarged vessels, erratic blood flow, microhemorrhaging, leaking of plasma into the tissue parenchyma, and abnormal levels of endothelial cell proliferation and apoptosis.[78]'[79]

Angiogenesis is induced surprisingly early during the multistage development of invasive cancers both in animal models and in humans. Histologic analyses of premalignant, noninvasive lesions, including dysplasias and in situ carcinomas arising in a variety of organs, have revealed the early tripping of the angiogenic switch.[70]'[80] Historically, angiogenesis was envisioned to be important only when rapidly growing macroscopic tumors had formed, but more recent data indicate that angiogenesis also contributes to the microscopic premalignant phase of neoplastic progression, further cementing its status as an integral hallmark of cancer.

Gradations of the Angiogenic Switch

Once angiogenesis has been activated, tumors exhibit diverse patterns of neovascularization. Some tumors, including highly aggressive types such as pancreatic ductal adenocarcinomas, are

hypovascularized and replete with stromal deserts that are largely avascular and indeed may even be actively antiangiogenic.[81] In contrast, many other tumors, including human renal and pancreatic neuroendocrine carcinomas, are highly angiogenic and, consequently, densely vascularized.[82],[83]

Collectively, such observations suggest an initial tripping of the angiogenic switch during tumor development, which is followed by a variable intensity of ongoing neovascularization, the latter being controlled by a complex biologic rheostat that involves both the cancer cells and the associated stromal microenvironment.[71],[72] Of note, the switching mechanisms can vary, even though the net result is a common inductive signal (e.g., VEGF). In some tumors, dominant oncogenes operating within tumor cells, such as *Ras* and *Myc*, can upregulate the expression of angiogenic factors, whereas in others, such inductive signals are produced indirectly by immune inflammatory cells, as will be discussed.

Endogenous Angiogenesis Inhibitors Present Natural Barriers to Tumor Angiogenesis

A variety of secreted proteins have been reported to have the capability to help shut off normally transitory angiogenesis, including thrombospondin-1 (TSP-1), fragments of plasmin (angiostatin) and type 18 collagen (endostatin), along with another dozen candidate antiangiogenic proteins.[77],[84],[85],[86],[87],[88] Most are proteins, and many are derived by proteolytic cleavage of structural proteins that are not themselves angiogenic regulators.

A number of these endogenous inhibitors of angiogenesis can be detected in the circulation of normal mice and humans. Genes that encode several endogenous angiogenesis inhibitors have been deleted from the mouse germ line without untoward developmental or physiologic effects; however, the growth of autochthonous and implanted tumors is enhanced as a consequence.[84],[85],[88] By contrast, if the circulating levels of an endogenous inhibitor are genetically increased (e.g., via overexpression in transgenic mice or in xenotransplanted tumors), tumor growth is impaired.[85],[88] Interestingly, wound healing and fat deposition are impaired or accelerated by elevated or ablated expression of such genes.[89],[90] The data suggest that, under normal conditions, endogenous angiogenesis inhibitors serve as physiologic regulators modulating the transitory angiogenesis that occurs during tissue remodeling and wound healing; they may also act as intrinsic barriers to the induction and/or persistence of angiogenesis by incipient neoplasias.

Pericytes are Important Components of the Tumor Neovasculature

Pericytes have long been known as supporting cells that are closely apposed to the outer surfaces of the endothelial tubes in normal tissue vasculature, where they provide important mechanical and physiologic support to the endothelial cells. Microscopic studies conducted in recent years have revealed that pericytes are associated, albeit loosely, with the neovasculature of most, if not all, tumors.[91],[92],[93] More importantly, mechanistic studies (discussed subsequently) have revealed that pericyte coverage is important for the maintenance of a functional tumor neovasculature.

A Variety of Bone Marrow-Derived Cells Contribute to Tumor Angiogenesis

It is now clear that a repertoire of cell types originating in the bone marrow play crucial roles in pathologic angiogenesis.[94],[95],[96],[97] These include cells of the innate immune system—notably, macrophages, neutrophils, mast cells, and myeloid progenitors—that assemble at the margins of such lesions or infiltrate deeply within them; the tumor-associated inflammatory cells can help to trip the angiogenic switch in quiescent tissue and sustain ongoing angiogenesis associated with tumor growth. In addition, they can help protect the vasculature from the effects of drugs targeting endothelial cell signaling.[98] Moreover, several types of bone marrow–derived *vascular progenitor cells* have been

observed to have migrated into neoplastic lesions and become intercalated into the existing neovasculature, where they assumed the roles of either pericytes or endothelial cells.[92],[99],[100]

Activating Invasion and Metastasis

The multistep process of invasion and metastasis has been schematized as a sequence of discrete steps, often termed the invasion–metastasis cascade.[101],[102] This depiction portrays a succession of cell-biologic changes, beginning with local invasion, then intravasation by cancer cells into nearby blood and lymphatic vessels, transit of cancer cells through the lymphatic and hematogenous systems, followed by the escape of cancer cells from the lumina of such vessels into the parenchyma of distant tissues (extravasation), the formation of small nests of cancer cells (micrometastases), and finally, the growth of micrometastatic lesions into macroscopic tumors, this last step being termed *colonization*. These steps have largely been studied in the context of carcinoma pathogenesis. Indeed, when viewed through the prism of the invasion–metastasis cascade, the diverse tumors of this class appear to behave in similar ways.

During the malignant progression of carcinomas, the neoplastic cells typically develop alterations in their shape as well as their attachment to other cells and to the extracellular matrix (ECM). The best-characterized alteration involves the loss by carcinoma cells of E-cadherin, a key epithelial cell-to-cell adhesion molecule. By forming adherens junctions between adjacent epithelial cells, E-cadherin helps to assemble epithelial cell sheets and to maintain the quiescence of the cells within these sheets. Moreover, increased expression of E-cadherin has been well established as an antagonist of invasion and metastasis, whereas a reduction of its expression is known to potentiate these behaviors. The frequently observed downregulation and occasional mutational inactivation of the E-cadherin–encoding gene, *CDH1,* in human carcinomas provides strong support for its role as a key suppressor of the invasion–metastasis hallmark capability.[103],[104]

Notably, the expression of genes encoding other cell-to-cell and cell-to-ECM adhesion molecules is also significantly altered in the cells of many highly aggressive carcinomas, with those favoring cytostasis typically being downregulated. Conversely, adhesion molecules normally associated with the cell migrations that occur during embryogenesis and inflammation are often upregulated. For example, N-cadherin, which is normally expressed in migrating neurons and mesenchymal cells during organogenesis, is upregulated in many invasive carcinoma cells, replacing the previously expressed E-cadherin.[104]

Research into the capability for invasion and metastasis has accelerated dramatically over the past decade as powerful new research tools, and refined experimental models have become available. Although still an emerging field replete with major unanswered questions, significant progress has been made in delineating important features of this complex hallmark capability. An admittedly incomplete representation of these advances is highlighted as follows.

The Epithelial-to-Mesenchymal Transition Program Broadly Regulates Invasion and Metastasis

A developmental regulatory program, termed the EMT, has become implicated as a prominent means by which neoplastic epithelial cells can acquire the abilities to invade, resist apoptosis, and disseminate.[105],[106],[107],[108],[109],[110] By co-opting a process involved in various steps of embryonic morphogenesis and wound healing, carcinoma cells can concomitantly acquire multiple attributes that enable invasion and metastasis. This multifaceted EMT program can be activated transiently or stably,

and to differing degrees, by carcinoma cells during the course of invasion and metastasis.

A set of pleiotropically acting transcriptional factors (TF), including Snail, Slug, Twist, and Zeb1/2, orchestrate the EMT and related migratory processes during embryogenesis; most were initially identified by developmental genetics. These transcriptional regulators are expressed in various combinations in a number of malignant tumor types. Some of these EMT-TFs have been shown in experimental models of carcinoma formation to be causally important for programming invasion; others have been found to elicit metastasis when experimentally expressed in primary tumor cells.[105],[111],[112],[113],[114] Included among the cell-biologic traits evoked by these EMT-TFs are loss of adherens junctions and associated conversion from a polygonal/epithelial to a spindly/fibroblastic morphology, concomitant with expression of secreted matrix-degrading enzymes, increased motility, and heightened resistance to apoptosis, which are implicated in the processes of invasion and metastasis. Several of these transcription factors can directly repress E-cadherin gene expression, thereby releasing neoplastic epithelial cells from this key suppressor of motility and invasiveness.[115]

The available data suggest that EMT-TFs regulate one another as well as overlapping sets of target genes. Results from developmental genetics indicate that contextual signals received from neighboring cells in the embryo are involved in triggering expression of these transcription factors in cells that are destined to pass through an EMT[111]; in an analogous fashion, heterotypic interactions of cancer cells with adjacent tumor-associated stromal cells have been shown to induce expression of the malignant cell phenotypes that are known to be choreographed by one or more of these EMT-TFs.[116],[117] Moreover, cancer cells at the invasive margins of certain carcinomas can be seen to have undergone an EMT, suggesting that these cancer cells are subject to microenvironmental stimuli distinct from those received by cancer cells located in the cores of these lesions.[118] Although the evidence is still incomplete, it would appear that EMT-TFs are able to orchestrate most steps of the invasion–metastasis cascade, except perhaps the final step of colonization, which involves adaptation of cells originating in one tissue to the microenvironment of a foreign, potentially inhospitable tissue.

We still know rather little about the various manifestations and temporal stability of the mesenchymal state produced by an EMT. Indeed, it seems increasingly likely that many human carcinoma cells only experience a *partial EMT*, in which they acquire mesenchymal markers while retaining many preexisting epithelial ones. Although the expression of EMT-TFs has been observed in certain nonepithelial tumor types, such as sarcomas and neuroectodermal tumors, their roles in programming malignant traits in these tumors are presently poorly documented. Additionally, it remains to be determined whether aggressive carcinoma cells invariably acquire their malignant capabilities through activation of components of the EMT program, or whether alternative regulatory programs can also enable expression of these traits.

Heterotypic Contributions of Stromal Cells to Invasion and Metastasis

As mentioned previously, cross-talk between cancer cells and cell types of the neoplastic stroma is involved in the acquired capabilities of invasiveness and metastasis.[94],[119],[120],[121] For example, mesenchymal stem cells (MSC) present in the tumor stroma have been found to secrete CCL5/RANTES in response to signals released by cancer cells; CCL5 then acts reciprocally on the cancer cells to stimulate invasive behavior.[122] In other work, carcinoma cells secreting IL-1 have been shown to induce MSCs to synthesize a spectrum of other cytokines that proceed thereafter to promote activation of the EMT program in the carcinoma cells; these effectors include IL-6, IL-8, growth-regulated oncogene alpha (GRO-α), and prostaglandin E2.[123]

Macrophages at the tumor periphery can foster local invasion by supplying matrix-degrading enzymes such as metalloproteinases and cysteine cathepsin proteases[76],[120],[124],[125]; in one model system, the invasion-promoting macrophages are activated by IL-4 produced by the cancer cells.[126] And in an experimental model of metastatic breast cancer, tumor-associated macrophages (TAM) supply epidermal growth factor (EGF) to breast cancer cells, while the cancer cells reciprocally stimulate the macrophages with colony stimulating factor 1 (CSF-1). Their concerted interactions facilitate intravasation into the circulatory system and metastatic dissemination of the cancer cells.[94],[127]

Observations like these indicate that the phenotypes of high-grade malignancy do not arise in a strictly cell-autonomous manner, and that their manifestation cannot be understood solely through analyses of signaling occurring within tumor cells. One important implication of the EMT model, still untested, is that the ability of carcinoma cells in primary tumors to negotiate most of the steps of the invasion–metastasis cascade may be acquired in certain tumors without the requirement that these cells undergo additional mutations beyond those that were needed for primary tumor formation.

Plasticity in the Invasive Growth Program

The role of contextual signals in inducing an invasive growth capability (often via an EMT) implies the possibility of reversibility, in that cancer cells that have disseminated from a primary tumor to more distant tissue sites may no longer benefit from the activated stroma and the EMT-inducing signals that they experienced while residing in the primary tumor. In the absence of ongoing exposure to these signals, carcinoma cells may revert in their new tissue environment to a noninvasive state. Thus, carcinoma cells that underwent an EMT during initial invasion and metastatic dissemination may reverse this metamorphosis, doing so via a mesenchymal-to-epithelial transition (MET). This plasticity may result in the formation of new tumor colonies of carcinoma cells exhibiting an organization and histopathology similar to those created by carcinoma cells in the primary tumor that never experienced an EMT.[128]

Distinct Forms of Invasion May Underlie Different Cancer Types

The EMT program regulates a particular type of invasiveness that has been termed *mesenchymal*. In addition, two other distinct modes of invasion have been identified and implicated in cancer cell invasion.[129],[130] *Collective invasion* involves phalanxes of cancer cells advancing en masse into adjacent tissues and is characteristic of, for example, squamous cell carcinomas. Interestingly, such cancers are rarely metastatic, suggesting that this form of invasion lacks certain functional attributes that facilitate metastasis. Less clear is the prevalence of an *amoeboid* form of invasion,[131],[132] in which individual cancer cells show morphologic plasticity, enabling them to slither through existing interstices in the ECM rather than clearing a path for themselves, as occurs in both the mesenchymal and collective forms of invasion. It is presently unresolved whether cancer cells participating in the collective and amoeboid forms of invasion employ components of the EMT program, or whether entirely different cell-biologic programs are responsible for choreographing these alternative invasion programs.

Another emerging concept, noted previously, involves the facilitation of cancer cell invasion by inflammatory cells that assemble at the boundaries of tumors, producing the ECM-degrading enzymes and other factors that enable invasive growth.[76],[94],[120],[133] These functions may obviate the need of invading cancer cells to produce these proteins through activation of EMT programs. Thus, rather than synthesizing these proteases themselves, cancer cells may secrete chemoattractants that recruit proinvasive inflammatory cells; the latter then proceed to produce matrix-degrading enzymes that enable invasive growth.

The Daunting Complexity of Metastatic Colonization

Metastasis can be broken down into two major phases: the physical dissemination of cancer cells from the primary tumor to distant tissues, and the adaptation of these cells to foreign tissue microenvironments that results in successful colonization (i.e., the growth of micrometastases into macroscopic tumors). The multiple steps of dissemination would seem to lie within the purview of the EMT and similarly acting migratory programs. Colonization, however, is not strictly coupled with physical dissemination, as evidenced by the presence in many patients of myriad micrometastases that have disseminated but never progress to form macroscopic metastatic tumors.[101],[102],[134],[135],[136]

In some types of cancer, the primary tumor may release systemic suppressor factors that render such micrometastases dormant, as revealed clinically by explosive metastatic growth soon after resection of the primary growth.[87],[137] In others, however, such as breast cancer and melanoma, macroscopic metastases may erupt decades after a primary tumor has been surgically removed or pharmacologically destroyed. These metastatic tumor growths evidently reflect dormant micrometastases that have solved, after much trial and error, the complex problem of adaptation to foreign tissue microenvironments, allowing subsequent tissue colonization.[135],[136],[138] Implicit here is the notion that most disseminated cancer cells are likely to be poorly adapted, at least initially, to the microenvironment of the tissue in which they have landed. Accordingly, each type of disseminated cancer cell may need to develop its own set of ad hoc solutions to the problem of thriving in the microenvironment of one or another foreign tissue.[139]

One can infer from such natural histories that micrometastases may lack certain hallmark capabilities necessary for vigorous growth, such as the ability to activate angiogenesis. Indeed, the inability of certain experimentally generated dormant micrometastases to form macroscopic tumors has been ascribed to their failure to activate tumor angiogenesis.[135],[140] Additionally, recent experiments have shown that nutrient starvation can induce intense autophagy that causes cancer cells to shrink and adopt a state of reversible dormancy. Such cells may exit this state and resume active growth and proliferation when permitted by changes in tissue microenvironment, such as increased availability of nutrients, inflammation from causes such as infection or wound healing, or other local abnormalities.[49],[141] Other mechanisms of micrometastatic dormancy may involve antigrowth signals embedded in normal tissue ECM[138] and tumor-suppressing actions of the immune system.[135],[142]

Metastatic dissemination has long been depicted as the last step in multistep primary tumor progression; indeed, for many tumors, that is likely the case, as illustrated by recent genome sequencing studies that provide genetic evidence for clonal evolution of pancreatic ductal adenocarcinoma to a metastatic stage.[143],[144],[145] Importantly, however, recent results have revealed that some cancer cells can disseminate remarkably early, dispersing from apparently noninvasive premalignant lesions in both mice and humans.[146],[147] Additionally, micrometastases can be spawned from primary tumors that are not obviously invasive but possess a neovasculature lacking in luminal integrity.[148] Although cancer cells can clearly disseminate from such preneoplastic lesions and seed the bone marrow and other tissues, their capability to colonize these sites and develop into pathologically significant macrometastases remains unproven. At present, we view this early metastatic dissemination as a demonstrable phenomenon in mice and humans, the clinical significance of which is yet to be established.

Having developed such a tissue-specific colonizing ability, the cells in metastatic colonies may proceed to disseminate further, not only to new sites in the body, but also back to the primary tumors in which their ancestors arose. Accordingly, tissue-specific colonization programs that are evident among certain cells within a primary tumor may originate not from classical tumor progression occurring entirely within

the primary lesion, but instead from immigrants that have returned home.[149] Such reseeding is consistent with the aforementioned studies of human pancreatic cancer metastasis.[143],[144],[145] Stated differently, the phenotypes and underlying gene expression programs in focal subpopulations of cancer cells within primary tumors may reflect, in part, the reverse migration of their distant metastatic progeny.

Implicit in this *self-seeding* process is another notion: The supportive stroma that arises in a primary tumor and contributes to its acquisition of malignant traits provides a hospitable site for reseeding and colonization by circulating cancer cells released from metastatic lesions.

Clarifying the regulatory programs that enable metastatic colonization represents an important agenda for future research. Substantial progress is being made, for example, in defining sets of genes (*metastatic signatures*) that correlate with and appear to facilitate the establishment of macroscopic metastases in specific tissues.[139],[146],[150],[151],[152] Importantly, metastatic colonization almost certainly requires the establishment of a permissive tumor microenvironment composed of critical stromal support cells. For these reasons, the process of colonization is likely to encompass a large number of cell-biologic programs that are, in aggregate, considerably more complex and diverse than the preceding steps of metastatic dissemination that allow carcinoma cells to depart from primary tumors to sites of lodging and extravasation throughout the body.

Reprogramming Energy Metabolism

The chronic and often uncontrolled cell proliferation that represents the essence of neoplastic disease involves not only deregulated control of cell proliferation but also corresponding adjustments of energy metabolism in order to fuel cell growth and division. Under aerobic conditions, normal cells process glucose, first to pyruvate via glycolysis in the cytosol and thereafter via oxidative phosphorylation to carbon dioxide in the mitochondria. Under anaerobic conditions, glycolysis is favored and relatively little pyruvate is dispatched to the oxygen-consuming mitochondria. Otto Warburg first observed an anomalous characteristic of cancer cell energy metabolism[153],[154],[155]: Even in the presence of oxygen, cancer cells can reprogram their glucose metabolism, and thus their energy production, leading to a state that has been termed *aerobic glycolysis*.

The existence of this metabolic specialization operating in cancer cells has been substantiated in the ensuing decades. A key signature of aerobic glycolysis is upregulation of glucose transporters, notably GLUT1, which substantially increases glucose import into the cytoplasm.[156],[157],[158] Indeed, markedly increased uptake and utilization of glucose has been documented in many human tumor types, most readily by noninvasively visualizing glucose uptake using positron-emission tomography (PET) with a radiolabeled analog of glucose (^{18}F-fluorodeoxyglucose [FDG]) as a reporter.

Glycolytic fueling has been shown to be associated with activated oncogenes (e.g., *RAS*, *MYC*) and mutant tumor suppressors (e.g., *TP53*),[18],[156],[157],[159] whose alterations in tumor cells have been selected primarily for their benefits in conferring the hallmark capabilities of cell proliferation, subversion of cytostatic controls, and attenuation of apoptosis. This reliance on glycolysis can be further accentuated under the hypoxic conditions that operate within many tumors: The hypoxia response system acts pleiotropically to upregulate glucose transporters and multiple enzymes of the glycolytic pathway.[156],[157],[160] Thus, both the Ras oncoprotein and hypoxia can independently increase the levels of the HIF1α and HIF2α hypoxia-response transcription factors, which in turn upregulate glycolysis.[160],[161],[162]

The reprogramming of energy metabolism is seemingly counterintuitive, in that cancer cells must compensate for the ~18-fold lower efficiency of ATP production afforded by glycolysis relative to mitochondrial oxidative phosphorylation. According to one long-forgotten[163] and a recently revived and refined hypothesis,[164] increased glycolysis allows the diversion of glycolytic intermediates into various biosynthetic pathways, including those generating nucleosides and amino acids. In turn, this facilitates the biosynthesis of the macromolecules and organelles required for assembling new cells. Moreover, Warburg-like metabolism seems to be present in many rapidly dividing embryonic tissues, once again suggesting a role in supporting the large-scale biosynthetic programs that are required for active cell proliferation.

Interestingly, some tumors have been found to contain two subpopulations of cancer cells that differ in their energy-generating pathways. One subpopulation consists of glucose-dependent (Warburg-effect) cells that secrete lactate, whereas cells of the second subpopulation preferentially import and utilize the lactate produced by their neighbors as their main energy source, employing part of the citric acid cycle to do so.[165],[166],[167],[168] These two populations evidently function symbiotically: The hypoxic cancer cells depend on glucose for fuel and secrete lactate as waste, which is imported and preferentially used as fuel by their better oxygenated brethren. Although this provocative mode of intratumoral symbiosis has yet to be generalized, the cooperation between lactate-secreting and lactate-utilizing cells to fuel tumor growth is in fact not an invention of tumors, but rather again reflects the co-opting of a normal physiologic mechanism, in this case one operative in muscle[165],[167],[168] and the brain.[169] Additionally, it is becoming apparent that oxygenation, ranging from normoxia to hypoxia, is not necessarily static in tumors, but instead fluctuates temporally and regionally,[170] likely as a result of the instability and chaotic organization of the tumor-associated neovasculature.

Finally, the notion of the Warburg effect needs to be refined for most if not all tumors exhibiting aerobic glycolysis. The effect does not involve a switching off oxidative phosphorylation concurrent with activation of glycolysis, the latter then serving as the sole source of energy. Rather, cancer cells become highly adaptive, utilizing both mitochondrial oxidative phosphorylation and glycolysis in varying proportions to generate fuel (ATP) and biosynthetic precursors needed for chronic cell proliferation. Finally, this capability for reprograming energy metabolism, dubbed to be an *emerging hallmark* in 2011,[2] is clearly intertwined with the hallmarks conveying deregulated proliferative signals and evasion of growth suppressors, as discussed earlier. As such, its status as a discrete, independently acquired hallmark remains unclear, despite growing appreciation of its importance as a crucial component of the neoplastic growth state.

Evading Immune Destruction

The eighth hallmark reflects the role played by the immune system in antagonizing the formation and progression of tumors. A long-standing theory of immune surveillance posited that cells and tissues are constantly monitored by an ever alert immune system, and that such immune surveillance is responsible for recognizing and eliminating the vast majority of incipient cancer cells and, thus, nascent tumors.[171],[172] According to this logic, clinical detectable cancers have somehow managed to avoid detection by the various arms of the immune system, or have been able to limit the extent of immunologic killing, thereby evading eradication.

The role of defective immunologic monitoring of tumors would seem to be validated by the striking increases of certain cancers in immune-compromised individuals.[173] However, the great majority of these are virus-induced cancers, suggesting that much of the control of this class of cancers normally depends on reducing viral burden in infected individuals, in part through eliminating virus-infected cells.

These observations, therefore, shed little light on the possible role of the immune system in limiting formation of the >80% of tumors of nonviral etiology. In recent years, however, an increasing body of evidence, both from genetically engineered mice and from clinical epidemiology, suggests that the immune system operates as a significant barrier to tumor formation and progression, at least in some forms of non–virus-induced cancer.[174],[175],[176],[177]

When mice genetically engineered to be deficient for various components of the immune system were assessed for the development of carcinogen-induced tumors, it was observed that tumors arose more frequently and/or grew more rapidly in the immunodeficient mice relative to immune-competent controls. In particular, deficiencies in the development or function of either $CD8^+$ cytotoxic T lymphocytes (CTL), $CD4^+$ T_H1 helper T cells, or natural killer (NK) cells, each led to demonstrable increases in tumor incidence. Moreover, mice with combined immunodeficiencies in both T cells and NK cells were even more susceptible to cancer development. The results indicated that, at least in certain experimental models, both the innate and adaptive cellular arms of the immune system are able to contribute significantly to immune surveillance and, thus, tumor eradication.[142],[178]

In addition, transplantation experiments have shown that cancer cells that originally arose in immunodeficient mice are often inefficient at initiating secondary tumors in syngeneic immunocompetent hosts, whereas cancer cells from tumors arising in immunocompetent mice are equally efficient at initiating transplanted tumors in both types of hosts.[142],[178] Such behavior has been interpreted as follows: Highly immunogenic cancer cell clones are routinely eliminated in immunocompetent hosts—a process that has been referred to as *immunoediting*—leaving behind only weakly immunogenic variants to grow and generate solid tumors. Such weakly immunogenic cells can thereafter successfully colonize both immunodeficient and immunocompetent hosts. Conversely, when arising in immunodeficient hosts, the immunogenic cancer cells are not selectively depleted and can, instead, prosper along with their weakly immunogenic counterparts. When cells from such nonedited tumors are serially transplanted into syngeneic recipients, the immunogenic cancer cells are rejected when they confront, for the first time, the competent immune systems of their secondary hosts.[179] (Unanswered in these particular experiments is the question of whether the chemical carcinogens used to induce such tumors are prone to generate cancer cells that are especially immunogenic.)

Clinical epidemiology also increasingly supports the existence of antitumoral immune responses in some forms of human cancer.[180],[181],[182] For example, patients with colon and ovarian tumors that are heavily infiltrated with CTLs and NK cells have a better prognosis than those who lack such abundant killer lymphocytes.[176],[177],[182],[183] The case for other cancers is suggestive but less compelling and is the subject of ongoing investigation. Additionally, some immunosuppressed organ transplant recipients have been observed to develop donor-derived cancers, suggesting that in ostensibly tumor-free organ donors, the cancer cells were held in check in a dormant state by a functional immune system,[184] only to launch into proliferative expansion once these *passenger cells* in the transplanted organ found themselves in immunocompromised patients who lack the physiologically important capabilities to mount immune responses that would otherwise hold latent cancer cells in check or eradicate them.

Still, the epidemiology of chronically immunosuppressed patients does not indicate significantly increased incidences of the major forms of nonviral human cancers, as noted previously. This might be taken as an argument against the importance of immune surveillance as an effective barrier to tumorigenesis and tumor progression. We note, however, that HIV and pharmacologically immunosuppressed patients are predominantly immunodeficient in the T- and B-cell compartments and thus do not present with the multicomponent immunologic deficiencies that have been produced in the

genetically engineered mutant mice lacking both NK cells and CTLs. This leaves open the possibility that such patients still have residual capability for mounting an anticancer immunologic defense that is mediated by NK and other innate immune cells.

In truth, the previous discussions of cancer immunology simplify tumor–host immunologic interactions, because highly immunogenic cancer cells may well succeed in evading immune destruction by disabling components of the immune system that have been dispatched to eliminate them. For example, cancer cells may paralyze infiltrating CTLs and NK cells by secreting TGF-β or other immunosuppressive factors.[32],[185],[186] Alternatively, cancer cells may express immunosuppressive cell-surface ligands, such as PD-L1, that prevent activation of the cytotoxic mechanisms of the CTLs. These PD-L1 molecules serve as ligands for the PD-1 receptors displayed by the CTLs, together exemplifying a system of *checkpoint* ligands and receptors that serve to constrain immune responses in order to avoid autoimmunity.[187],[188],[189] Yet other localized immunosuppressive mechanisms operate through the recruitment of inflammatory cells that can actively suppress CTL activity, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC).[174],[190],[191],[192],[193]

In summary, these eight hallmarks each contribute qualitatively distinct capabilities that seem integral to most lethal forms of human cancer. Certainly, the balance and relative importance of their respective contributions to disease pathogenesis will vary among cancer types, and some hallmarks may be absent or of minor importance in some cases. Still, there is reason to postulate their generality and, thus, their applicability to understanding the biology of human cancer. Next, we turn to the question of how these capabilities are acquired during the multistep pathways through which cancers develop, focusing on two facilitators that are commonly involved.

Two Ubiquitous Characteristics Facilitate the Acquisition of Hallmark Capabilities

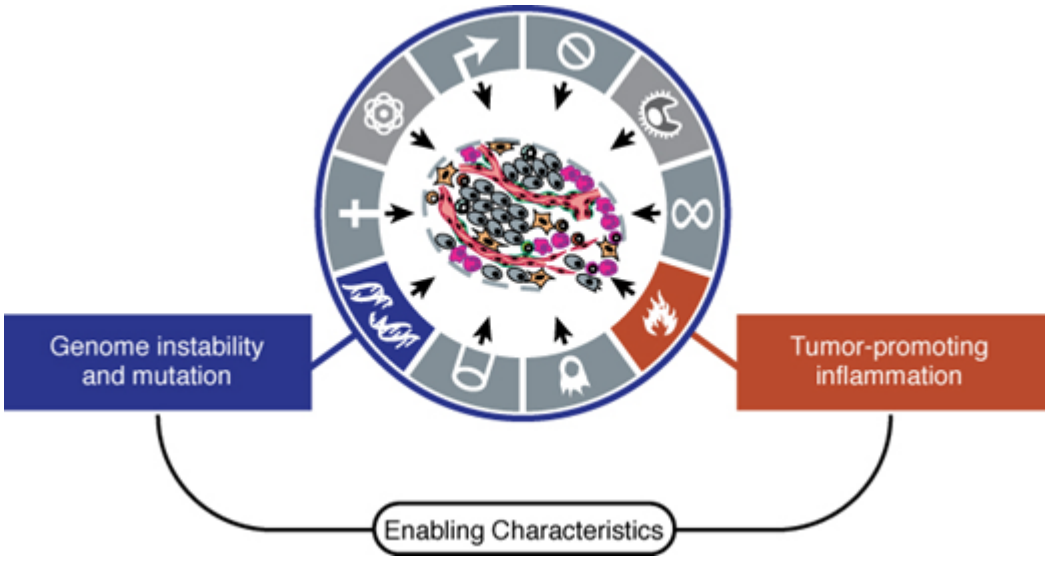
We have defined the hallmarks of cancer as acquired functional capabilities that allow cancer cells to survive, proliferate, and disseminate. Their acquisition is made possible by two *enabling characteristics* (**Fig. 2.2**). Most prominent is the development of genomic instability in cancer cells, which generates random mutations, including chromosomal rearrangements, among which are rare genetic changes that can orchestrate individual hallmark capabilities. A second enabling characteristic involves the inflammatory state of premalignant and frankly malignant lesions. A variety of cells of the innate and adaptive immune system infiltrate neoplasias, some of which serve to promote tumor progression through various means.

FIGURE 2.2

Enabling characteristics.

Two ostensibly generic characteristics of cancer cells and the neoplasias they create are involved in the acquisition of the hallmark capabilities. First and foremost, the impairment of genome maintenance systems in aberrantly proliferating cancer cells enables the generation of mutations in genes that contribute to multiple hallmarks. Secondarily, neoplasias invariably attract cells of the innate immune system that are programmed to heal wounds and fight infections; these cells, including macrophages, neutrophils, and partially differentiated myeloid cells, can contribute functionally to acquisition of many of the hallmark capabilities.

(Adapted from Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–674.)



An Enabling Characteristic: Genome Instability and Mutation

Acquisition of the multiple hallmarks enumerated previously depends in large part on a succession of alterations in the genomes of neoplastic cells. Basically, certain mutant genotypes can confer selective advantage to particular subclones among proliferating nests of incipient cancer cells, enabling their outgrowth and eventual dominance in a local tissue environment. Accordingly, multistep tumor progression can be portrayed as a succession of clonal expansions, most of which are triggered by the chance acquisition of an enabling mutation.

Indeed, it is apparent that virtually every human cancer cell genome carries mutant alleles of one or several growth-regulating genes, underscoring the central importance of these genetic alterations in driving malignant progression.[194] Still, we note that many heritable phenotypes—including, notably, inactivation of tumor suppressor genes—can be acquired through epigenetic mechanisms, such as DNA methylation and histone modifications.[195],[196],[197],[198] Thus, many clonal expansions may also be triggered by heritable nonmutational changes affecting the regulation of gene expression. At present, the relative importance of genetic versus heritable epigenetic alterations to the various clonal expansions remains unclear, and likely, varies broadly amongst the catalog of human cancer types.

The extraordinary ability of genome maintenance systems to detect and resolve defects in the DNA ensures that rates of spontaneous mutation in normal cells of the body are typically very low, both in quiescent cells and during cell division. The genomes of most cancer cells, by contrast, are replete with these alterations, reflecting loss of genomic integrity with concomitantly increased rates of mutation. This heightened mutability appears to accelerate the generation of variant cells, facilitating the selection of those cells whose advantageous phenotypes enable their clonal expansion.[199],[200] This mutability is achieved through increased sensitivity to mutagenic agents, through a breakdown in one or several components of the genomic maintenance machinery, or both. In addition, the accumulation of mutations can be accelerated by aberrations that compromise the surveillance systems that normally monitor genomic integrity and force such genetically damaged cells into either quiescence, senescence, or apoptosis.[201],[202],[203] The role of TP53 is central here, leading to its being called the *guardian of the genome*.[204]

A diverse array of defects affecting various components of the DNA-maintenance machinery, referred to as the *caretakers* of the genome,[205] have been documented. The catalog of defects in these caretaker genes includes those whose products are involved in (1) detecting DNA damage and activating the

repair machinery, (2) directly repairing damaged DNA, and (3) inactivating or intercepting mutagenic molecules before they have damaged the DNA.[199],[201],[202],[206],[207],[208] From a genetic perspective, these caretaker genes behave much like tumor suppressor genes, in that their functions are often lost during the course of tumor progression, with such losses being achieved either through inactivating mutations or via epigenetic repression. Mutant copies of many of these caretaker genes have been introduced into the mouse germ line, resulting, not unexpectedly, in increased cancer incidence, thus supporting their involvement in human cancer development.[209]

In addition, research over the past decade has revealed another major source of tumor-associated genomic instability. As described earlier, the loss of telomeric DNA in many tumors generates karyotypic instability and associated amplification and deletion of chromosomal segments.[58] When viewed in this light, telomerase is more than an enabler of the hallmark capability for unlimited replicative potential. It must also be added to the list of critical caretakers responsible for maintaining genome integrity.

Advances in the molecular–genetic analysis of cancer cell genomes have provided the most compelling demonstrations of function-altering mutations and of ongoing genomic instability during tumor progression. One type of analysis—comparative genomic hybridization (CGH)—documents the gains and losses of gene copy number across the cell genome. In many tumors, the pervasive genomic aberrations revealed by CGH provide clear evidence for loss of control of genome integrity. Importantly, the recurrence of specific aberrations (both amplifications and deletions) at particular locations in the genome indicates that such sites are likely to harbor genes whose alteration favors neoplastic progression.[210]

More recently, with the advent of efficient and economical DNA sequencing technologies, higher resolution analyses of cancer cell genomes have become possible. Early studies are revealing distinctive patterns of DNA mutations in different tumor types (see: **http://cancergenome.nih.gov/**). In the not-too-distant future, the sequencing of entire cancer cell genomes promises to clarify the importance of ostensibly random mutations scattered across cancer cell genomes.[194] Thus, the use of whole genome resequencing offers the prospect of revealing recurrent genetic alterations (i.e., those found in multiple independently arising tumors) that in aggregate represent only minor proportions of the tumors of a given type. The recurrence of such mutations, despite their infrequency, may provide clues about the regulatory pathways playing causal roles in the pathogenesis of the tumors under study.

These surveys of cancer cell genomes have shown that the specifics of genome alteration vary dramatically between different tumor types. Nonetheless, the large number of already documented genome maintenance and repair defects, together with abundant evidence of widespread destabilization of gene copy number and nucleotide sequence, persuade us that instability of the genome is inherent to the cancer cells forming virtually all types of human tumors. This leads, in turn, to the conclusion that the defects in genome maintenance and repair are selectively advantageous and, therefore, instrumental for tumor progression, if only because they accelerate the rate at which evolving premalignant cells can accumulate favorable genotypes. As such, genome instability is clearly an *enabling characteristic* that is causally associated with the acquisition of hallmark capabilities.

An Enabling Characteristic: Tumor-Promoting Inflammation

Among the cells recruited to the stroma of carcinomas are a variety of cell types of the immune system that mediate various inflammatory functions. Pathologists have long recognized that some (but not all) tumors are densely infiltrated by cells of both the innate and adaptive arms of the immune system, thereby mirroring inflammatory conditions arising in nonneoplastic tissues.[211] With the advent of better

markers for accurately identifying the distinct cell types of the immune system, it is now clear that virtually every neoplastic lesion contains immune cells present at densities ranging from subtle infiltrations detectable only with cell type–specific antibodies to gross inflammations that are apparent even by standard histochemical staining techniques.[183] Historically, such immune responses were largely thought to reflect an attempt by the immune system to eradicate tumors, and indeed, there is increasing evidence for antitumoral responses to many tumor types with an attendant pressure on the tumor to evade immune destruction,[174],[176],[177],[183] as discussed earlier.

By 2000, however, there were also clues that tumor-associated inflammatory responses can have the unanticipated effect of facilitating multiple steps of tumor progression, thereby helping incipient neoplasias to acquire hallmark capabilities. In the ensuing years, research on the intersections between inflammation and cancer pathogenesis has blossomed, producing abundant and compelling demonstrations of the functionally important tumor-promoting effects that immune cells—largely of the innate immune system—have on neoplastic progression.[19],[53],[94],[174],[212],[213] Inflammatory cells can contribute to multiple hallmark capabilities by supplying signaling molecules to the tumor microenvironment, including growth factors that sustain proliferative signaling; survival factors that limit cell death; proangiogenic factors; extracellular matrix-modifying enzymes that facilitate angiogenesis, invasion, and metastasis; and inductive signals that lead to activation of EMT and other hallmark-promoting programs.[53],[94],[116],[212],[213]

Importantly, localized inflammation is often apparent at the earliest stages of neoplastic progression and is demonstrably capable of fostering the development of incipient neoplasias into full-blown cancers.[94],[214] Additionally, inflammatory cells can release chemicals—notably, reactive oxygen species—that are actively mutagenic for nearby cancer cells, thus accelerating their genetic evolution toward states of heightened malignancy.[53] As such, inflammation by selective cell types of the immune system is demonstrably an *enabling characteristic* for its contributions to the acquisition of hallmark capabilities. The cells responsible for this enabling characteristic are described in the following section.

The Constituent Cell Types of the Tumor Microenvironment

Over the past 2 decades, tumors have increasingly been recognized as tissues whose complexity approaches and may even exceed that of normal healthy tissues. This realization contrasts starkly with the earlier, reductionist view of a tumor as nothing more than a collection of relatively homogeneous cancer cells, whose entire biology could be understood by elucidating the cell-autonomous properties of these cells (**Fig. 2.3A**). Rather, assemblages of diverse cell types associated with malignant lesions are increasingly documented to be functionally important for the manifestation of symptomatic disease (**Fig. 2.3B**). When viewed from this perspective, the biology of a tumor can only be fully understood by studying the individual specialized cell types within it. We enumerate as follows a set of accessory cell types recruited directly or indirectly by neoplastic cells into tumors, where they contribute in important ways to the biology of many tumors, and we discuss the regulatory mechanisms that control their individual and collective functions. Most of these observations stem from the study of carcinomas, in which the neoplastic epithelial cells constitute a compartment (the parenchyma) that is clearly distinct from the mesenchymal cells forming the tumor-associated stroma.

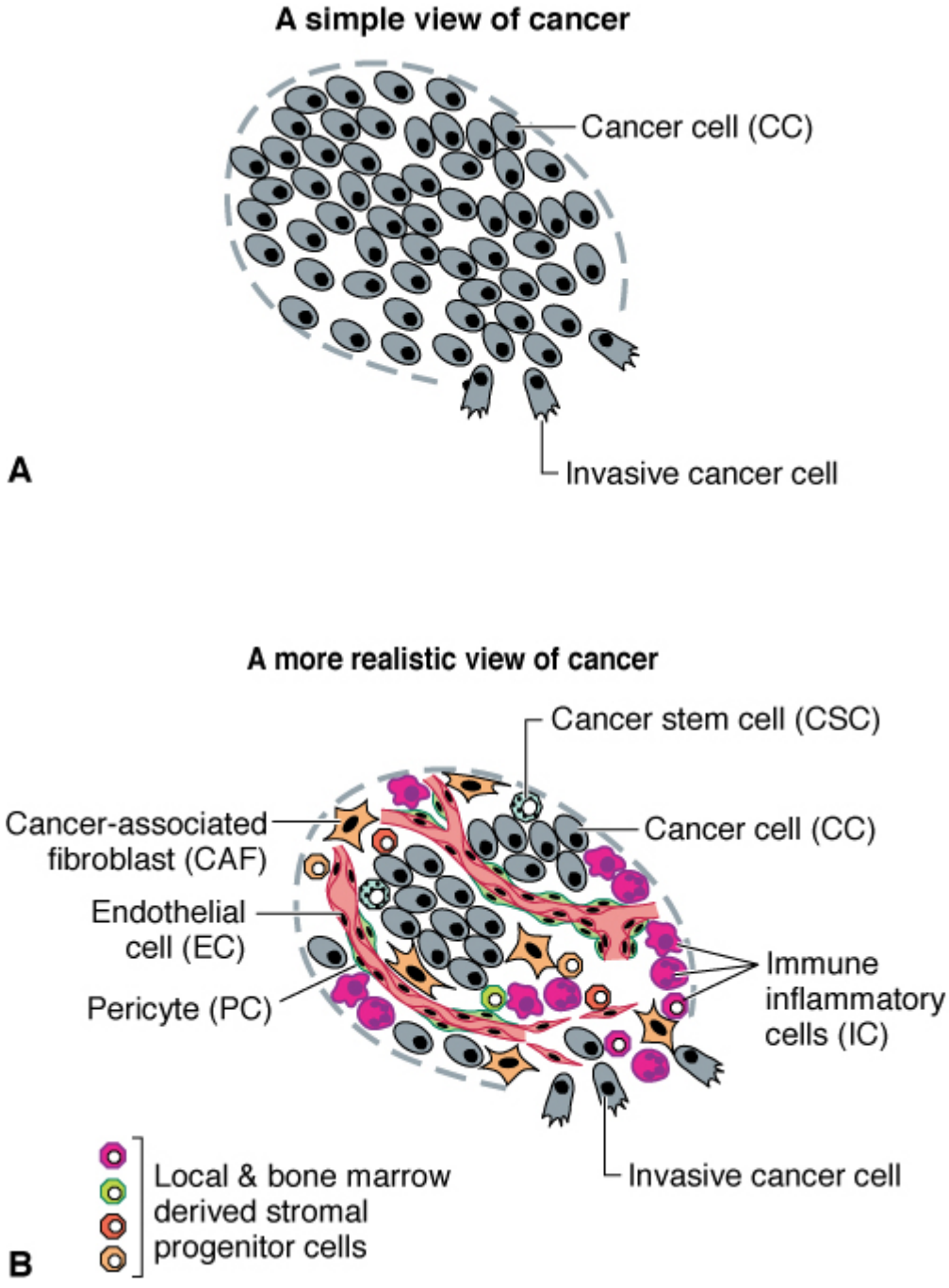
FIGURE 2.3

Tumors as outlaw organs.

Research aimed at understanding the biology of tumors has historically focused on the cancer cells, which constitute the drivers of neoplastic disease. This view of tumors as nothing more than masses of

cancer cells **(A)** ignores an important reality, that cancer cells recruit and corrupt a variety of normal cell types that form the tumor-associated stroma. Once formed, the stroma acts reciprocally on the cancer cells, affecting almost all of the traits that define the neoplastic behavior of the tumor as a whole **(B).** The assemblage of heterogeneous populations of cancer cells and stromal cells is often referred to as the tumor microenvironment (TME).

(Adapted from Hanahan D, Weinberg R. The hallmarks of cancer. *Cell* 2000;100:57–70; Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–674.)



Cancer-Associated Fibroblasts

Fibroblasts are found in various proportions across the spectrum of carcinomas, in many cases constituting the preponderant cell population of the tumor stroma. The term *cancer-associated fibroblasts* (CAFs) subsumes at least two distinct cell types: (1) cells with similarities to the fibroblasts that create the structural foundation supporting most normal epithelial tissues, and (2) myofibroblasts,

whose biologic roles and properties differ markedly from those of the widely distributed tissue-derived fibroblasts. Myofibroblasts are identifiable by their expression of α-smooth muscle actin (αSMA). They are rare in most healthy epithelial tissues, although certain tissues, such as the liver and pancreas, contain appreciable numbers of αSMA-expressing cells. Myofibroblasts transiently increase in abundance in wounds and are also found in sites of chronic inflammation. Although beneficial to tissue repair, myofibroblasts are problematic in chronic inflammation, in that they contribute to the pathologic fibrosis observed in tissues such as the lung, kidney, and liver.

Recruited myofibroblasts and variants of normal tissue-derived fibroblastic cells have been demonstrated to enhance tumor phenotypes, notably cancer cell proliferation, angiogenesis, invasion, and metastasis. Their tumor-promoting activities have largely been defined by transplantation of cancer-associated fibroblasts admixed with cancer cells into mice, and more recently by genetic and pharmacologic perturbation of their functions in tumor-prone mice.[8],[121],[133],[215],[216],[217],[218],[219] Because they secrete a variety of ECM components, cancer-associated fibroblasts are implicated in the formation of the desmoplastic stroma that characterizes many advanced carcinomas. The full spectrum of functions contributed by both subtypes of cancer-associated fibroblasts to tumor pathogenesis remains to be elucidated.

Endothelial Cells

Prominent among the stromal constituents of the TME are the endothelial cells forming the tumor-associated vasculature. Quiescent tissue capillary endothelial cells are activated by *angiogenic* regulatory factors to produce a neovasculature that sustains tumor growth concomitant with continuing endothelial cell proliferation and vessel morphogenesis. A network of interconnected signaling pathways involving ligands of signal-transducing receptors (e.g., the Angiopoeitin-1/2, Notch ligands, Semaphorin, Neuropilin, Robo, and Ephrin-A/B) is now known to be involved in regulating quiescent versus activated angiogenic endothelial cells, in addition to the aforementioned counterbalancing VEGF and TSP signals. This network of signaling pathways has been functionally implicated in developmental and tumor-associated angiogenesis, further illustrating the complex regulation of endothelial cell phenotypes.[220],[221],[222],[223],[224]

Other avenues of research are revealing distinctive gene expression profiles of tumor-associated endothelial cells and identifying cell-surface markers displayed on the luminal surfaces of normal versus tumor endothelial cells.[78],[225],[226] Differences in signaling, in transcriptome profiles, and in vascular *ZIP codes* will likely prove to be important for understanding the conversion of normal endothelial cells into tumor-associated endothelial cells. Such knowledge may lead, in turn, to opportunities to develop novel therapies that exploit these differences in order to selectively target tumor-associated endothelial cells. Additionally, the activated (*angiogenic*) tumor vasculature has been revealed as a barrier to efficient intravasation and a functional suppressor of cytotoxic T cells,[227] and thus, tumor endothelial cells can contribute to the hallmark capability for evading immune destruction. As such, another emerging concept is to normalize rather than ablate them, so as to improve immunotherapy[190] as well as delivery of chemotherapy.[228]

Closely related to the endothelial cells of the circulatory system are those forming lymphatic vessels.[229] Their role in the tumor-associated stroma, specifically in supporting tumor growth, is poorly understood. Indeed, because of high interstitial pressure within solid tumors, intratumoral lymphatic vessels are typically collapsed and nonfunctional; in contrast, however, there are often functional, actively growing (*lymphangiogenic*) lymphatic vessels at the periphery of tumors and in the adjacent normal tissues that cancer cells invade. These associated lymphatics likely serve as channels for the

seeding of metastatic cells in the draining lymph nodes that are commonly observed in a number of cancer types. Recent results that are yet to be generalized suggest an alternative role for the activated (i.e., lymphangiogenic) lymphatic endothelial cells associated with tumors, not in supporting tumor growth like the blood vessels, but in inducing (via VEGF-C–mediated signaling) a lymphatic tissue microenvironment that suppresses immune responses ordinarily marshaled from the draining lymph nodes.[230] As such, the real value to a tumor from activating the signaling circuit involving the ligand VEGF-C and its receptor VEGFR3 may be to facilitate the evasion of antitumor immunity by abrogating the otherwise immunostimulatory functions of draining lymphatic vessels and lymph nodes, with the collateral effect of inducing lymphatic endothelial cells to form the new lymphatic vessels that are commonly detected in association with tumors.

Pericytes

Pericytes represent a specialized mesenchymal cell type that are closely related to smooth muscle cells, with fingerlike projections that wrap around the endothelial tubing of blood vessels. In normal tissues, pericytes are known to provide paracrine support signals to the quiescent endothelium. For example, Ang-1 secreted by pericytes conveys antiproliferative stabilizing signals that are received by the Tie2 receptors expressed on the surface of endothelial cells. Some pericytes also produce low levels of VEGF that serve a trophic function in endothelial homeostasis.[93],[231] Pericytes also collaborate with the endothelial cells to synthesize the vascular basement membrane that anchors both pericytes and endothelial cells and helps vessel walls to withstand the hydrostatic pressure created by the blood.

Genetic and pharmacologic perturbation of the recruitment and association of pericytes has demonstrated the functional importance of these cells in supporting the tumor endothelium.[93],[217],[231] For example, the pharmacologic inhibition of signaling through the platelet-derived growth factor (PDGF) receptor expressed by tumor pericytes and bone marrow–derived pericyte progenitors results in reduced pericyte coverage of tumor vessels, which in turn destabilizes vascular integrity and function.[91],[217],[231] Interestingly, and in contrast, the pericytes of normal vessels are not prone to such pharmacologic disruption, providing another example of the differences in the regulation of normal quiescent and tumor vasculature. An intriguing hypothesis, still to be fully substantiated, is that tumors with poor pericyte coverage of their vasculature may be more prone to permit cancer cell intravasation into the circulatory system, thereby enabling subsequent hematogenous dissemination.[91],[148]

Immune Inflammatory Cells

Infiltrating cells of the immune system are increasingly accepted to be generic constituents of tumors. These inflammatory cells operate in conflicting ways: Both tumor-antagonizing and tumor-promoting leukocytes can be found in various proportions in most, if not all, neoplastic lesions. Evidence began to accumulate in the late 1990s that the infiltration of neoplastic tissues by cells of the immune system serves, perhaps counterintuitively, to promote tumor progression. Such work traced its conceptual roots back to the observed association of tumor formation with sites of chronic inflammation. Indeed, this led some to liken tumors to “wounds that do not heal.”[211],[232] In the course of normal wound healing and the resolution of infections, immune inflammatory cells appear transiently and then disappear, in contrast to their persistence in sites of chronic inflammation, where their presence has been associated with a variety of tissue pathologies, including fibrosis, aberrant angiogenesis, and as mentioned, neoplasia.[53],[233]

We now know that immune cells play diverse and critical roles in fostering tumorigenesis. The roster of

tumor-promoting inflammatory cells includes macrophage subtypes, mast cells, and neutrophils, as well as T and B lymphocytes.[96],[97],[119],[133],[212],[234],[235] Studies of these cells are yielding a growing list of tumor-promoting signaling molecules that they release, which include the tumor growth factor EGF, the angiogenic growth factors VEGF-A/-C, other proangiogenic factors such as FGF2, plus chemokines and cytokines that amplify the inflammatory state. In addition, these cells may produce proangiogenic and/or proinvasive matrix-degrading enzymes, including MMP-9 and other MMPs, cysteine cathepsin proteases, and heparanase.[94],[96] Consistent with the expression of these diverse signals, tumor-infiltrating inflammatory cells have been shown to induce and help sustain tumor angiogenesis, to stimulate cancer cell proliferation, to facilitate tissue invasion, and to support the metastatic dissemination and seeding of cancer cells.[94],[96],[97],[119],[120],[234],[235],[236],[237]

In addition to fully differentiated immune cells present in tumor stroma, a variety of partially differentiated myeloid progenitors have been identified in tumors.[96] Such cells represent intermediaries between circulating cells of bone marrow origin and the differentiated immune cells typically found in normal and inflamed tissues. Importantly, these progenitors, like their more differentiated derivatives, have demonstrable tumor-promoting activity. Of particular interest, a class of tumor-infiltrating myeloid cells has been shown to suppress CTL and NK cell activity, having been identified as MDSCs that function to block the attack on tumors by the adaptive (i.e., CTL) and innate (i.e., NK) arms of the immune system.[94],[133],[193] Hence, recruitment of certain myeloid cells may be doubly beneficial for the developing tumor, by directly promoting angiogenesis and tumor progression, while at the same time affording a means of evading immune destruction.

These conflicting roles of the immune system in confronting tumors would seem to reflect similar situations that arise routinely in normal tissues. Thus, the immune system detects and targets infectious agents through cells of the adaptive immune response. Cells of the innate immune system, in contrast, are involved in wound healing and in clearing dead cells and cellular debris. The balance between the conflicting immune responses within particular tumor types (and indeed in individual patients' tumors) is likely to prove critical in determining the characteristics of tumor growth and the stepwise progression to stages of heightened aggressiveness (i.e., invasion and metastasis). Moreover, there is increasing evidence supporting the proposition that this balance can be modulated for therapeutic purposes in order to redirect or reprogram the immune response to focus its functional capabilities on destroying tumors.[133],[238],[239]

Stem and Progenitor Cells of the Tumor Stroma

The various stromal cell types that constitute the tumor microenvironment may be recruited from adjacent normal tissue—the most obvious reservoir of such cell types. However, in recent years, bone marrow (BM) has increasingly been implicated as a key source of tumor-associated stromal cells.[99],[100],[240],[241],[242],[243] Thus, mesenchymal stem and progenitor cells can be recruited into tumors from BM, where they may subsequently differentiate into the various well-characterized stromal cell types. Some of these recent arrivals may also persist in an undifferentiated or partially differentiated state, exhibiting functions that their more differentiated progeny lack.

The BM origins of stromal cell types have been demonstrated using tumor-bearing mice in which the BM cells (and thus their disseminated progeny) have been selectively labeled with reporters such as green fluorescent protein (GFP). Although immune inflammatory cells have been long known to derive from BM, more recently progenitors of endothelial cells, pericytes, and several subtypes of cancer-associated fibroblasts have also been shown to originate from BM in various mouse models of cancer.[100],[240],[241],[242],[243] The prevalence and functional importance of endothelial progenitors for tumor

angiogenesis is, however, currently unresolved.[99],[242] Taken together, these various lines of evidence indicate that tumor-associated stromal cells may be supplied to growing tumors by the proliferation of preexisting stromal cells or via recruitment of BM-derived stem/progenitor cells.

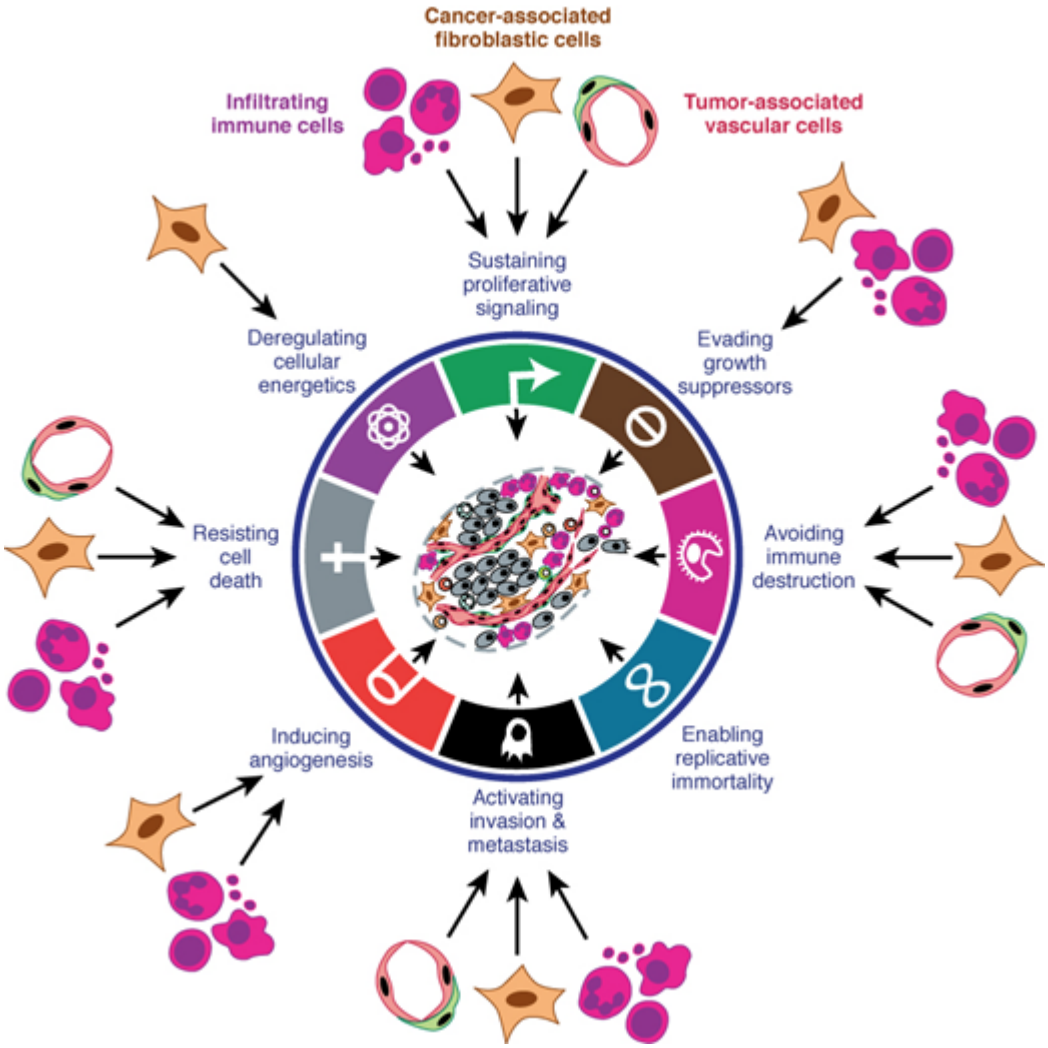
In summary, it is evident that virtually all cancers, including even the *liquid tumors* of hematopoietic malignancies, depend not only on neoplastic cells for their pathogenic effects, but also on diverse cell types recruited from local and distant tissue sources to assemble specialized, supporting tumor microenvironments. Importantly, the composition of stromal cell types supporting a particular cancer evidently varies considerably from one tumor type to another; even within a particular type, the patterns and abundance can be informative about malignant grade and prognosis. The inescapable conclusion is that cancer cells are not fully autonomous, and rather depend to various degrees on stromal cells of the tumor microenvironment, which can contribute functionally to seven of the eight hallmarks of cancer (**Fig. 2.4**).

FIGURE 2.4

Diverse contributions of stromal cells to the hallmarks of cancer.

Of the eight hallmark capabilities acquired by cancer cells, seven depend on contributions by stromal cells forming the tumor microenvironment.[2],[213] The stromal cells can be divided into three general classes: infiltrating immune cells, cancer-associated fibroblastic cells, and tumor-associated vascular cells. The association of these corrupted cell types with the acquisition of individual hallmark capabilities has been documented through a variety of experimental approaches that are often supported by descriptive studies in human cancers. The relative importance of each of these stromal cell classes to a particular hallmark varies according to tumor type and stage of progression.

(Adapted from Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* 2012;21:309–322.)



Heterotypic Signaling Orchestrates the Cells of the Tumor Microenvironment

Every cell in our bodies is governed by an elaborate intracellular signaling circuit—in effect, its own microcomputer. In cancer cells, key subcircuits in this integrated circuit are reprogrammed so as to activate and sustain hallmark capabilities. These changes are induced by mutations in the cells' genomes, by epigenetic alterations affecting gene expression, and by the receipt of a diverse array of signals from the tumor microenvironment. **Figure 2.5A** illustrates some of the circuits that are reprogrammed to enable cancer cells to proliferate chronically, to avoid proliferative brakes and cell death, and to become invasive and metastatic. Similarly, the intracellular integrated circuits that regulate the actions of stromal cells are also evidently reprogrammed. Current evidence suggests that stromal cell reprogramming is primarily affected by extracellular cues and epigenetic alterations in gene expression, rather than gene mutation.

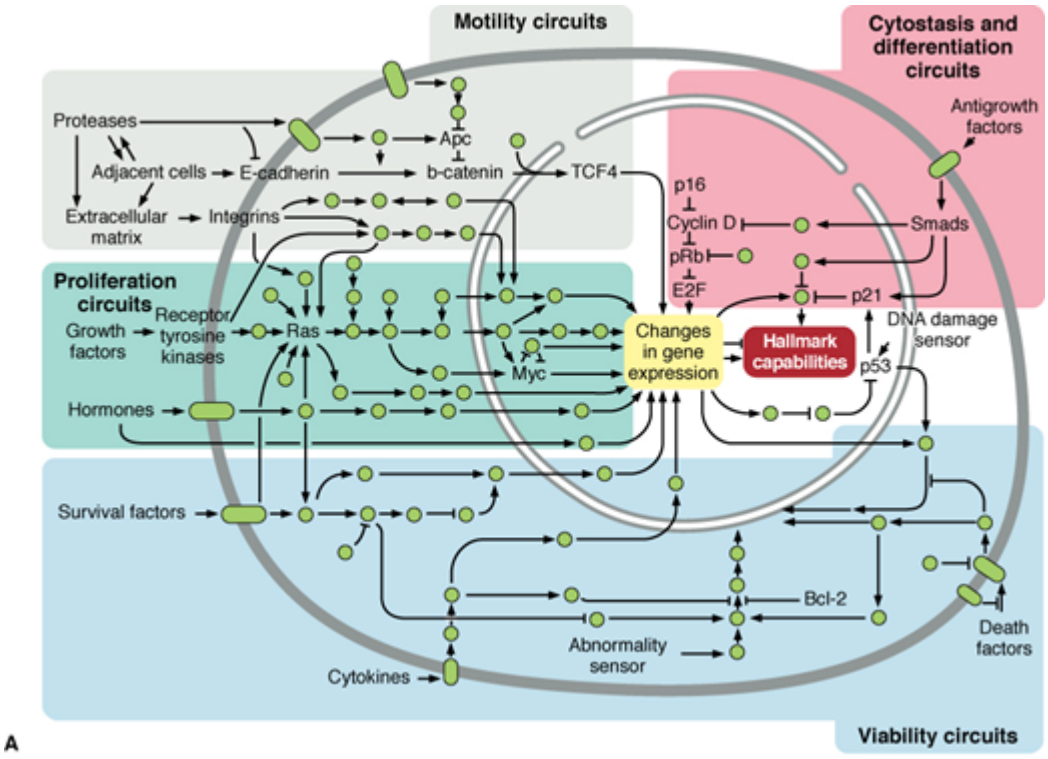
FIGURE 2.5

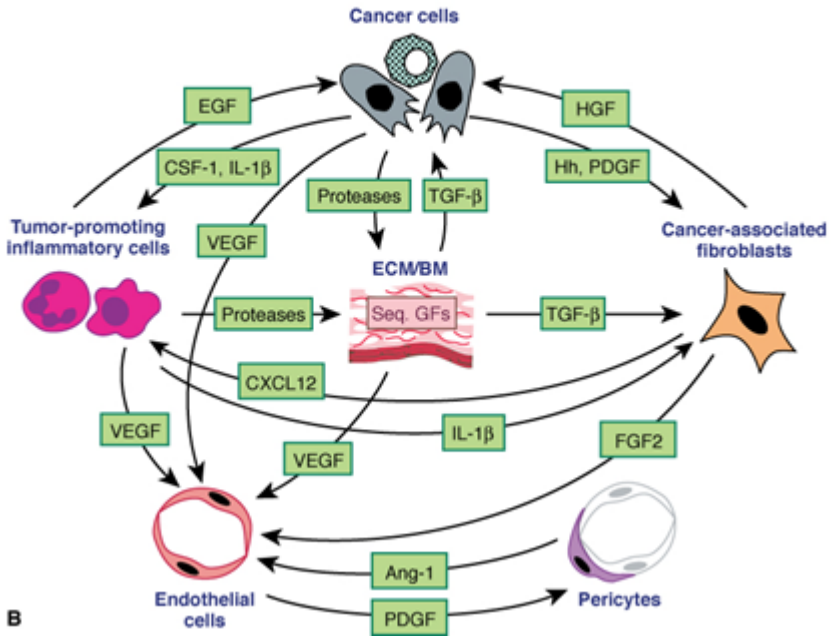
Reprogramming intracellular circuits and cell-to-cell signaling pathways dictates tumor inception and progression.

An elaborate integrated circuit operating within normal cells is reprogrammed to regulate the hallmark capabilities acquired by cancer cells **(A)** and by associated stromal cells. Separate subcircuits, depicted here in differently colored fields, are specialized to orchestrate distinct capabilities. At one level, this depiction is simplistic, because there is considerable cross-talk between such subcircuits. More broadly, the integrated circuits operating inside cancer cells and stromal cells are interconnected via a complex network of signals transmitted by the various cells in the tumor microenvironment (in some cases via the

extracellular matrix *[ECM]* and basement membranes *[BM]* they synthesize), of which a few signals are exemplified **(B).** HGF, hepatocyte growth factor for the cMet receptor; Hh, hedgehog ligand for the Patched (PTCH) receptor; Seq. GF, growth factors sequested in the ECM/BM.

(Adapted from Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–674.)





Given the alterations in the signaling within both neoplastic cells and their stromal neighbors, a tumor can be depicted as a network of interconnected (cellular) microcomputers. This dictates that a complete elucidation of a particular tumor's biology will require far more than an elucidation of the aberrantly functioning integrated circuits within its neoplastic cells. Accordingly, the rapidly growing catalog of the function-enabling genetic mutations within cancer cell genomes[194] provides only one dimension to this problem. A reasonably complete, graphical depiction of the network of microenvironmental signaling

interactions remains far beyond our reach, because the great majority of signaling molecules and their circuitry are still to be identified. Instead, we provide a hint of such interactions in **Figure 2.5B**. These few well-established examples are intended to exemplify a signaling network of remarkable complexity that is of critical importance to tumor pathogenesis.

Coevolution of the Tumor Microenvironment During Carcinogenesis

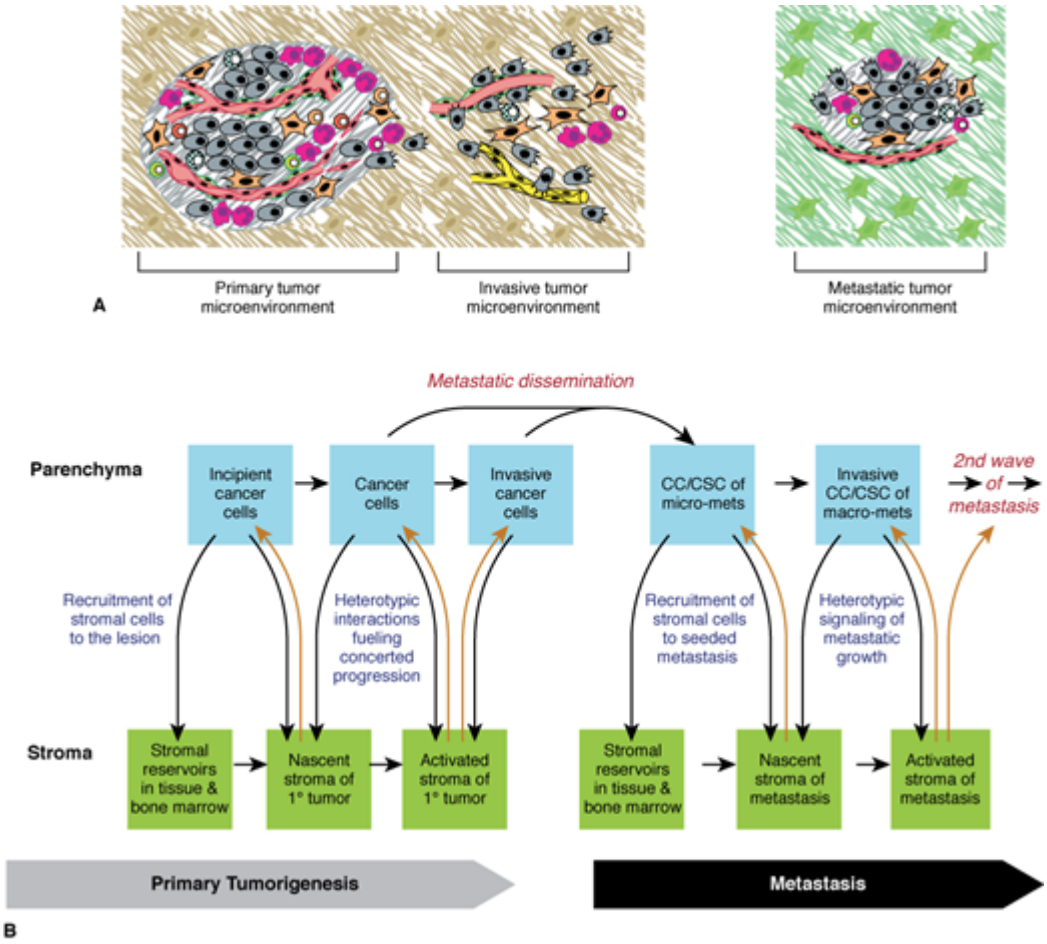
The tumor microenvironment described previously is not static during multistage tumor development and progression, thus creating another dimension of complexity. Rather, the abundance and functional contributions of the stromal cells populating neoplastic lesions will likely vary during progression in two respects. First, as the neoplastic cells evolve, there will be a parallel coevolution occurring in the stroma, as indicated by the shifting composition of stroma-associated cell types. Second, as cancer cells enter into different locations, they encounter distinct stromal microenvironments. Thus, the microenvironment in the interior of a primary tumor will likely be distinct both from locally invasive breakout lesions and from the one encountered by disseminated cells in distant organs (**Fig. 2.6A**). This dictates that the observed histopathologic progression of a tumor reflects underlying changes in heterotypic signaling between tumor parenchyma and stroma.

FIGURE 2.6

The dynamic variation and coevolution of the tumor microenvironment during the lesional progression of cancer.

(A) Interactions between multiple stromal cell types and heterogeneously evolving mutant cancer cells create a succession of tumor microenvironments that change dynamically as tumors are initiated, invade normal tissues, and thereafter seed and colonize distant tissues. The abundance, histologic organization, and characteristics of the stromal cell types and associated extracellular matrix *(hatched background)* evolves during progression, thereby enabling primary, invasive, and then metastatic growth. **(B)** Importantly, the signaling networks depicted in **Figure 2.5** involving cancer cells and their stromal collaborators change during tumor progression as a result of reciprocal signaling interactions between these various cells. CC, cancer cell; CSC, cancer stem cell; mets, metastases.

(Adapted from Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–674.)



We envision back-and-forth reciprocal interactions between the neoplastic cells and the supporting stromal cells that change during the course of multistep tumor development and progression, as depicted in **Figure 2.6B**. Thus, incipient neoplasias begin the interplay by recruiting and activating stromal cell types that assemble into an initial preneoplastic stroma, which in turn responds reciprocally by enhancing the neoplastic phenotypes of the nearby cancer cells. The cancer cells, in response, may then undergo further genetic evolution, causing them to feed signals back to the stroma. Ultimately, signals originating in the stroma of primary tumors enable cancer cells to invade normal adjacent tissues and disseminate, seeding distant tissues and, with low efficiency, metastatic colonies (see **Fig. 2.6B**).

The circulating cancer cells that are released from primary tumors leave a microenvironment supported by this coevolved stroma. Upon landing in a distant organ, however, disseminated cancer cells must find a means to grow in a quite different tissue microenvironment. In some cases, newly seeded cancer cells must survive and expand in naïve, fully normal tissue microenvironments. In other cases, the newly encountered tissue microenvironments may already be supportive of such disseminated cancer cells, having been preconditioned prior to their arrival. Such permissive sites have been referred to as *premetastatic niches*.[146],[244],[245] These supportive niches may already preexist in distant tissues for various physiologic reasons,[101] including the actions of circulating factors dispatched systemically by primary tumors.[245]

The fact that signaling interactions between cancer cells and their supporting stroma are likely to evolve during the course of multistage primary tumor development and metastatic colonization clearly complicates the goal of fully elucidating the mechanisms of cancer pathogenesis. For example, this complexity poses challenges to systems biologists seeking to chart the crucial regulatory networks that orchestrate malignant progression, because much of the critical signaling is not intrinsic to cancer cells and instead operates through the interactions that these cells establish with their neighbors.

Cancer Cells, Cancer Stem Cells, and Intratumoral Heterogeneity

Cancer cells are the foundation of the disease. They initiate neoplastic development and drive tumor progression forward, having acquired the oncogenic and tumor suppressor mutations that define cancer as a genetic disease. Traditionally, the cancer cells within tumors have been portrayed as reasonably homogeneous cell populations until relatively late in the course of tumor progression, when hyperproliferation combined with increased genetic instability spawn genetically distinct clonal subpopulations. Reflecting such clonal heterogeneity, many human tumors are histopathologically diverse, containing regions demarcated by various degrees of differentiation, proliferation, vascularity, and invasiveness. In recent years, however, evidence has accumulated pointing to the existence of a new dimension of intratumor heterogeneity and a hitherto unappreciated subclass of neoplastic cells within tumors, termed cancer stem cells (CSC).

CSCs were initially implicated in the pathogenesis of hematopoietic malignancies,[246],[247] and years later, were identified in solid tumors, in particular breast carcinomas and neuroectodermal tumors.[248],[249] The fractionation of cancer cells on the basis of cell-surface markers has yielded subpopulations of neoplastic cells with a greatly enhanced ability, relative to the corresponding majority populations of non-CSCs, to seed new tumors upon implantation in immunodeficient mice. These, often rare, tumor-initiating cells have proven to share transcriptional profiles with certain normal tissue stem cells, thus justifying their designation as stemlike.

Although the evidence is still fragmentary, CSCs may prove to be a constituent of many, if not most tumors, albeit being present with highly variable abundance. CSCs are defined operationally through their ability to efficiently seed new tumors upon implantation into recipient host mice.[250],[251],[252],[253] This functional definition is often complemented by profiling the expression of certain CSC-associated markers that are typically expressed by the normal stem cells in the corresponding normal tissues of origin.[249] Importantly, recent in vivo lineage-tracing experiments have provided an additional functional test of CSCs by demonstrating their ability to spawn large numbers of progeny, including non-CSCs within tumors.[250] At the same time, these experiments have provided the most compelling evidence to date that CSCs exist, and that they can be defined functionally through tests that do not depend on the implantation of tumor cells into appropriate mouse hosts.

The origins of CSCs within a solid tumor have not been clarified and, indeed, may well vary from one tumor type to another.[250],[251],[254] In some tumors, normal tissue stem cells may serve as the cells of origin that undergo oncogenic transformation to yield CSCs; in others, partially differentiated transit-amplifying cells, also termed progenitor cells, may suffer the initial oncogenic transformation, thereafter assuming more stemlike characters. Once primary tumors have formed, the CSCs, like their normal counterparts, may self-renew as well as spawn more differentiated derivatives. In the case of neoplastic CSCs, these descendant cells form the great bulk of many tumors and thus are responsible for creating many tumor-associated phenotypes. It remains to be established whether multiple distinct classes of increasingly neoplastic stem cells form during the inception and subsequent multistep progression of tumors, ultimately yielding the CSCs that have been described in fully developed cancers.

Recent research has interrelated the acquisition of CSC traits with the EMT transdifferentiation program discussed previously.[250],[255] The induction of this program in certain model systems can induce many of the defining features of stem cells, including self-renewal ability and the antigenic phenotypes associated with both normal and cancer stem cells. This concordance suggests that the EMT program may not only enable cancer cells to physically disseminate from primary tumors, but can also confer on such cells the self-renewal capability that is crucial to their subsequent role as founders of new

neoplastic colonies at sites of dissemination.[256] If generalized, this connection raises an important corollary hypothesis: The heterotypic signals that trigger an EMT, such as those released by an activated, inflammatory stroma, may also be important in creating and maintaining CSCs.

An increasing number of human tumors are reported to contain subpopulations with the properties of CSCs, as defined operationally through their efficient tumor-initiating capabilities upon xenotransplantation into mice. Nevertheless, the importance of CSCs as a distinct phenotypic subclass of neoplastic cells remains a matter of debate, as does their oft cited rarity within tumors.[254],[257],[258],[259] Indeed, it is plausible that the phenotypic plasticity operating within tumors may produce bidirectional interconversion between CSCs and non-CSCs, resulting in dynamic variation in the relative abundance of CSCs.[250],[260] Such plasticity could complicate a definitive measurement of their characteristic abundance. Analogous plasticity is already implicated in the EMT program, which can be engaged reversibly.[261]

These complexities notwithstanding, it is already evident that this new dimension of tumor heterogeneity holds important implications for successful cancer therapies. Increasing evidence in a variety of tumor types suggests that cells exhibiting the properties of CSCs are more resistant to various commonly used chemotherapeutic treatments.[255],[262],[263] Their persistence following initial treatment may help to explain the almost inevitable disease recurrence occurring after apparently successful debulking of human solid tumors by radiation and various forms of chemotherapy. Moreover, CSCs may well prove to underlie certain forms of tumor dormancy, whereby latent cancer cells persist for years or even decades after initial surgical resection or radio/chemotherapy, only to suddenly erupt and generate life-threatening disease. Hence, CSCs represent a double threat in that they are more resistant to therapeutic killing, and at the same time, are endowed with the ability to regenerate a tumor once therapy has been halted.

This phenotypic plasticity implicit in the CSC state may also enable the formation of functionally distinct subpopulations within a tumor that support overall tumor growth in various ways. Thus, an EMT can convert epithelial carcinoma cells into mesenchymal, fibroblast-like cancer cells that may well assume the duties of CAFs in some tumors (e.g., pancreatic ductal adenocarcinoma).[264] Intriguingly, several recent reports that have yet to be thoroughly validated in terms of generality, functional importance, or prevalence have documented the ability of glioblastoma cells (or possibly their associated CSC subpopulations) to transdifferentiate into endothelial-like cells that can substitute for bona fide host-derived endothelial cells in forming a tumor-associated neovasculature.[265],[266],[267] These examples suggest that certain tumors may induce some of their own cancer cells to undergo various types of metamorphoses in order to generate stromal cell types needed to support tumor growth and progression, rather than relying on recruited host cells to provide the requisite hallmark-enabling functions.

Another form of phenotypic variability resides in the genetic heterogeneity of cancer cells within a tumor. Genomewide sequencing of cancer cells microdissected from different sectors of the same tumor[145] has revealed striking intratumoral genetic heterogeneity. Some of this genetic diversity may be reflected in the long recognized histologic heterogeneity within individual human tumors. Thus, genetic diversification may produce subpopulations of cancer cells that contribute distinct and complementary capabilities, which then accrue to the common benefit of overall tumor growth, progression, and resistance to therapy, as described earlier. Alternatively, such heterogeneity may simply reflect the genetic chaos that arises as tumor cell genomes become increasingly destabilized.

Therapeutic Targeting of the Hallmarks of Cancer

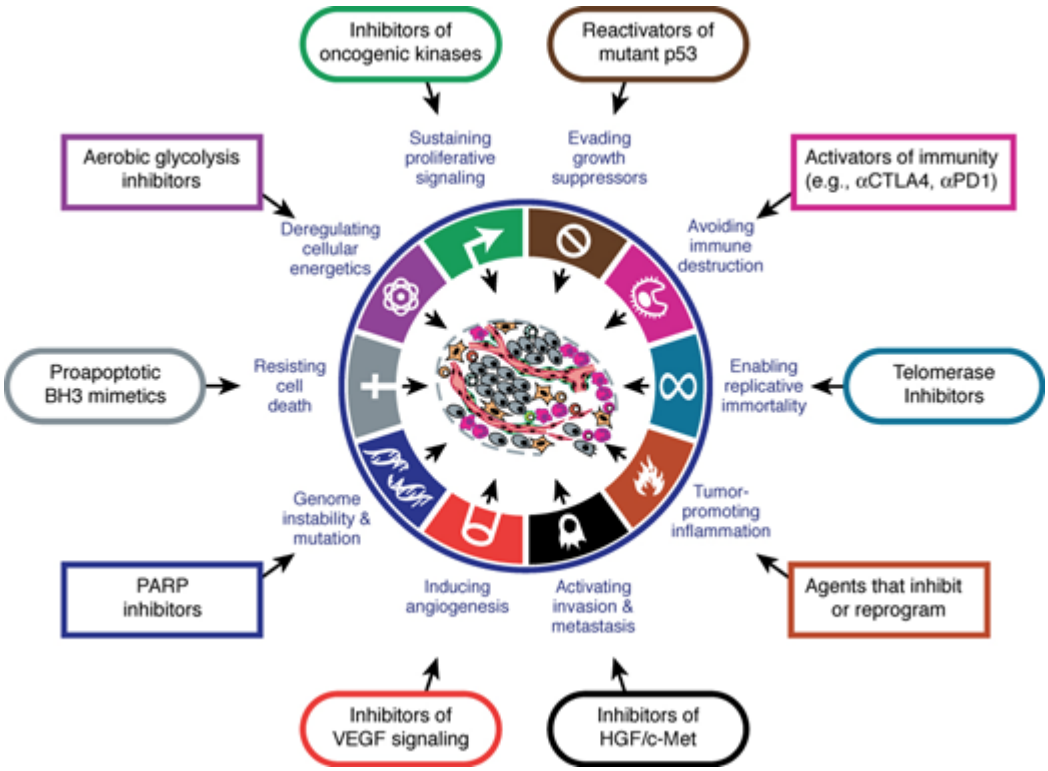
We do not attempt here to enumerate the myriad therapies that are currently under development or have been introduced of late into the clinic. Instead, we consider how the description of hallmark principles is likely to inform therapeutic development at present and may increasingly do so in the future. Thus, the rapidly growing armamentarium of therapeutics directed against specific molecular targets can be categorized according to their respective effects on one or more hallmark capabilities, as illustrated in the examples presented in **Figure 2.7**. Indeed, the observed efficacy of these drugs represents, in each case, a validation of a particular capability: If a capability is truly critical to the biology of tumors, then its inhibition should impair tumor growth and progression.

FIGURE 2.7

Therapeutic targeting of the hallmarks of cancer.

Drugs that interfere with each of the hallmark capabilities and hallmark-enabling processes have been developed and are in preclinical and/or clinical testing, and in some cases, approved for use in treating certain forms of human cancer. A focus on antagonizing specific hallmark capabilities is likely to yield insights into developing novel, highly effective therapeutic strategies. PARP, poly ADP ribose polymerase.

(Adapted from Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–674.)



Unfortunately, however, the clinical responses elicited by these targeted therapies have generally been transitory, being followed all too often by relapse. One interpretation, which is supported by growing experimental evidence, is that each of the core hallmark capabilities is regulated by a set of partially redundant signaling pathways. Consequently, a targeted therapeutic agent inhibiting one key pathway in a tumor may not completely eliminate a hallmark capability, allowing some cancer cells to survive with residual function until they or their progeny eventually adapt to the selective pressure imposed by the initially applied therapy. Such adaptation can reestablish the expression of the functional capability, permitting renewed tumor growth and clinical relapse. Because the number of parallel signaling pathways supporting a given hallmark must be limited, it may become possible to therapeutically

cotarget all of these supporting pathways, thereby preventing the development of adaptive resistance.

Another dimension of the plasticity of tumors under therapeutic attack is illustrated by the unanticipated responses to antiangiogenic therapy, in which cancer cells reduce their dependence on this hallmark capability by increasing their dependence on another. Thus, many observers anticipated that potent inhibition of angiogenesis would starve tumors of vital nutrients and oxygen, forcing them into dormancy and possibly leading to their dissolution.[86],[87],[268] Instead, the clinical responses to antiangiogenic therapies have been found to be transitory, followed by relapse, implicating adaptive or evasive resistance mechanisms.[220],[269],[270],[271] One such mechanism of evasive resistance, observed in certain preclinical models of antiangiogenic therapy, involves reduced dependence on continuing angiogenesis by increasing the activity of two other capabilities: invasiveness and metastasis.[269],[270],[271] By invading nearby and distant tissues, initially hypoxic cancer cells gain access to normal, preexisting tissue vasculature. The initial clinical validation of this adaptive/evasive resistance is apparent in the increased invasion and local metastasis seen when human glioblastomas are treated with antiangiogenic therapies.[272],[273],[274] The applicability of this lesson to other human cancers has yet to be established.

Analogous adaptive shifts in dependence on other hallmark traits may also limit the efficacy of analogous hallmark-targeting therapies. For example, the deployment of apoptosis-inducing drugs may induce cancer cells to hyperactivate mitogenic signaling, enabling them to compensate for the initial attrition triggered by such treatments. Such considerations suggest that drug development and the design of treatment protocols will benefit from incorporating the concepts of functionally discrete hallmark capabilities and of the multiple biochemical pathways involved in supporting each of them. For these reasons, we envisage that attacking multiple hallmark capabilities with hallmark-targeting drugs (see **Fig. 2.7**), in carefully considered combinations, sequences, and temporal regimens,[275] will result in increasingly effective therapies that produce more durable clinical responses.

Conclusion and a Vision for the Future

Looking ahead, we envision significant advances in our understanding of invasion and metastasis during the coming decade. Similarly, the role of altered energy metabolism in malignant growth will be elucidated, including a resolution of whether this metabolic reprogramming is a discrete capability separable from the core hallmark of chronically sustained proliferation. We are excited about the new frontier of immunotherapy, which will be empowered to leverage detailed knowledge about the regulation of immune responses in order to develop pharmacologic tools that can modulate them therapeutically for the purpose of effectively and sustainably attacking tumors and, most importantly, their metastases.

Other areas are currently in rapid flux. In recent years, elaborate molecular mechanisms controlling transcription through chromatin modifications have been uncovered, and there are clues that specific shifts in chromatin configuration occur during the acquisition of certain hallmark capabilities.[195],[196] Functionally significant epigenetic alterations seem likely to be factors not only in the cancer cells, but also in the altered cells of the tumor-associated stroma. At present, it is unclear whether an elucidation of these epigenetic mechanisms will materially change our overall understanding of the means by which hallmark capabilities are acquired, or simply add additional detail to the regulatory circuitry that is already known to govern them.

Similarly, the discovery of hundreds of distinct regulatory microRNAs has already led to profound changes in our understanding of the molecular control mechanisms that operate in health and disease. By now, dozens of microRNAs have been implicated in various tumor phenotypes.[276],[277] Still, these

only scratch the surface of the true complexity, because the functions of hundreds of microRNAs known to be present in our cells and to be altered in expression levels in different forms of cancer remain total mysteries. Here again, we are unclear whether future progress will cause fundamental shifts in our understanding of the pathogenic mechanisms of cancer, or only add detail to the elaborate regulatory circuits that have already been mapped out.

Finally, the existing diagrams of heterotypic interactions between the multiple distinct cell types that collaborate to produce malignant tumors are still rudimentary. We anticipate that, in another decade, the signaling pathways describing the intercommunication between these various cell types within tumors will be charted in far greater detail and clarity, eclipsing our current knowledge. And, as before,[1],[2] we continue to foresee cancer research as an increasingly logical science, in which myriad phenotypic complexities are manifestations of an underlying organizing principle.

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