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Review

Hallmarks of Cancer: The Next Generation

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The hallmarks of cancer comprise six biological capabilities acquired 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, and activating invasion and metastasis. Underlying these hallmarks are genome instability, which generates the genetic diversity that expedites their acquisition, and inflammation, which fosters multiple hallmark functions. Conceptual progress in the last decade has added two emerging hallmarks of potential generality to this list—reprogramming of energy metabolism and evading immune destruction. 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 affect the development of new means to treat human cancer.

INTRODUCTION

We have proposed that six hallmarks of cancer together constitute an organizing principle that provides a logical framework for understanding the remarkable diversity of neoplastic diseases (Hanahan and Weinberg, 2000). 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 could be rationalized by the need of incipient cancer cells to acquire the traits that 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 cell types 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. During the ensuing decade this notion has been solidified and extended, revealing 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 the course of remarkable progress in cancer research subsequent to this publication, new observations have served both to clarify and to modify the original formulation of the hallmark capabilities. In addition, yet other observations have raised questions and highlighted mechanistic concepts that were not integral to our original elaboration of the hallmark traits. Motivated by these developments, we now revisit the original hallmarks, consider new ones that might be included in this roster, and expand upon the functional roles and contributions made by recruited stromal cells to tumor biology.

HALLMARK CAPABILITIES—CONCEPTUAL PROGRESS

The six hallmarks of cancer—distinctive and complementary capabilities that enable tumor growth and metastatic dissemination—continue to provide a solid foundation for understanding the biology of cancer (Figure 1; see the Supplemental Information for downloadable versions of the figures for presentations). In the first section of this Review, we summarize the essence of each hallmark as described in the original presentation in 2000, followed by selected illustrations (demarcated by subheadings in italics) of the conceptual progress made over the past decade in understanding their mechanistic underpinnings. In subsequent sections we address new developments that broaden the scope of the conceptualization, describing in turn two enabling characteristics crucial to the acquisition of the six hallmark capabilities, two new emerging hallmark capabilities, the constitution and signaling interactions of the tumor microenvironment crucial to cancer phenotypes, and we finally discuss the new frontier of therapeutic application of these concepts.

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 into and progression through the cell growth-and-division cycle, thereby ensuring a homeostasis of cell

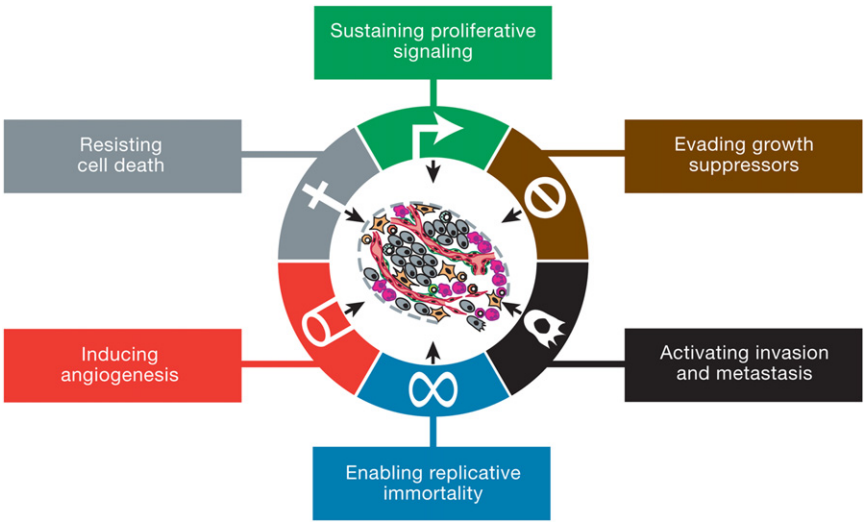

Figure 1. The Hallmarks of Cancer
This illustration encompasses the six hallmark capabilities originally proposed in our 2000 perspective. The past decade has witnessed remarkable progress toward understanding the mechanistic underpinnings of each hallmark.

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, increases in cell size); often these signals influence yet other cell-biological properties, such as cell survival and energy metabolism.

Remarkably, the precise identities and sources of the proliferative signals operating within normal tissues were poorly understood a decade ago and in general remain so. Moreover, we still know relatively little about the mechanisms controlling the release of these mitogenic signals. In part, the understanding of these mechanisms is complicated by the fact that the growth factor signals controlling cell number and position within 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 sequestration in the pericellular space and extracellular matrix, and by the actions of a complex network of proteases, sulfatases, and possibly other enzymes that liberate and activate them, apparently in a highly specific and localized fashion.

The mitogenic signaling in cancer cells is, in contrast, better understood (Lemmon and Schlessinger, 2010; Witsch et al., 2010; Hynes and MacDonald, 2009; Perona, 2006). 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 respond via the expression of cognate receptors, resulting in autocrine proliferative stimulation. Alternatively, cancer cells may send signals to stimulate normal cells within the supporting tumor-associated stroma, which reciprocate by supplying the cancer cells with various growth factors (Cheng et al., 2008; Bhowmick et al., 2004). Receptor 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 ligand; the same outcome can result from structural alterations in the receptor molecules that facilitate ligand-independent firing.

Growth factor independence may also derive from the constitutive activation of components of signaling pathways operating downstream of these receptors, obviating the need to stimulate these pathways by ligand-mediated receptor activation. Given that a number of distinct downstream signaling pathways radiate from a ligand-stimulated receptor, the activation of one or another of these downstream pathways, for example, the one responding to the Ras signal transducer, may only recapitulate a subset of the regulatory instructions transmitted by an activated receptor.

Somatic Mutations Activate Additional Downstream Pathways

High-throughput DNA sequencing analyses of cancer cell genomes have revealed somatic mutations in certain human tumors that predict constitutive activation of signaling circuits usually triggered by activated growth factor receptors. Thus, 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 mitogen-activated protein (MAP)-kinase pathway (Davies and Samuels 2010). Similarly, mutations in the catalytic subunit of phosphoinositide 3-kinase (PI3-kinase) isoforms are being detected in an array of tumor types, which serve to hyperactivate the PI3-kinase signaling circuitry, including its key Akt/PKB signal transducer (Jiang and Liu, 2009; Yuan and Cantley, 2008). The advantages to tumor cells of activating upstream (receptor) versus downstream (transducer) signaling remain obscure, as does the functional impact of crosstalk between the multiple pathways radiating from growth factor receptors.

Disruptions of Negative-Feedback Mechanisms that Attenuate Proliferative Signaling

Recent results have 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 (Wertz and Dixit, 2010; Cabrita and Christofori, 2008; Amit et al., 2007; Mosesson et al., 2008). Defects in these feedback mechanisms are capable of enhancing proliferative signaling. The prototype of this type of regulation involves the Ras oncoprotein: the oncogenic effects of Ras do not result from a hyperactivation of its signaling powers; instead, the oncogenic mutations affecting *ras* genes compromise Ras GTPase activity, which

operates as an intrinsic negative-feedback mechanism that normally ensures that active signal transmission is transitory.

Analogous negative-feedback mechanisms operate at multiple nodes within the proliferative signaling circuitry. A prominent example involves the PTEN phosphatase, which counteracts PI3-kinase by degrading its product, phosphatidylinositol (3,4,5) trisphosphate (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 promoter methylation (Jiang and Liu, 2009; Yuan and Cantley, 2008).

Yet another example involves the mTOR kinase, a 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. Thus, when mTOR is pharmacologically inhibited in such cancer cells (such as by the drug rapamycin), the associated loss of negative feedback results in increased activity of PI3K and its effector Akt/PKB, thereby blunting the antiproliferative effects of mTOR inhibition (Sudarsanam and Johnson, 2010; O'Reilly et al., 2006). It is likely that compromised negative-feedback loops in this and other signaling pathways will prove to be widespread among human cancer cells and serve as an important means by which these cells can achieve proliferative independence. Moreover, disruption of such self-attenuating signaling may contribute to the development of adaptive resistance toward 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 manifested in their protein products would result in correspondingly increased cancer cell proliferation and thus tumor growth. More recent research has undermined this notion, in that excessively elevated signaling by oncoproteins such as RAS, MYC, and RAF can provoke counteracting responses from cells, specifically induction of cell senescence and/or apoptosis (Collado and Serrano, 2010; Evan and d'Adda di Fagagna, 2009; Lowe et al., 2004). For example, cultured cells expressing high levels of the Ras oncoprotein may enter into the nonproliferative but viable state called senescence; in contrast, cells expressing lower levels of this protein may avoid senescence and proliferate.

Cells with morphological features of senescence, including enlarged cytoplasm, the absence of proliferation markers, and expression of the senescence-induced β-galactosidase enzyme, are abundant in the tissues of mice engineered to overexpress certain oncogenes (Collado and Serrano, 2010; Evan and d'Adda di Fagagna, 2009) and are prevalent in some cases of human melanoma (Mooi and Peeper, 2006). These ostensibly paradoxical responses seem to reflect intrinsic cellular defense mechanisms designed to eliminate cells experiencing excessive levels of certain types of signaling. Accordingly, the relative intensity of oncogenic signaling in cancer cells may represent compromises between maximal mitogenic stimulation and avoidance of these antiproliferative 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 growth and proliferation have been discovered through their characteristic 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 suppressors encode the RB (retinoblastoma-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, activate senescence and apoptotic programs.

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 (Burkhart and Sage, 2008; Deshpande et al., 2005; Sherr and McCormick, 2002). Cancer cells with defects in 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 originate largely outside of the cell, TP53 receives inputs from stress and abnormality sensors that function within the cell's intracellular operating systems: if the degree of damage to the genome is excessive, or if the levels of nucleotide pools, growth-promoting signals, glucose, or oxygenation are suboptimal, 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 subsystems, TP53 can trigger apoptosis. Notably, the various 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 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 loss of RB function would allow continuous firing of the cell division cycle in 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 was in the development of pituitary tumors late in life (Lipinski and Jacks, 1999). Similarly, *TP53* null mice develop normally, show largely proper cell and tissue homeostasis, and again develop abnormalities later in life, in the form of leukemias and sarcomas (Ghebranious and Donehower, 1998). Both examples must reflect the operations of redundantly acting mechanisms that serve to constrain inappropriate replication of cells lacking these key proliferation suppressors.

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 propagated in two-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, one 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.

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 via 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 adhesivity of cadherin-mediated cell-to-cell attachments. Additionally, by sequestering growth factor receptors, Merlin limits their ability to efficiently emit mitogenic signals (Curto et al., 2007; Okada et al., 2005).

A second mechanism of contact inhibition involves the LKB1 epithelial polarity protein, which organizes epithelial structure and helps maintain tissue integrity. LKB1 can, for example, overrule the mitogenic effects of the powerful Myc oncogene when the latter is upregulated in organized, quiescent epithelial structures; in contrast, when LKB1 expression is suppressed, epithelial integrity is destabilized, and epithelial cells become susceptible to Myc-induced transformation (Partanen et al., 2009; Hezel and Bardeesy, 2008). *LKB1* has also been identified as a tumor suppressor gene that is lost in certain human malignancies (Shaw, 2009), possibly reflecting its normal function as a suppressor of inappropriate proliferation. It remains to be seen how frequently these two mechanisms of contact-mediated growth suppression are compromised in human cancers; no doubt yet other contact-induced proliferative barriers are yet to be discovered. Clearly mechanisms like these that enable cells to construct and maintain architecturally complex tissues represent important means of suppressing and counterbalancing inappropriate proliferative signals.

Corruption of the TGF-β Pathway Promotes Malignancy

TGF-β is best known for its antiproliferative effects, and evasion by cancer cells of these effects is now appreciated to be far more elaborate than simple shutdown of its signaling circuitry (Ikushima and Miyazono, 2010; Massagué, 2008; Bierie and Moses, 2006). In many late-stage tumors, 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), that confers on cancer cells traits associated with high-grade malignancy, as discussed in further detail below.

Resisting Cell Death

The concept that programmed cell death by apoptosis serves as a natural barrier to cancer development has been established by compelling functional studies conducted over the last two decades (Adams and Cory, 2007; Lowe et al., 2004: Evan and Littlewood, 1998). Elucidation of the signaling circuitry governing the apoptotic program has revealed how apoptosis is triggered in response to various physiologic stresses that cancer cells experience 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, as mentioned earlier, and DNA damage associated with hyperproliferation. Yet other research has revealed how apoptosis is attenuated in those tumors that succeed in progressing to states of high-grade malignancy and resistance to therapy (Adams and Cory, 2007; Lowe et al., 2004).

The apoptotic machinery is composed of both upstream regulators and downstream effector components (Adams and Cory, 2007). The regulators, in turn, 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 culminates in activation of a normally latent protease (caspases 8 and 9, respectively), which proceeds to initiate a cascade of proteolysis involving effector caspases responsible for the execution phase of apoptosis, in which the 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 "apoptotic trigger" that conveys signals between the regulators and effectors is controlled by counterbalancing pro- and antiapoptotic members of the Bcl-2 family of regulatory proteins (Adams and Cory, 2007). The archetype, Bcl-2, along with its closest relatives (Bcl-x_L, 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 Bak disrupt the integrity of the outer mitochondrial membrane, causing the release of proapoptotic signaling proteins, the most important of which is cytochrome *c*. The released cytochrome *c* activates, in turn, a cascade of caspases that act via their proteolytic activities to induce the multiple cellular changes associated with the apoptotic program. Bax and Bak share protein-protein interaction domains, termed BH3 motifs, with the antiapoptotic Bcl-2-like proteins that mediate their various physical interactions. The activities of a subfamily of related proteins, each of which contains a single such BH3 motif, are coupled to a variety of sensors of cellular abnormality; these "BH3-only" proteins act either by interfering with antiapoptotic Bcl-2 proteins or by directly stimulating the proapoptotic members of this family (Adams and Cory, 2007; Willis and Adams, 2005).

Although the cellular conditions that trigger apoptosis remain to be fully enumerated, several abnormality sensors that play key roles in tumor development have been identified (Adams and Cory, 2007; Lowe et al., 2004). Most notable is a DNA-damage sensor that functions via the TP53 tumor suppressor (Junttila and Evan, 2009); TP53 induces apoptosis by upregulating expression of the Noxa and Puma BH3-only proteins, doing so in response to substantial levels of DNA breaks and other chromosomal abnormalities. Alternatively, insufficient survival

factor signaling (for instance inadequate levels of interleukin-3 in lymphocytes or of insulin-like growth factor 1/2 [Igf1/2] in epithelial cells) can elicit apoptosis through a BH3-only protein called Bim. Yet another condition leading to cell death involves hyperactive signaling by certain oncoproteins, such as Myc, which triggers apoptosis (in part via Bim and other BH3-only proteins) unless counterbalanced by antiapoptotic factors (Junttila and Evan, 2009; Lowe et al., 2004).

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 expression of antiapoptotic regulators (Bcl-2, Bcl-x_L) or of survival signals (Igf1/2), by downregulating proapoptotic 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 to the malignant state.

The structure of the apoptotic machinery and program, and the strategies used by cancer cells to evade its actions, were widely appreciated by the beginning of the last decade. The most notable conceptual advances since then have involved other forms of cell death that broaden the scope of ''programmed cell death'' as a barrier to cancer.

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 (Levine and Kroemer, 2008; Mizushima, 2007). 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 intracellular organelles and then fuse with lysosomes wherein 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.

Like apoptosis, the autophagy machinery has both regulatory and effector components (Levine and Kroemer, 2008; Mizushima, 2007). 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 the PI3-kinase, AKT, and mTOR kinases, 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 (Levine and Kroemer, 2008; Sinha and Levine, 2008; Mathew et al., 2007).

Another interconnection between these two programs resides in the Beclin-1 protein, which has been shown by genetic studies to be necessary for induction of autophagy (Levine and Kroemer, 2008; Sinha and Levine, 2008; Mizushima, 2007). Beclin-1 is a member of the BH3-only subfamily of apoptotic regulatory proteins, and its BH3 domain allows it to bind the Bcl-2/Bcl-x_L proteins. Stress-sensor-coupled BH3 proteins can displace Beclin-1 from its association with Bcl-2/Bcl-x_L, enabling the liberated Beclin-1 to trigger autophagy, much as they can release proapoptotic Bax and Bak to trigger apoptosis. Hence, stress-transducing BH3 proteins (e.g., Bid, Bad, Puma, et al.) can induce apoptosis and/or autophagy depending on the physiologic state of the cell.

Mice bearing inactivated alleles of the *Beclin-1* gene or of certain other components of the autophagy machinery exhibit increased susceptibility to cancer (White and DiPaola, 2009: Levine and Kroemer, 2008). These results suggest that induction of autophagy can serve as a barrier to tumorigenesis that may operate independently of or in concert with apoptosis. Accordingly, autophagy appears to represent yet another barrier that needs to be circumvented during tumor development (White and DiPaola, 2009).

Perhaps paradoxically, nutrient starvation, radiotherapy, and certain cytotoxic drugs can induce elevated levels of autophagy that are apparently cytoprotective for cancer cells, impairing rather than accentuating the killing actions of these stress-inducing situations (White and DiPaola, 2009; Apel et al., 2009; Amaravadi and Thompson, 2007; Mathew et al., 2007). Moreover, severely stressed cancer cells have been shown to shrink via autophagy to a state of reversible dormancy (White and DiPaola, 2009; Lu et al., 2008). This survival response may enable the persistence and eventual regrowth of some late-stage tumors following treatment with potent anticancer agents. Thus, in analogy to TGF-β signaling, which can be tumor suppressing at early stages of tumorigenesis and tumor promoting later on, autophagy seems to have conflicting effects on tumor cells and thus tumor progression (Apel et al., 2009; White and DiPaola, 2009). An important agenda for future research will involve clarifying the genetic and cell-physiologic conditions that dictate when and how autophagy enables cancer cells to survive or 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 neighbors, necrotic cells become bloated and explode, releasing their contents into the local tissue microenvironment. Although necrosis has historically been viewed much like organismic death, as a form of system-wide exhaustion and breakdown, the conceptual landscape is changing: cell death by necrosis is clearly under genetic control in some circumstances, rather than being a random and undirected process (Galluzzi and Kroemer, 2008; Zong and Thompson, 2006).

Perhaps more important, necrotic cell death releases proinflammatory signals into the surrounding tissue microenvironment, in contrast to apoptosis and autophagy, which do not. As a consequence, necrotic cells can recruit inflammatory cells of the immune system (Grivennikov et al., 2010; White et al., 2010; Galluzzi and Kroemer, 2008), 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, given that such cells are capable

of fostering angiogenesis, cancer cell proliferation, and invasiveness (see below). Additionally, necrotic cells can release bioactive regulatory factors, such as IL-1α, which can directly stimulate neighboring viable cells to proliferate, with the potential, once again, to facilitate neoplastic progression (Grivennikov et al., 2010). Consequently, necrotic cell death, while seemingly beneficial in counterbalancing cancer-associated hyperproliferation, may ultimately do more damage than good. Accordingly, incipient neoplasias and potentially invasive and metastatic tumors may gain an advantage by tolerating some degree of necrotic cell death, doing so in order to recruit tumor-promoting inflammatory cells that bring growth-stimulating factors to the surviving cells within these growths.

Enabling Replicative Immortality

By 2000, it was widely accepted that 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 able to pass through only a limited number of successive cell growth-and-division cycles. This limitation has been associated with two distinct barriers to proliferation: 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 senescence and then, for those cells that succeed in circumventing this barrier, to a 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 (Blasco, 2005; Shay and Wright, 2000). The telomeres, composed of multiple tandem hexanucleotide repeats, shorten progressively in nonimmortalized cells propagated in culture, eventually losing the ability to protect the ends of chromosomal DNAs from end-to-end fusions; such fusions generate unstable dicentric chromosomes whose resolution results in a scrambling of karyotype 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, triggering entrance into crisis.

Telomerase, the specialized DNA polymerase that adds telomere repeat segments to the ends of telomeric DNA, is almost absent in nonimmortalized cells but expressed at functionally significant levels in the vast 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, 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—senescence and crisis/apoptosis—have been rationalized as crucial anticancer defenses that are hard-wired into our cells, being 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 one or the other 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 senescence or apoptosis, achieved most commonly by upregulating expression of telomerase or, less frequently, via an alternative recombination-based telomere maintenance mechanism. 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

Whereas telomere maintenance has been increasingly substantiated as a condition critical to the neoplastic state, the concept of replication-induced senescence as a general barrier requires refinement and reformulation. (Differences in telomere structure and function in mouse versus human cells have also complicated investigation of the roles of telomeres and telomerase in replicative senescence.) 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 able to proliferate unimpeded in culture up the point of crisis and the associated induction of apoptosis triggered by critically shortened telomeres (Ince et al., 2007; Passos et al., 2007; Zhang et al., 2004; Sherr and DePinho, 2000). In contrast, experiments in mice engineered to lack telomerase indicate that the consequently shortened 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 (Artandi and DePinho, 2010). Such telomerase null mice with highly eroded telomeres exhibit multiorgan dysfunction and abnormalities that include evidence for both senescence and apoptosis, perhaps analogous to the senescence and apoptosis observed in cell culture (Artandi and DePinho, 2010; Feldser and Greider, 2007).

Of note, and as discussed earlier, a morphologically similar form of cell senescence induced by excessive or unbalanced oncogene signaling is now well documented as a protective mechanism against neoplasia; the possible interconnections of this form of senescence with telomerase and telomeres remain to be ascertained. Thus, cell senescence is emerging conceptually as a protective barrier to neoplastic expansion that can be triggered by various proliferation-associated abnormalities, including high levels of oncogenic signaling and, apparently, subcritical shortening of telomeres.

Delayed Activation of Telomerase May Both Limit and Foster Neoplastic Progression

There is now evidence that clones of incipient cancer cells often 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 (Kawai et al., 2007; Hansel et al., 2006). These results also suggest that such 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 succeed in becoming macroscopic, frankly neoplastic growths.

In contrast, the absence of TP53-mediated surveillance of genomic integrity may permit other incipient neoplasias to survive initial telomere erosion and attendant chromosomal breakage-fusion-bridge (BFB) cycles. The genomic alterations resulting from these BFB cycles, including deletions and amplifications of chromosomal segments, evidently serve to increase the mutability of the genome, thereby accelerating the acquisition of mutant oncogenes and tumor suppressor genes. The realization that impaired telomere function can actually foster tumor progression has come from the study of mutant mice that lack both p53 and telomerase function (Artandi and DePinho, 2010, 2000). The proposition that these two defects can cooperatively enhance human tumorigenesis has not yet been directly documented.

Circumstantial support for the importance of transient telomere deficiency in facilitating malignant progression has come, in addition, from comparative analyses of premalignant and malignant lesions in the human breast (Raynaud et al., 2010; Chin et al., 2004). The premalignant lesions did not express significant levels of telomerase and were marked by telomere shortening and nonclonal chromosomal aberrations. In contrast, overt carcinomas exhibited telomerase expression concordantly with the reconstruction of longer telomeres and the fixation (via clonal outgrowth) 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 activation stabilizes the mutant genome and confers the unlimited replicative capacity that cancer cells require in order to generate clinically apparent tumors.

New Functions of Telomerase

Telomerase was discovered because of its ability to elongate and maintain telomeric DNA, and almost all telomerase research has been posited on the notion that its functions are confined to this crucial function. However, in recent years it has become apparent that telomerase exerts functions that are relevant to cell proliferation but unrelated to telomere maintenance. The noncanonical roles of telomerase, and in particular its protein subunit TERT, have been revealed by functional studies in mice and cultured cells; in some cases novel functions have been demonstrated in conditions where the telomerase enzymatic activity has been eliminated (Cong and Shay, 2008). Among the growing list of telomere-independent functions of TERT/telomerase is the ability of TERT to amplify signaling by the Wnt pathway, by serving as a cofactor of the β-catenin/LEF transcription factor complex (Park et al., 2009). Other ascribed telomere-independent effects include demonstrable enhancement of cell proliferation and/or resistance to apoptosis (Kang et al., 2004), involvement in DNA-damage repair (Masutomi et al., 2005), and RNA-dependent RNA polymerase function (Maida et al., 2009). Consistent with these broader roles, TERT can be found associated with chromatin at multiple sites along the chromosomes, not just at the telomeres (Park et al., 2009; Masutomi et al., 2005). Hence, telomere maintenance is proving to be the most prominent of a diverse series of functions to which TERT contributes. The contributions of these additional functions of telomerase to tumorigenesis remain to be fully elucidated.

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 (Hanahan and Folkman, 1996).

A compelling body of evidence indicates that the angiogenic switch is governed by countervailing factors that either induce or oppose angiogenesis (Baeriswyl and Christofori, 2009; Bergers and Benjamin, 2003). 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, and then in homeostatic survival of endothelial cells, as well as in physiological and pathological situations in the adult. VEGF signaling via three receptor tyrosine kinases (VEGFR-1–3) is regulated at multiple levels, reflecting this complexity of purpose. Thus, VEGF gene expression can by upregulated both by hypoxia and by oncogene signaling (Ferrara, 2009; Mac Gabhann and Popel, 2008; Carmeliet, 2005). 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., MMP-9; Kessenbrock et al., 2010). In addition, other proangiogenic signals, such as members of the fibroblast growth factor (FGF) family, have been implicated in sustaining tumor angiogenesis when their expression is chronically upregulated (Baeriswyl and Christofori, 2009). TSP-1, a key counterbalance in the

angiogenic switch, also binds transmembrane receptors displayed by endothelial cells and thereby evokes suppressive signals that can counteract proangiogenic stimuli (Kazerounian et al., 2008).

The blood vessels produced within tumors by chronically activated angiogenesis and 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, leakiness, and abnormal levels of endothelial cell proliferation and apoptosis (Nagy et al., 2010; Baluk et al., 2005).

Angiogenesis is induced surprisingly early during the multistage development of invasive cancers both in animal models and in humans. Histological 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 (Raica et al., 2009; Hanahan and Folkman, 1996). 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.

The past decade has witnessed an astonishing outpouring of research on angiogenesis. Amid this wealth of new knowledge, we highlight several advances of particular relevance to tumor physiology.

Gradations of the Angiogenic Switch

Once angiogenesis has been activated, tumors exhibit diverse patterns of neovascularization. Some tumors, including such highly aggressive types as pancreatic ductal adenocarcinomas, are hypovascularized and replete with stromal ''deserts'' that are largely avascular and indeed may even be actively antiangiogenic (Olive et al., 2009). Many other tumors, including human renal and pancreatic neuroendocrine carcinomas, are highly angiogenic and consequently densely vascularized (Zee et al., 2010; Turner et al., 2003).

Collectively, such observations suggest an initial tripping of the angiogenic switch during tumor development that is followed by a variable intensity of ongoing neovascularization, the latter being controlled by a complex biological rheostat that involves both the cancer cells and the associated stromal microenvironment (Baeriswyl and Christofori, 2009; Bergers and Benjamin, 2003). Of note, the switching mechanism can vary in its form, 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 expression of angiogenic factors, whereas in others, such inductive signals are produced indirectly by immune inflammatory cells, as discussed below. The direct induction of angiogenesis by oncogenes that also drive proliferative signaling illustrates the important principle that distinct hallmark capabilities can be coregulated by the same transforming agents.

Endogenous Angiogenesis Inhibitors Present Natural Barriers to Tumor Angiogenesis

Research in the 1990s revealed that TSP-1 as well as fragments of plasmin (angiostatin) and type 18 collagen (endostatin) can act as endogenous inhibitors of angiogenesis (Ribatti, 2009; Kazerounian, et al., 2008; Folkman, 2006, 2002; Nyberg et al., 2005). The last decade has seen reports of another dozen such agents (Ribatti, 2009; Folkman, 2006; Nyberg et al., 2005). 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. The genes encoding several endogenous angiogenesis inhibitors have been deleted from the mouse germline without untoward physiological effects; the growth of autochthonous and implanted tumors, however, is enhanced as a consequence (Ribatti, 2009; Nyberg et al., 2005). 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 (Ribatti, 2009; Nyberg et al., 2005); interestingly, wound healing and fat deposition are impaired or accelerated by elevated or ablated expression of such genes (Cao, 2010; Seppinen et al., 2008). The data suggest that such endogenous angiogenesis inhibitors serve under normal circumstances as physiologic regulators that modulate transitory angiogenesis during tissue remodeling and wound healing; they may also act as intrinsic barriers to 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. Tumor-associated vasculature, in contrast, was portrayed as lacking appreciable coverage by these auxiliary cells. However, careful microscopic studies conducted in recent years have revealed that pericytes are associated, albeit loosely, with the neovasculature of most if not all tumors (Raza et al., 2010; Bergers and Song, 2005). More importantly, mechanistic studies discussed below 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 pathological angiogenesis (Qian and Pollard, 2010; Zumsteg and Christofori, 2009; Murdoch et al., 2008; De Palma et al., 2007). These include cells of the innate immune system—notably macrophages, neutrophils, mast cells, and myeloid progenitors—that infiltrate premalignant lesions and progressed tumors and assemble at the margins of such lesions; the peri-tumoral inflammatory cells help to trip the angiogenic switch in previously quiescent tissue and to sustain ongoing angiogenesis associated with tumor growth, in addition to facilitating local invasion, as noted below. In addition, they can help protect the vasculature from the effects of drugs targeting endothelial cell signaling (Ferrara, 2010). Additionally, several types of bone marrow-derived ''vascular progenitor cells'' have been observed in certain cases to have migrated into neoplastic lesions and become intercalated into the neovasculature as pericytes or endothelial cells (Patenaude et al., 2010; Kovacic and Boehm, 2009; Lamagna and Bergers, 2006).

Activating Invasion and Metastasis

In 2000, the mechanisms underlying invasion and metastasis were largely an enigma. It was clear that as carcinomas arising from epithelial tissues progressed to higher pathological grades of malignancy, reflected in local invasion and distant metastasis, the associated cancer cells typically developed alterations in their shape as well as in their attachment to other cells and to the extracellular matrix (ECM). The best characterized alteration involved the loss by carcinoma cells of E-cadherin, a key cell-to-cell adhesion molecule. By forming adherens junctions with adjacent epithelial cells, E-cadherin helps to assemble epithelial cell sheets and maintain the quiescence of the cells within these sheets. Increased expression of E-cadherin was well established as an antagonist of invasion and metastasis, whereas reduction of its expression was known to potentiate these phenotypes. The frequently observed downregulation and occasional mutational inactivation of E-cadherin in human carcinomas provided strong support for its role as a key suppressor of this hallmark capability (Berx and van Roy, 2009; Cavallaro and Christofori, 2004).

Additionally, expression of genes encoding other cell-to-cell and cell-to-ECM adhesion molecules is demonstrably altered in some 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. Beyond the gain and loss of such cell-cell/matrix attachment proteins, the master regulators of invasion and metastasis were largely unknown or, when suspected, lacking in functional validation (Cavallaro and Christofori, 2004).

The multistep process of invasion and metastasis has been schematized as a sequence of discrete steps, often termed the invasion-metastasis cascade (Talmadge and Fidler, 2010; Fidler, 2003). This depiction envisions 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 escape of cancer cells from the lumina of such vessels into the parenchyma of distant tissues (extravasation), the formation of small nodules of cancer cells (micrometastases), and finally the growth of micrometastatic lesions into macroscopic tumors, this last step being termed ''colonization.''

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, and as critical regulatory genes were identified. While 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 below.

The EMT Program Broadly Regulates Invasion and Metastasis

A developmental regulatory program, referred to as the ''epithelial-mesenchymal transition'' (EMT), has become prominently implicated as a means by which transformed epithelial cells can acquire the abilities to invade, to resist apoptosis, and to disseminate (Klymkowsky and Savagner, 2009; Polyak and Weinberg, 2009; Thiery et al., 2009; Yilmaz and Christofori, 2009; Barrallo-Gimeno and Nieto, 2005). 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, 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 and have been shown in experimental models of carcinoma formation to be causally important for programming invasion; some have been found to elicit metastasis when ectopically overexpressed (Micalizzi et al., 2010; Taube et al., 2010; Schmalhofer et al., 2009; Yang and Weinberg, 2008). Included among the cell-biological traits evoked by such transcription factors are loss of adherens junctions and associated conversion from a polygonal/epithelial to a spindly/fibroblastic morphology, expression of matrix-degrading enzymes, increased motility, and heightened resistance to apoptosis—all traits implicated in the processes of invasion and metastasis. Several of these transcription factors can directly repress E-cadherin gene expression, thereby depriving neoplastic epithelial cells of this key suppressor of motility and invasiveness (Peinado et al., 2004).

The available evidence suggests that these transcription factors regulate one another as well as overlapping sets of target genes. No rules have yet been established to describe their interactions and the conditions that govern their expression. Evidence from developmental genetics indicates that contextual signals received from neighboring cells in the embryo are involved in triggering expression of these transcription factors in those cells destined to pass through an EMT (Micalizzi et al., 2010); in an analogous fashion, increasing evidence suggests that heterotypic interactions of cancer cells with adjacent tumor-associated stromal cells can induce expression of the malignant cell phenotypes that are known to be choreographed by one or more of these transcriptional regulators (Karnoub and Weinberg, 2006–2007; Brabletz et al., 2001). 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 (Hlubek et al., 2007).

Although the evidence is still incomplete, it would appear that EMT-inducing transcription factors are able to orchestrate most steps of the invasion-metastasis cascade save the final step of colonization. We still know rather little about the various manifestations and temporal stability of the mesenchymal state produced by an EMT. Although expression of EMT-inducing transcription factors 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 invasive carcinoma cells necessarily acquire their capability through activation of parts of the EMT program, or whether alternative regulatory programs can also enable this capability.

Heterotypic Contributions of Stromal Cells to Invasion and Metastasis

It is increasingly apparent that crosstalk between cancer cells and cells of the neoplastic stroma is involved in the acquired capability for invasive growth and metastasis (Egeblad et al., 2010; Qian and Pollard, 2010; Joyce and Pollard, 2009; Kalluri and Zeisberg, 2006). Such signaling may impinge on carcinoma cells and act to alter their hallmark capabilities as suggested above. For example, mesenchymal stem cells (MSCs) 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 (Karnoub et al., 2007).

Macrophages at the tumor periphery can foster local invasion by supplying matrix-degrading enzymes such as metalloproteinases and cysteine cathepsin proteases (Kessenbrock et al., 2010; Joyce and Pollard, 2009; Palermo and Joyce, 2008; Mohamed and Sloane, 2006); in one model system, the invasion-promoting macrophages are activated by IL-4 produced by the cancer cells (Gocheva et al., 2010). And in an experimental model of metastatic breast cancer, tumor-associated macrophages (TAMs) supply epidermal growth factor (EGF) to breast cancer cells, while the cancer cells reciprocally stimulate the macrophages with CSF-1; their concerted interactions facilitate intravasation into the circulatory system and metastatic dissemination of the cancer cells (Qian and Pollard, 2010; Wyckoff et al., 2007).

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 tumor cell genomes. One important implication, still untested, is that the ability to negotiate most of the steps of the invasion-metastasis cascade may be acquired in certain tumors without the requirement that the associated cancer 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 a more distant tissue site may no longer benefit from the activated stroma and invasion/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 homes to a noninvasive state. Thus, carcinoma cells that have undergone an EMT during initial invasion and metastatic dissemination may pass through the reverse process, termed the mesenchymal-epithelial transition (MET). This plasticity may result in the formation of new tumor colonies of carcinoma cells exhibiting a histopathology similar to those of carcinoma cells in the primary tumor that never underwent an EMT (Hugo et al., 2007). Moreover, the notion that cancer cells routinely pass through a complete EMT program is likely to be simplistic; instead, in many cases, cancer cells may enter into an EMT program only partially, thereby acquiring new mesenchymal traits while continuing to express residual epithelial traits.

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 (Friedl and Wolf, 2008, 2010). "Collective invasion" involves nodules 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 (Madsen and Sahai, 2010; Sabeh et al., 2009), in which individual cancer cells show morphological plasticity, enabling them to slither through existing interstices in the extracellular matrix 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-biological programs are responsible for choreographing these alternative invasion programs.

Another emerging concept, noted above, involves the facilitation of cancer cell invasion by inflammatory cells that assemble at the boundaries of tumors, producing the extracellular matrix-degrading enzymes and other factors that enable invasive growth (Kessenbrock et al., 2010; Qian and Pollard, 2010; Joyce and Pollard, 2009); these functions may obviate the need of cancer cells to produce these proteins through activation of EMT programs. Thus, cancer cells may secrete the chemoattractants that recruit the proinvasive inflammatory cells rather than producing the matrix-degrading enzymes themselves.

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 be in 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 successfully disseminated but never progress to macroscopic metastatic tumors (Talmadge and Fidler, 2010; McGowan et al., 2009; Aguirre-Ghiso, 2007; Townson and Chambers, 2006; Fidler, 2003).

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 (Demicheli et al., 2008; Folkman, 2002). 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 tissue colonization (Barkan, et al., 2010; Aguirre-Ghiso, 2007; Townson and Chambers, 2006).

One can infer from such natural histories that micrometastases may lack other 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 (Naumov et al., 2008; Aguirre-Ghiso, 2007). 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 changes in tissue microenvironment, such as access to more nutrients, permit (Kenific et al., 2010; Lu et al., 2008). Other mechanisms of micrometastatic dormancy may involve anti-growth signals embedded in normal tissue extracellular matrix (Barkan et al., 2010) and tumor-suppressing actions of the immune system (Teng et al., 2008; Aguirre-Ghiso, 2007).

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 (Gupta et al., 2005). These adaptations might require hundreds of distinct colonization programs, each dictated by the type of disseminating cancer cell and the nature of the tissue microenvironment in which colonization is proceeding. As further discussed below, however, certain tissue microenviroments may be preordained to be intrinsically hospitable to disseminated cancer cells (Peinado et al., 2011; Talmadge and Fidler, 2010).

Metastatic dissemination has long been depicted as the last step in multistep primary tumor progression, and indeed for many tumors that is likely the case, as illustrated by recent genome sequencing studies that present genetic evidence for clonal evolution of pancreatic ductal adenocarcinoma to metastasis (Campbell et al., 2010; Luebeck, 2010; Yachida et al., 2010). On the other hand, evidence has recently emerged indicating that cells can disseminate remarkably early, dispersing from ostensibly noninvasive premalignant lesions in both mice and humans (Coghlin and Murray, 2010; Klein, 2009). Additionally, micrometastases can be spawned from primary tumors that are not obviously invasive but possess a neovasculature lacking in lumenal integrity (Gerhardt and Semb, 2008). Although cancer cells can clearly disseminate from such pre-neoplastic 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 whose clinical significance is yet to be established.

Beyond the timing of their dissemination, it also remains unclear when and where cancer cells develop the ability to colonize foreign tissues as macroscopic tumors. This capability may arise during primary tumor formation as a result of a tumor's particular developmental path prior to any dissemination, such that primary tumor cells entering the circulation are fortuitously endowed with the ability to colonize certain distant tissue sites (Talmadge and Fidler, 2010). Alternatively, the ability to colonize specific tissues may only develop in response to the selective pressure on already disseminated cancer cells to adapt to growth in foreign tissue microenvironments.

Having developed such 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 cells within a primary tumor may originate not from classical tumor progression occurring within the primary lesion but instead from emigrants that have returned home (Kim et al., 2009). Such reseeding is consistent with the aforementioned studies of human pancreatic cancer metastasis (Campbell et al., 2010; Luebeck, 2010; Yachida et al., 2010). Stated differently, the phenotypes and underlying gene expression programs of the populations of cancer cells (and of the cancer stem cells discussed below) within primary tumors may be significantly modified by 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 may intrinsically provide a hospitable site for reseeding and colonization by circulating cancer cells emanating 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 (Coghlin and Murray, 2010; Bos et al., 2009; Olson et al., 2009; Nguyen et al., 2009; Gupta et al., 2005). The challenge is considerable, given the apparent multitude of distinct colonization programs cited above. Moreover, colonization is unlikely to depend exclusively on cell-autonomous processes. Instead, it 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-biological programs that are, in aggregate, considerably more complex and diverse than the preceding steps of metastatic dissemination.

Programming of Hallmark Capabilities by Intracellular Circuitry

In 2000, we presented a metaphor, in which the numerous signaling molecules affecting cancer cells operate as nodes and branches of elaborate integrated circuits that are reprogrammed derivatives of the circuits operating in normal cells. The ensuing decade has both solidified the original depiction of these circuits and expanded the catalog of signals and the interconnections of their signaling pathways. It is difficult if not impossible to graphically portray this circuit comprehensively and coherently, as was already the case in 2000.

We now suggest a portrayal of this circuitry that is aligned with individual hallmarks of cancer. Thus, the intracellular integrated

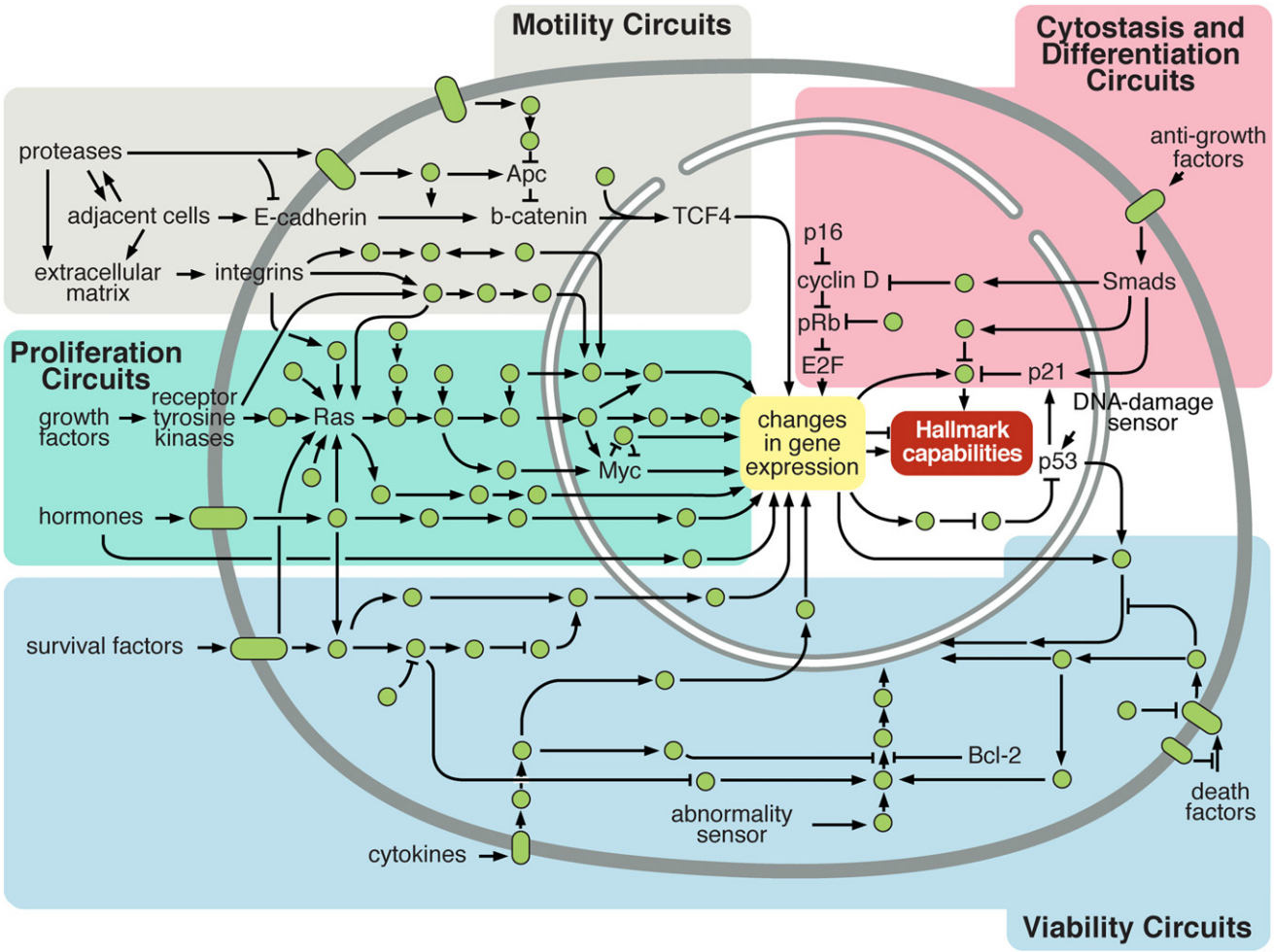

Figure 2. Intracellular Signaling Networks Regulate the Operations of the Cancer Cell
An elaborate integrated circuit operates within normal cells and is reprogrammed to regulate hallmark capabilities within cancer cells. Separate subcircuits, depicted here in differently colored fields, are specialized to orchestrate the various capabilities. At one level, this depiction is simplistic, as there is considerable crosstalk between such subcircuits. In addition, because each cancer cell is exposed to a complex mixture of signals from its microenvironment, each of these subcircuits is connected with signals originating from other cells in the tumor microenvironment, as outlined in Figure 5.

circuit can be segmented into distinct subcircuits, each of which is specialized to support a discrete cell-biological property in normal cells and is reprogrammed in order to implement a hallmark capability in cancer cells (Figure 2). Only a subset of hallmark capabilities are addressed in this figure, either because their underlying control circuits remain poorly understood or because they overlap extensively with those portrayed here.

An additional dimension of complexity involves considerable interconnections and thus crosstalk between the individual subcircuits. For example, certain oncogenic events can affect multiple capabilities, as illustrated by the diverse effects that prominent oncogenes, such as mutant *RAS* and upregulated *MYC*, have on multiple hallmark capabilities (e.g., proliferative signaling, energy metabolism, angiogenesis, invasion, and survival). We anticipate that future renditions of this integrated circuit will encompass subcircuits and associated hallmark capabilities that are still not addressed here.

ENABLING CHARACTERISTICS AND EMERGING HALLMARKS

We have defined the hallmarks of cancer as acquired functional capabilities that allow cancer cells to survive, proliferate, and disseminate; these functions are acquired in different tumor types via distinct mechanisms and at various times during the course of multistep tumorigenesis. Their acquisition is made possible by two *enabling characteristics*. Most prominent is the development of genomic instability in cancer cells, which generates random mutations including chromosomal rearrangements; among these are the rare genetic changes that can orchestrate hallmark capabilities. A second enabling characteristic involves the inflammatory state of premalignant and frankly malignant lesions that is driven by cells of the immune system, some of which serve to promote tumor progression through various means.

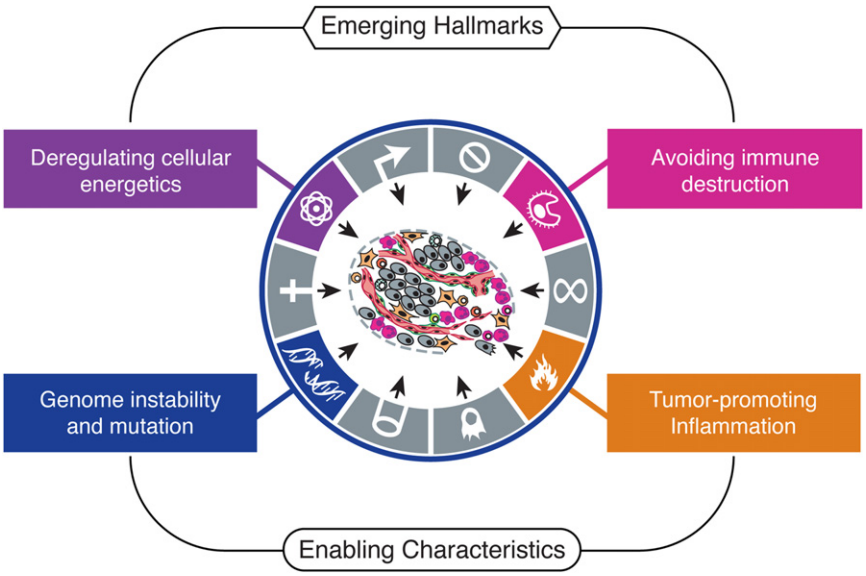

Figure 3. Emerging Hallmarks and Enabling Characteristics

An increasing body of research suggests that two additional hallmarks of cancer are involved in the pathogenesis of some and perhaps all cancers. One involves the capability to modify, or reprogram, cellular metabolism in order to most effectively support neoplastic proliferation. The second allows cancer cells to evade immunological destruction, in particular by T and B lymphocytes, macrophages, and natural killer cells. Because neither capability is yet generalized and fully validated, they are labeled as emerging hallmarks. Additionally, two consequential characteristics of neoplasia facilitate acquisition of both core and emerging hallmarks. Genomic instability and thus mutability endow cancer cells with genetic alterations that drive tumor progression. Inflammation by innate immune cells designed to fight infections and heal wounds can instead result in their inadvertent support of multiple hallmark capabilities, thereby manifesting the now widely appreciated tumor-promoting consequences of inflammatory responses.

Yet other distinct attributes of cancer cells have been proposed to be functionally important for the development of cancer and might therefore be added to the list of core hallmarks (Negrini et al., 2010; Luo et al., 2009; Colotta et al., 2009). Two such attributes are particularly compelling. The first involves major reprogramming of cellular energy metabolism in order to support continuous cell growth and proliferation, replacing the metabolic program that operates in most normal tissues and fuels the physiological operations of the associated cells. The second involves active evasion by cancer cells from attack and elimination by immune cells; this capability highlights the dichotomous roles of an immune system that both antagonizes and enhances tumor development and progression. Both of these capabilities may well prove to facilitate the development and progression of many forms of human cancer and therefore can be considered to be emerging hallmarks of cancer. These enabling characteristics and *emerging hallmarks,* depicted in Figure 3, are discussed individually below.

An Enabling Characteristic: Genome Instability and Mutation

Acquisition of the multiple hallmarks enumerated above depends in large part on a succession of alterations in the genomes of neoplastic cells. Simply depicted, certain mutant genotypes confer selective advantage on subclones of 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, each of which is triggered by the chance acquisition of an enabling mutant genotype. Because heritable phenotypes, e.g., inactivation of tumor suppressor genes, can also be acquired through epigenetic mechanisms such as DNA methylation and histone modifications (Berdasco and Esteller, 2010; Esteller, 2007; Jones and Baylin, 2007), some clonal expansions may well be triggered by nonmutational changes affecting the regulation of gene expression.

The extraordinary ability of genome maintenance systems to detect and resolve defects in the DNA ensures that rates of spontaneous mutation are usually very low during each cell generation. In the course of acquiring the roster of mutant genes needed to orchestrate tumorigenesis, cancer cells often increase the rates of mutation (Negrini et al., 2010; Salk et al., 2010). 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 compromising the surveillance systems that normally monitor genomic integrity and force genetically damaged cells into either senescence or apoptosis (Jackson and Bartek, 2009; Kastan, 2008; Sigal and Rotter, 2000). The role of TP53 is central here, leading to its being called the "guardian of the genome" (Lane, 1992).

A diverse array of defects affecting various components of the DNA-maintenance machinery—often referred to as the "caretakers" of the genome (Kinzler and Vogelstein, 1997)—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 (Negrini et al., 2010; Ciccia and Elledge, 2010; Jackson and Bartek, 2009; Kastan, 2008; Harper and Elledge, 2007; Friedberg et al., 2006). From a genetic perspective, these caretaker genes behave much like tumor suppressor genes, in that their functions can be 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 germline and result, predictably, in increased cancer incidence, supporting their potential involvement in human cancer development (Barnes and Lindahl, 2004).

In the decade since we first enumerated the cancer hallmarks, another major source of tumor-associated genomic instability has been uncovered: as described earlier, the loss of telomeric DNA in many tumors generates karyotypic instability and associated amplification and deletion of chromosomal segments (Artandi and DePinho, 2010). When viewed in this light, telomerase is more than an enabler of the hallmark capability for unlimited replicative potential and 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 sites in the genome indicates that such sites are likely to harbor genes whose alteration favors neoplastic progression (Korkola and Gray, 2010).

More recently, with the advent of efficient and economical DNA-sequencing technologies, higher-resolution analyses 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 prevalence of ostensibly random mutations scattered across cancer cell genomes. Thus, recurring genetic alterations may point to a causal role of particular mutations in tumor pathogenesis.

Although the specifics of genome alteration vary dramatically between different tumor types, the large number of genome maintenance and repair defects that have already been documented in human tumors, 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 great majority of human cancer cells. 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

Pathologists have long recognized that some tumors are densely infiltrated by cells of both the innate and adaptive arms of the immune system and thereby mirror inflammatory conditions arising in non-neoplastic tissues (Dvorak, 1986). 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 (Pagès et al., 2010). 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, as discussed below.

By 2000, there were already clues that the tumor-associated inflammatory response had the unanticipated, paradoxical effect of enhancing tumorigenesis and progression, in effect helping incipient neoplasias to acquire hallmark capabilities. In the ensuing decade, 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 (DeNardo et al., 2010; Grivennikov et al., 2010; Qian and Pollard, 2010; Colotta et al., 2009). Inflammation can contribute to multiple hallmark capabilities by supplying bioactive 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-facilitating programs (DeNardo et al., 2010; Grivennikov et al., 2010; Qian and Pollard, 2010; Karnoub and Weinberg, 2006–2007).

Importantly, inflammation is in some cases evident at the earliest stages of neoplastic progression and is demonstrably capable of fostering the development of incipient neoplasias into full-blown cancers (Qian and Pollard, 2010; de Visser et al., 2006). Additionally, inflammatory cells can release chemicals, notably reactive oxygen species, that are actively mutagenic for nearby cancer cells, accelerating their genetic evolution toward states of heightened malignancy (Grivennikov et al., 2010). As such, inflammation can be considered an enabling characteristic for its contributions to the acquisition of core hallmark capabilities. The cells responsible for this enabling characteristic are described in the section below on the tumor microenvironment.

An Emerging Hallmark: 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 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 (Warburg, 1930, 1956a, 1956b): even in the presence of oxygen, cancer cells can reprogram their glucose metabolism, and thus their energy production, by limiting their energy metabolism largely to glycolysis, leading to a state that has been termed ''aerobic glycolysis.''

The existence of this metabolic switch in cancer cells has been substantiated in the ensuing decades. Such 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. They do so in part by upregulating glucose transporters, notably GLUT1, which substantially increases glucose import into the cytoplasm (Jones and Thompson, 2009; DeBerardinis et al., 2008; Hsu and Sabatini, 2008). Indeed, markedly increased uptake and utilization of glucose have 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*) (DeBerardinis et al., 2008; Jones and Thompson, 2009), whose alterations in tumor cells have been selected primarily for their benefits in conferring the hallmark capabilities of cell proliferation, avoidance 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 (Semenza, 2010a; Jones and Thompson, 2009; DeBerardinis et al., 2008). Thus, both the Ras oncoprotein and hypoxia can independently increase the levels of the HIF1α and HIF2α transcription factors, which in turn upregulate glycolysis (Semenza, 2010a, 2010b; Kroemer and Pouyssegur, 2008).

A functional rationale for the glycolytic switch in cancer cells has been elusive, given the relatively poor efficiency of generating ATP by glycolysis relative to mitochondrial oxidative phosphorylation. According to one long-forgotten (Potter, 1958) and recently revived and refined hypothesis (Vander Heiden et al., 2009), increased glycolysis allows the diversion of glycolytic intermediates into various biosynthetic pathways, including those generating nucleosides and amino acids; this facilitates, in turn, 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 (Kennedy and Dewhirst, 2010; Feron, 2009; Semenza, 2008). 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 cooption of a normal physiological mechanism, in this case one operating in muscle (Kennedy and Dewhirst, 2010; Feron, 2009; Semenza, 2008). Additionally, it is becoming apparent that oxygenation, ranging from normoxia to hypoxia, is not necessarily static in tumors but instead fluctuates temporally and regionally (Hardee et al., 2009), likely as a result of the instability and chaotic organization of the tumor-associated neovasculature.

Altered energy metabolism is proving to be as widespread in cancer cells as many of the other cancer-associated traits that have been accepted as hallmarks of cancer. This realization raises the question of whether deregulating cellular energy metabolism is therefore a core hallmark capability of cancer cells that is as fundamental as the six well-established core hallmarks. In fact, the redirection of energy metabolism is largely orchestrated by proteins that are involved in one way or another in programming the core hallmarks of cancer. When viewed in this way, aerobic glycolysis is simply another phenotype that is programmed by proliferation-inducing oncogenes.

Interestingly, activating (gain-of-function) mutations in the isocitrate dehydrogenase 1/2 (IDH) enzymes have been reported in glioma and other human tumors (Yen et al., 2010). Although these mutations may prove to have been clonally selected for their ability to alter energy metabolism, there is confounding data associating their activity with elevated oxidation and stability of the HIF-1 transcription factors (Reitman and Yan, 2010), which could in turn affect genome stability and angiogenesis/invasion, respectively, thus blurring the lines of phenotypic demarcation. Currently, therefore, the designation of reprogrammed energy metabolism as an emerging hallmark seems most appropriate, to highlight both its evident importance as well as the unresolved issues surrounding its functional independence from the core hallmarks.

An Emerging Hallmark: Evading Immune Destruction

A second, still-unresolved issue surrounding tumor formation involves the role that the immune system plays in resisting or eradicating formation and progression of incipient neoplasias, late-stage tumors, and micrometastases. The long-standing theory of immune surveillance proposes 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. According to this logic, solid tumors that do appear have somehow managed to avoid detection by the various arms of the immune system or have been able to limit the extent of immunological killing, thereby evading eradication.

The role of defective immunological monitoring of tumors would seem to be validated by the striking increases of certain cancers in immunocompromised individuals (Vajdic and van Leeuwen, 2009). 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, seem to 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.

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 immunocompetent controls. In particular, deficiencies in the development or function of $CD8^+$ cytotoxic T lymphocytes (CTLs), $CD4^+$ 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 (Teng et al., 2008; Kim et al., 2007).

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 (Teng et al., 2008; Kim et al., 2007). 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 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 (Smyth et al., 2006). (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 (Bindea et al., 2010; Ferrone and Dranoff, 2010; Nelson, 2008). For example, patients with colon and ovarian tumors that are heavily infiltrated with CTLs and NK cells have a better prognosis than those that lack such abundant killer lymphocytes (Pagès et al., 2010; Nelson, 2008); 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 the ostensibly tumor-free donors, the cancer cells were held in check, in a dormant state, by a fully functional immune system (Strauss and Thomas, 2010).

Still, the epidemiology of chronically immunosuppressed patients does not indicate significantly increased incidences of the major forms of nonviral human cancer, as noted above. 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 immunological 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 an immunological defense against cancer that is mounted by NK and other innate immune cells.

In truth, the above discussions of cancer immunology simplify tumor-host immunological interactions, as highly immunogenic cancer cells may well evade 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 (Yang et al., 2010; Shields et al., 2010). More subtle mechanisms operate through the recruitment of inflammatory cells that are actively immunosuppressive, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). Both can suppress the actions of cytotoxic lymphocytes (Mougiakakos et al., 2010; Ostrand-Rosenberg and Sinha, 2009).

In light of these considerations and the still-rudimentary demonstrations of antitumor immunity as a significant barrier to tumor formation and progression in humans, we present immunoevasion as another emerging hallmark, whose generality as a core hallmark capability remains to be firmly established.

THE TUMOR MICROENVIRONMENT

Over the past decade, tumors have increasingly been recognized as organs whose complexity approaches and may even exceed that of normal healthy tissues. When viewed from this perspective, the biology of a tumor can only be understood by studying the individual specialized cell types within it (Figure 4, upper) as well as the "tumor microenvironment" that they construct during the course of multistep tumorigenesis (Figure 4, lower). This depiction 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. We enumerate here a set of cell types known to contribute in important ways to the biology of many tumors and discuss the regulatory signaling that controls 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.

Cancer Cells and Cancer Stem Cells

Cancer cells are the foundation of the disease; they initiate tumors and drive tumor progression forward, carrying the oncogenic and tumor suppressor mutations that define cancer as a genetic disease. Traditionally, the cancer cells within tumors

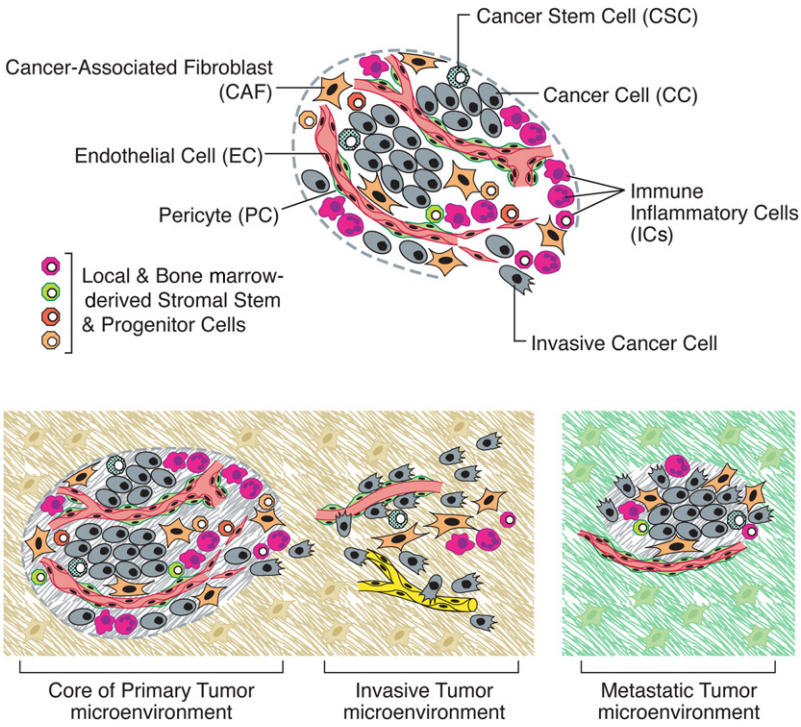

Figure 4. The Cells of the Tumor Microenvironment

(Upper) An assemblage of distinct cell types constitutes most solid tumors. Both the parenchyma and stroma of tumors contain distinct cell types and subtypes that collectively enable tumor growth and progression. Notably, the immune inflammatory cells present in tumors can include both tumor-promoting as well as tumor-killing subclasses.

(Lower) The distinctive microenvironments of tumors. The multiple stromal cell types create a succession of tumor microenvironments that change as tumors invade normal tissue and thereafter seed and colonize distant tissues. The abundance, histologic organization, and phenotypic characteristics of the stromal cell types, as well as of the extracellular matrix (hatched background), evolve during progression, thereby enabling primary, invasive, and then metastatic growth. The surrounding normal cells of the primary and metastatic sites, shown only schematically, likely also affect the character of the various neoplastic microenvironments. (Not shown are the premalignant stages in tumorigenesis, which also have distinctive microenvironments that are created by the abundance and characteristics of the assembled cells.)

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 distinct clonal subpopulations. Reflecting such clonal heterogeneity, many human tumors are histopathologically diverse, containing regions demarcated by various degrees of differentiation, proliferation, vascularity, inflammation, and/or 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 (CSCs).

Although the evidence is still fragmentary, CSCs may prove to be a common constituent of many if not most tumors, albeit being present with widely varying abundance. CSCs are defined operationally through their ability to efficiently seed new tumors upon inoculation into recipient host mice (Cho and Clarke, 2008; Lobo et al., 2007). This functional definition is often complemented by including the expression in CSCs of markers that are also expressed by the normal stem cells in the tissue-of-origin (Al-Hajj et al., 2003).

CSCs were initially implicated in the pathogenesis of hematopoietic malignancies (Reya et al., 2001; Bonnet and Dick, 1997) and then years later were identified in solid tumors, in particular breast carcinomas and neuroectodermal tumors (Gilbertson and Rich, 2007; Al-Hajj et al., 2003). Fractionation of cancer cells on the basis of displayed cell-surface markers has yielded subpopulations of neoplastic cells with a greatly enhanced ability, relative to the corresponding majority populations, to seed new tumors upon implantation in immunodeficient mice. These often-rare tumor-initiating cells proved to share transcriptional profiles with certain normal tissue stem cell populations, motivating their designation as stem-like.

The origins of CSCs within a solid tumor have not been clarified and indeed may well vary from one tumor type to another. 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 stem-like character. 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. It remains to be established whether multiple distinct classes of increasingly neoplastic stem cells form during 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 above (Singh and Settleman, 2010; Mani et al., 2008; Morel et al., 2008). 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 not only may enable cancer cells to physically disseminate from primary tumors but also can confer on such cells the self-renewal capability that is crucial to their subsequent clonal expansion at sites of dissemination (Brabletz et al., 2005). 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 (Boiko et al., 2010; Gupta et al., 2009; Quintana et al., 2008). 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. Such plasticity could complicate definitive measurement of their prevalence. Analogous plasticity is already implicated in the EMT program, which can be engaged reversibly (Thiery and Sleeman, 2006).

These complexities notwithstanding, it is 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 with properties of CSCs are more resistant to various commonly used chemotherapeutic treatments (Singh and Settleman, 2010; Creighton et al., 2009; Buck et al., 2007). Their persistence may help to explain the almost-inevitable disease recurrence following apparently successful debulking of human solid tumors by radiation and various forms of chemotherapy. Indeed, CSCs may well prove to underlie certain forms of tumor dormancy, whereby latent cancer cells persist for years or even decades after surgical resection or radio/chemotherapy, only to suddenly erupt and generate life-threatening disease. Hence, CSCs may represent a double-threat, in that they are more resistant to therapeutic killing and, at the same time, endowed with the ability to regenerate a tumor once therapy has been halted.

This phenotypic plasticity implicit in CSC state may also enable the formation of functionally distinct subpopulations within a tumor that support overall tumor growth in various ways. For example, an EMT can convert epithelial carcinoma cells into mesenchymal, fibroblast-like cancer cells that may well assume the duties of cancer-associated fibroblasts (CAFs) in some tumors. Remarkably, several recent reports 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 (Soda et al., 2011; El Hallani et al., 2010; Ricci-Vitiani et al., 2010; Wang et al., 2010). Observations like these indicate that certain tumors may acquire stromal support by inducing some of their own cancer cells to undergo various types of metamorphosis to produce stromal cell types rather than relying on recruited host cells to provide their functions.

The discovery of CSCs and biological plasticity in tumors indicates that a single, genetically homogeneous population of cells within a tumor may nevertheless be phenotypically heterogeneous due to the presence of cells in distinct states of differentiation. However, an equally important source of phenotypic variability may derive from the genetic heterogeneity within a tumor that accumulates as cancer progression proceeds. Thus, elevated genetic instability operating in later stages of tumor progression may drive rampant genetic diversification that outpaces the process of Darwinian selection, generating genetically distinct subpopulations far more rapidly than they can be eliminated.

Such thinking is increasingly supported by in-depth sequence analysis of tumor cell genomes, which has become practical due to recent major advances in DNA (and RNA) sequencing technology. Thus the sequencing of the genomes of cancer cells microdissected from different sectors of the same tumor (Yachida et al., 2010) has revealed striking intratumoral genetic heterogeneity. Some of this genetic diversity may be reflected in the long-recognized histological heterogeneity within individual human tumors. Alternatively, this genetic diversification may enable functional specialization, producing subpopulations of cancer cells that contribute distinct, complementary capabilities, which then accrue to the common benefit of overall tumor growth as described above.

Endothelial Cells

Much of the cellular heterogeneity within tumors is found in their stromal compartments. Prominent among the stromal constituents are the cells forming the tumor-associated vasculature. Mechanisms of development, differentiation, and homeostasis of endothelial cells composing the arteries, veins, and capillaries were already well understood in 2000. So too was the concept of the "angiogenic switch," which activates quiescent endothelial cells, causing them to enter into a cell-biological program that allows them to construct new blood vessels (see above). Over the last decade, a network of interconnected signaling pathways involving ligands of signal-transducing receptors displayed by endothelial cells (e.g., Notch, Neuropilin, Robo, and Eph-A/B) has been added to the already-prominent VEGF, angiopoietin, and FGF signals. These newly characterized pathways have been functionally implicated in developmental and tumor-associated angiogenesis and illustrate the complex regulation of endothelial cell phenotypes (Pasquale, 2010; Ahmed and Bicknell, 2009; Dejana et al., 2009; Carmeliet and Jain, 2000).

Other avenues of research are revealing distinctive gene expression profiles of tumor-associated endothelial cells and identifying cell-surface markers displayed on the lumenal surfaces of normal versus tumor endothelial cells (Nagy et al., 2010; Ruoslahti et al., 2010; Ruoslahti, 2002). 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.

Closely related to the endothelial cells of the general circulation are those forming lymphatic vessels (Tammela and Alitalo, 2010). 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 peripheries of tumors and in the adjacent normal tissues that cancer cells

invade. These associated lymphatics likely serve as channels for the seeding of metastases in the draining lymph nodes that are commonly observed in a number of cancer types.

Pericytes

As noted earlier, pericytes represent a specialized mesenchymal cell type (related to smooth muscle cells) with finger-like projections that wrap around the endothelial tubing of blood vessels. In normal tissues, pericytes are known to provide paracrine support signals to the normally 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 (Gaengel et al., 2009; Bergers and Song, 2005). 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 of blood flow.

Genetic and pharmacological perturbation of the recruitment and association of pericytes has demonstrated the functional importance of these cells in supporting the tumor endothelium (Pietras and Ostman, 2010; Gaengel et al., 2009; Bergers and Song, 2005). For example, pharmacological inhibition of signaling through the 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 (Pietras and Ostman, 2010; Raza et al., 2010; Gaengel et al., 2009); interestingly, and in contrast, the pericytes of normal vessels are not prone to such pharmacological disruption, providing another example of the differences in 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, enabling subsequent hematogenous dissemination (Raza et al., 2010; Gerhardt and Semb, 2008).

Immune Inflammatory Cells

As also discussed above, 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. Although the presence of tumor-antagonizing CTLs and NK cells is not surprising, the prevalence of immune cells that functionally enhance hallmark capabilities was largely unanticipated. 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 association of sites of chronic inflammation with tumor formation, and to the observation that tumors could be portrayed as wounds that never heal (Schäfer and Werner, 2008: Dvorak, 1986). In the course of normal wound healing and fighting 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 various tissue pathologies, including fibrosis, aberrant angiogenesis, and neoplasia (Grivennikov et al., 2010; Karin et al., 2006).

Over the past decade, the manipulation of genes involved in the determination or effector functions of various immune cell types, together with pharmacological inhibitors of such cells or their functions, has shown them to play diverse and critical roles in fostering tumorigenesis. The roster of tumor-promoting inflammatory cells now includes macrophage subtypes, mast cells, and neutrophils, as well as T and B lymphocytes (Coffelt et al., 2010; DeNardo et al., 2010; Egeblad et al., 2010; Johansson et al., 2008; Murdoch et al., 2008; DePalma et al., 2007). Such studies are yielding a growing list of signaling molecules released by inflammatory cells that serve as effectors of their tumor-promoting actions. These include the tumor growth factor EGF, the angiogenic growth factor VEGF, other proangiogenic factors such as FGF2, 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 matrix metalloproteinases, cysteine cathepsin proteases, and heparanase (Qian and Pollard, 2010; Murdoch et al., 2008). Consistent with their expression of these diverse effectors, tumor-infiltrating inflammatory cells have been shown to induce and help sustain tumor angiogenesis, to stimulate cancer cell proliferation, to facilitate, via their presence at the margins of tumors, tissue invasion, and to support the metastatic dissemination and seeding of cancer cells (Coffelt et al., 2010; Egeblad et al., 2010; Qian and Pollard, 2010; Mantovani, 2010; Joyce and Pollard, 2009; Mantovani et al., 2008; Murdoch et al., 2008; DePalma et al., 2007).

In addition to fully differentiated immune cells present in tumor stroma, a variety of partially differentiated myeloid progenitors have been identified in tumors (Murdoch et al., 2008). 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 (defined as coexpressing the macrophage marker CD11b and the neutrophil marker Gr1) has been shown to suppress CTL and NK cell activity, having been independently identified as MDSCs (Qian and Pollard, 2010; Ostrand-Rosenberg and Sinha, 2009). This attribute raises the possibility that 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 to evade immune destruction.

The counterintuitive existence of both tumor-promoting and tumor-antagonizing immune cells can be rationalized by invoking the diverse roles of the immune system: On the one hand, the immune system specifically detects and targets infectious agents with the adaptive immune response, which is supported by cells of the innate immune system. On the other, the innate immune system is involved in wound healing and clearing dead cells and cellular debris. These specialized tasks are accomplished by distinct subclasses of inflammatory cells, namely a class of conventional macrophages and neutrophils (engaged in supporting adaptive immunity), and subclasses of "alternatively activated" macrophages, neutrophils, and

myeloid progenitors that are engaged in wound healing and tissue housecleaning (Egeblad et al., 2010; Mantovani, 2010; Qian and Pollard, 2010; Johansson et al., 2008). The latter subtypes of immune cells are one of the major sources of the angiogenic, epithelial, and stromal growth factors and matrix-remodeling enzymes that are needed for wound healing, and it is these cells that are recruited and subverted to support neoplastic progression. Similarly, subclasses of B and T lymphocytes may facilitate the recruitment, activation, and persistence of such wound-healing and tumor-promoting macrophages and neutrophils (DeNardo et al., 2010; Egeblad et al., 2010; Biswas and Mantovani, 2010). Of course, other subclasses of B and T lymphocytes and innate immune cell types can mount demonstrable tumor-killing responses. The balance between the conflicting inflammatory responses in tumors is likely to prove instrumental in prognosis and, quite possibly, in therapies designed to redirect these cells toward tumor destruction.

Cancer-Associated Fibroblasts

Fibroblasts are found in various proportions across the spectrum of carcinomas, constituting in many cases the preponderant cell population of the tumor stroma. The term ''cancer-associated fibroblast'' 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 biological roles and properties differ markedly from those of tissue-derived fibroblasts. Myofibroblasts are identifiable by their expression of α-smooth muscle actin (SMA). They are rare in most healthy epithelial tissues, although certain tissues, such as the liver and pancreas, contain appreciable numbers of α-SMA-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, contributing to the pathological fibrosis observed in tissues such as lung, kidney, and liver.

Recruited myofibroblasts and reprogrammed variants of normal tissue-derived fibroblastic cells have been demonstrated to enhance tumor phenotypes, notably cancer cell proliferation, angiogenesis, and 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 (Dirat et al., 2010; Pietras and Ostman, 2010; Räsänen and Vaheri, 2010; Shimoda et al., 2010; Kalluri and Zeisberg, 2006; Bhowmick et al., 2004). Because they secrete a variety of extracellular matrix 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.

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, the bone marrow has increasingly been implicated as a key source of tumor-associated stromal cells (Bergfeld and DeClerck, 2010; Fang and Salven, 2011; Giaccia and Schipani, 2010; Patenaude et al., 2010; Lamagna and Bergers, 2006). Mesenchymal stem and progenitor cells have been found to transit into tumors from the marrow, where they may 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 bone marrow origins of stromal cell types have been demonstrated using tumor-bearing mice in which the bone marrow cells and thus their disseminated progeny have been selectively labeled with reporters such as green fluorescent protein (GFP). While immune inflammatory cells have been long known to derive from the bone marrow, more recently the progenitors of pericytes and of various subtypes of cancer-associated fibroblasts originating from the bone marrow have been described in various mouse models of cancer (Bergfeld and DeClerck, 2010; Fang and Salven, 2011; Giaccia and Schipani, 2010; Lamagna and Bergers, 2006); the prevalence and functional importance of endothelial progenitors for tumor angiogenesis is currently unresolved (Fang and Salven, 2011; Patenaude et al., 2010). Taken together, these various lines of evidence indicate that tumor-associated stromal cells may be supplied to growing tumors by proliferation of preexisting stromal cells, by differentiation in situ of local stem/progenitor cells originating in the neighboring normal tissue, or via recruitment of bone marrow-derived stem/progenitor cells.

Heterotypic Signaling Orchestrates the Cells of the Tumor Microenvironment

Depictions of the intracellular circuitry governing cancer cell biology (e.g., Figure 2) will need to be complemented by similar diagrams charting the complex interactions between the neoplastic and stromal cells within a tumor and the dynamic extracellular matrix that they collectively erect and remodel (Egeblad et al., 2010; Kessenbrock et al., 2010; Pietras and Ostman, 2010; Polyak et al., 2009). A reasonably complete, graphic depiction of the network of microenvironmental signaling interactions is still far beyond our reach, as the great majority of signaling molecules and pathways remain to be identified. We provide instead a hint of such interactions in Figure 5, upper. These few well-established examples are intended to exemplify a signaling network of remarkable complexity that is of critical importance to tumor pathogenesis.

Another dimension of complexity is not represented in this simple schematic: both neoplastic cells and the stromal cells around them change progressively during the multistep transformation of normal tissues into high-grade malignancies. This histopathological progression must reflect underlying changes in heterotypic signaling between tumor parenchyma and stroma.

Such stepwise progression is likely to depend on back-and-forth reciprocal interactions between the neoplastic cells and the supporting stromal cells, as depicted in Figure 5, lower. 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

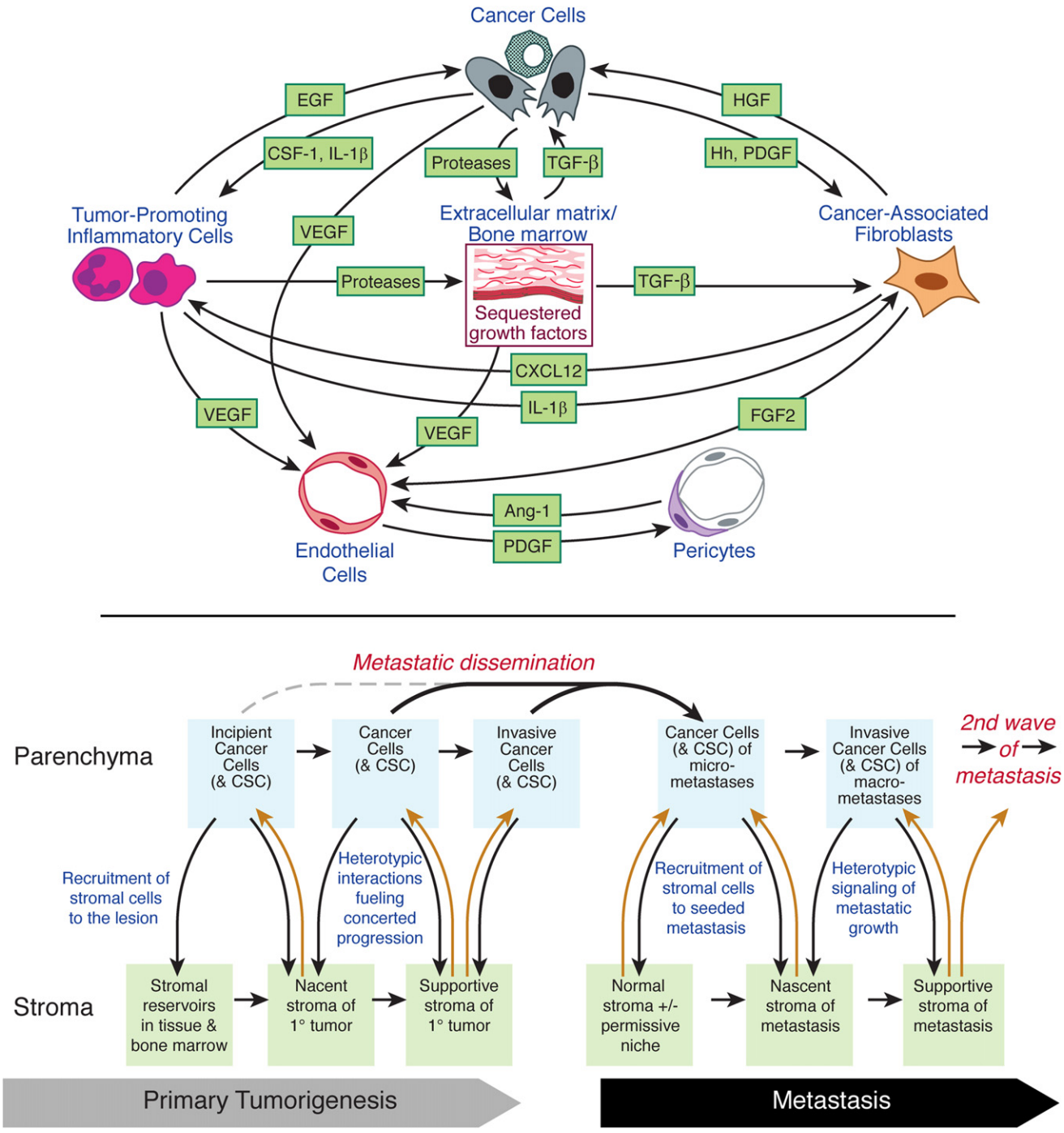

Figure 5. Signaling Interactions in the Tumor Microenvironment during Malignant Progression

(Upper) The assembly and collective contributions of the assorted cell types constituting the tumor microenvironment are orchestrated and maintained by reciprocal heterotypic signaling interactions, of which only a few are illustrated.

(Lower) The intracellular signaling depicted in the upper panel within the tumor microenvironment is not static but instead changes during tumor progression as a result of reciprocal signaling interactions between cancer cells of the parenchyma and stromal cells that convey the increasingly aggressive phenotypes that underlie growth, invasion, and metastatic dissemination. Importantly, the predisposition to spawn metastatic lesions can begin early, being influenced by the differentiation program of the normal cell-of-origin or by initiating oncogenic lesions. Certain organ sites (sometimes referred to as "fertile soil" or "metastatic niches") can be especially permissive for metastatic seeding and colonization by certain types of cancer cells, as a consequence of local properties that are either intrinsic to the normal tissue or induced at a distance by systemic actions of primary tumors. Cancer stem cells may be variably involved in some or all of the different stages of primary tumorigenesis and metastasis.

the neoplastic phenotypes of the nearby cancer cells. The cancer cells, which may further evolve genetically, again feed signals back to the stroma, continuing the reprogramming of normal stromal cells to serve the budding neoplasm; ultimately signals originating in the tumor stroma enable cancer cells to invade normal adjacent tissues and disseminate.

This model of reciprocal heterotypic signaling must be extended to encompass the final stage of multistep tumor progression—metastasis (Figure 5, lower right). The circulating cancer cells that are released from primary tumors leave a microenvironment created by the supportive stroma of such tumors. However, upon landing in a distant organ, these cancer cells encounter a naive, fully normal, tissue microenvironment. Consequently, many of the heterotypic signals that shaped their phenotype while they resided within primary tumors may be absent in sites of dissemination, constituting a barrier to growth of the seeded cancer cells. Thus, the succession of reciprocal cancer cell to stromal cell interactions that defined multistep progression in the primary tumor now must be repeated anew in distant tissues as disseminated cancer cells proceed to colonize their newfound organ sites.

Although this logic applies in some cases of metastasis, in others, as mentioned earlier, certain tissue microenvironments may, for various reasons, already be supportive of freshly seeded cancer cells; such permissive sites have been referred to as "metastatic niches" (Peinado et al., 2011; Coghlin and Murray, 2010). Implicit in this term is the notion that cancer cells seeded in such sites may not need to begin by inducing a supportive stroma because it already preexists, at least in part. Such permissivity may be intrinsic to the tissue site (Talmadge and Fidler, 2010) or preinduced by circulating factors released by the primary tumor (Peinado et al., 2011). The most well-documented components of induced premetastatic niches are tumor-promoting inflammatory cells, although other cell types and the ECM may well prove to play important roles in different metastatic contexts.

The likelihood that signaling interactions between cancer cells and their supporting stroma evolve during the course of multistage tumor development clearly complicates the goal of fully elucidating the mechanisms of cancer pathogenesis. For example, this reality poses challenges to systems biologists seeking to chart the crucial regulatory networks than orchestrate malignant progression. Moreover, it seems likely that understanding these dynamic variations will become crucial to the development of novel therapies designed to successfully target both primary and metastatic tumors.

THERAPEUTIC TARGETING

The introduction of mechanism-based targeted therapies to treat human cancers has been heralded as one of the fruits of three decades of remarkable progress of research into the mechanisms of cancer pathogenesis. We do not attempt here to enumerate the myriad therapies that are under development or have been introduced of late into the clinic. Instead, we consider how the description of hallmark principles is beginning to inform therapeutic development at present and may increasingly do so in the future.

The rapidly growing armamentarium of targeted therapeutics can be categorized according to their respective effects on one or more hallmark capabilities, as illustrated in the examples presented in Figure 6. Indeed, the observed efficacy of these drugs represents, in each case, a validation of a particular capability: if a capability is truly important for the biology of tumors, then its inhibition should impair tumor growth and progression.

We note that most of the hallmark-targeting cancer drugs developed to date have been deliberately directed toward specific molecular targets that are involved in one way or another in enabling particular capabilities. Such specificity of action has been considered a virtue, as it presents inhibitory activity against a target while having, in principle, relatively fewer off-target effects and thus less nonspecific toxicity. In fact, resulting clinical responses have generally been transitory, being followed by almost-inevitable relapses.

One interpretation of this history, supported by growing experimental evidence, is that each of the core hallmark capabilities is regulated by partially redundant signaling pathways. Consequently, a targeted therapeutic agent inhibiting one key pathway in a tumor may not completely shut off 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 therapy being applied. Such adaptation, which can be accomplished by mutation, epigenetic reprogramming, or remodeling of the stromal microenvironment, can reestablish the functional capability, permitting renewed tumor growth and clinical relapse. Given that the number of parallel signaling pathways supporting a given hallmark must be limited, it may become possible to target all of these supporting pathways therapeutically, thereby preventing the development of adaptive resistance.

In response to therapy, cancer cells may also reduce their dependence on a particular hallmark capability, becoming more dependent on another; this represents a quite different form of acquired drug resistance. This concept is exemplified by recent discoveries of unexpected responses to antiangiogenic therapies. Some have anticipated that effective inhibition of angiogenesis would render tumors dormant and might even lead to their dissolution (Folkman and Kalluri, 2004). Instead, the clinical responses to antiangiogenic therapies have been found to be transitory (Azam et al., 2010; Ebos et al., 2009; Bergers and Hanahan, 2008).

In certain preclinical models, where potent angiogenesis inhibitors succeed in suppressing this hallmark capability, tumors adapt and shift from a dependence upon continuing angiogenesis to heightening the activity of another instead—invasiveness and metastasis (Azam et al., 2010: Ebos et al., 2009; Bergers and Hanahan, 2008). By invading nearby tissues, initially hypoxic cancer cells evidently gain access to normal, preexisting tissue vasculature. 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 (Ellis and Reardon, 2009; Norden et al., 2009; Verhoeff et al., 2009). 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 efficacy of analogous hallmark-targeting

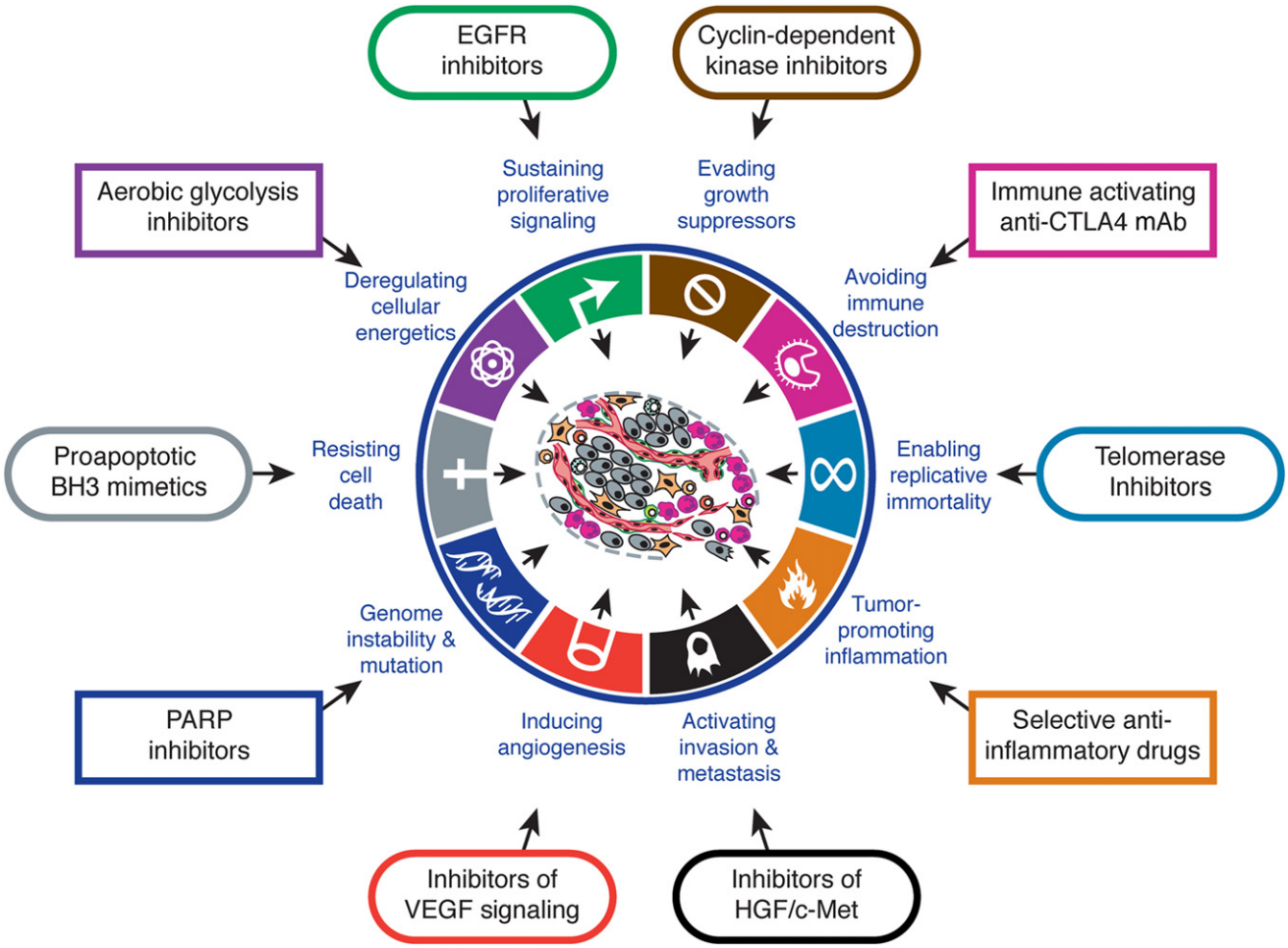

Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

Drugs that interfere with each of the acquired capabilities necessary for tumor growth and progression have been developed and are in clinical trials or in some cases approved for clinical use in treating certain forms of human cancer. Additionally, the investigational drugs are being developed to target each of the enabling characteristics and emerging hallmarks depicted in Figure 3, which also hold promise as cancer therapeutics. The drugs listed are but illustrative examples; there is a deep pipeline of candidate drugs with different molecular targets and modes of action in development for most of these hallmarks.

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. Thus, in particular, we can envisage that selective cotargeting of multiple core and emerging hallmark capabilities and enabling characteristics (Figure 6) in mechanism-guided combinations will result in more effective and durable therapies for human cancer.

CONCLUSION AND FUTURE VISION

We have sought here to revisit, refine, and extend the concept of cancer hallmarks, which has provided a useful conceptual framework for understanding the complex biology of cancer. The six acquired capabilities—the hallmarks of cancer—have stood the test of time as being integral components of most forms of cancer. Further refinement of these organizing principles will surely come in the foreseeable future, continuing the remarkable conceptual progress of the last decade.

Looking ahead, we envision significant advances during the coming decade in our understanding of invasion and metastasis. Similarly, the role of aerobic glycolysis 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 remain perplexed as to whether immune surveillance is a barrier that virtually all tumors must circumvent, or only an idiosyncrasy of an especially immunogenic subset of them; this issue too will be resolved in one way or another.

Yet 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 (Berdasco and Esteller, 2010). 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. It is unclear at present 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 genetic control mechanisms that operate in health and disease. By now dozens of microRNAs have been implicated in various tumor phenotypes (Garzon et al., 2010), and yet these only scratch the surface of the real complexity, as the functions of hundreds of microRNAs known to be present in our cells and altered in expression in different forms of cancer remain total mysteries. Here again, we are unclear as to whether future progress will cause fundamental shifts in our understanding of the pathogenetic mechanisms of cancer or only add detail to the elaborate regulatory circuits that have already been mapped out.

Finally, the circuit diagrams of heterotypic interactions between the multiple distinct cell types that assemble and collaborate to produce different forms and progressively malignant stages of cancer are currently rudimentary. In another decade, we anticipate that the signaling circuitry describing the intercommunication between these various cells within tumors will be charted in far greater detail and clarity, eclipsing our current knowledge. And, as before (Hanahan and Weinberg, 2000), we continue to foresee cancer research as an increasingly logical science, in which myriad phenotypic complexities are manifestations of a small set of underlying organizing principles.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures that are downloadable for presentations and can be found with this article online at doi:10.1016/j.cell.2011.02.013.

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