# Hallmarks of Cancer

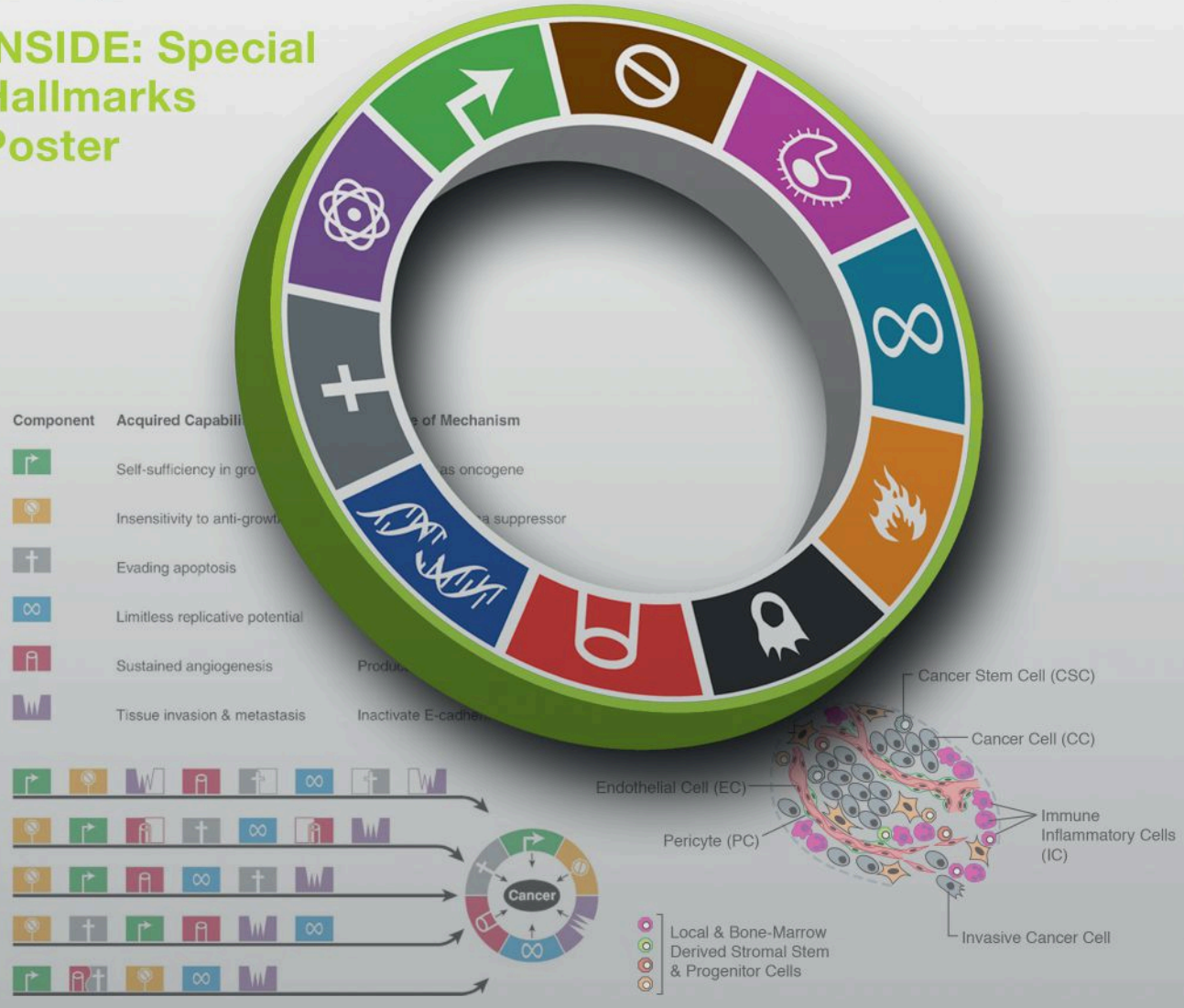
## A Cell Press Collection

The Next-Generation Review

A Perspective on Tumor Stroma

Six SnapShots

**INSIDE: Special Hallmarks Poster**

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# Foreword

In *Hallmarks of Cancer*, the now classic *Cell* Review, Douglas Hanahan and Robert Weinberg defined six core features that distinguish cancer from normal tissue: self-sufficiency in growth signals, insensitivity to antigrowth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis, and evading cell death. This ambitious synthesis has proven both enduring and greatly influential, its effectiveness deriving from the unique way in which it tackles a massive body of literature, distilling the complexities of the disease down to a few key principles and cell behaviors.

In recognition of the tremendous progress in the past decade in understanding the mechanisms of cancer progression, Hanahan and Weinberg revisited and expanded their classic framework in *Hallmarks of Cancer: The Next Generation* to include four emerging hallmarks: reprogramming of energy metabolism, evading immune destruction, genome instability, and tumor-promoting inflammation.

By providing a common frame of reference, the Hallmarks of Cancer Reviews have facilitated communication between bench researchers in diverse disciplines. And in an era of mechanism-based therapeutics, the hallmarks concepts are increasingly valuable to drug-discovery efforts and to clinicians. Many of the new therapies coming on line specifically target a particular molecular pathway that gives rise to one of cancer's hallmarks. Efforts to develop combination therapies, motivated by the propensity of most cancers to develop resistance to treatment, are also taking advantage of the hallmarks framework to figure out which sets of attributes may be therapeutically beneficial to target simultaneously.

In concert with these efforts in basic cancer research, we present this Cell Press collection, featuring *Hallmarks of Cancer: The Next Generation* and a Review by Douglas Hanahan and Lisa Coussens on the contribution of stromal cells to hallmark capabilities. You will also find recent SnapShots and a poster that depict key aspects of cancer cell proliferation, survival, and dissemination.

Finally, we are grateful for the generosity of NanoString Technologies Inc., who helped to make this reprint collection possible.

**Robert P. Kruger**
Deputy Editor, *Cell*

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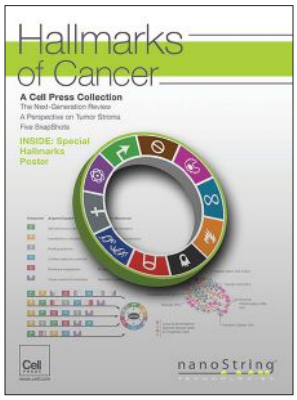
# Hallmarks of Cancer






**On the cover:** In *Hallmarks of Cancer: The Next Generation*, featured in this special collection, Douglas Hanahan and Robert Weinberg define ten core characteristics of cancer. The symbols in the central ring represent these hallmarks (moving clockwise from the top): evading growth suppressors, avoiding immune destruction, enabling replicative immortality, tumor-promoting inflammation, activating invasion and metastasis, inducing angiogenesis, genome instability and mutation, resisting cell death, deregulating cellular energetics, and sustaining proliferative signaling.

Cell

Leading Edge
Review

# Hallmarks of Cancer: The Next Generation

Douglas Hanahan[1,2,*] and Robert A. Weinberg[3,*]
[1]The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL, Lausanne CH-1015, Switzerland
[2]The Department of Biochemistry & Biophysics, UCSF, San Francisco, CA 94158, USA
[3]Whitehead Institute for Biomedical Research, Ludwig/MIT Center for Molecular Oncology, and MIT Department of Biology, Cambridge, MA 02142, USA
*Correspondence: dh@epfl.ch (D.H.), weinberg@wi.mit.edu (R.A.W.)
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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

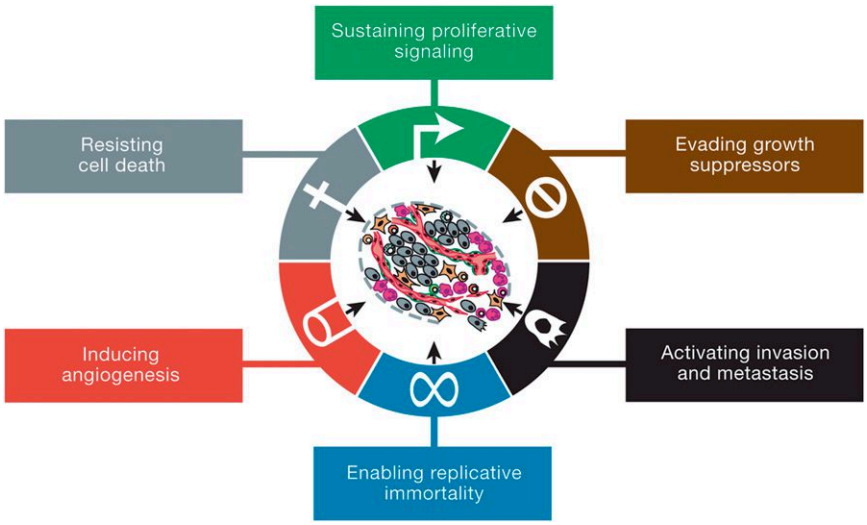


**Figure 1. The Hallmarks of Cancer**
This illustration encompasses the six hallmark capabilities originally proposed in our 2000 perspective. The past decade has witnessed remarkable progress toward understanding the mechanistic underpinnings of each hallmark.

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-1$\alpha$, 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

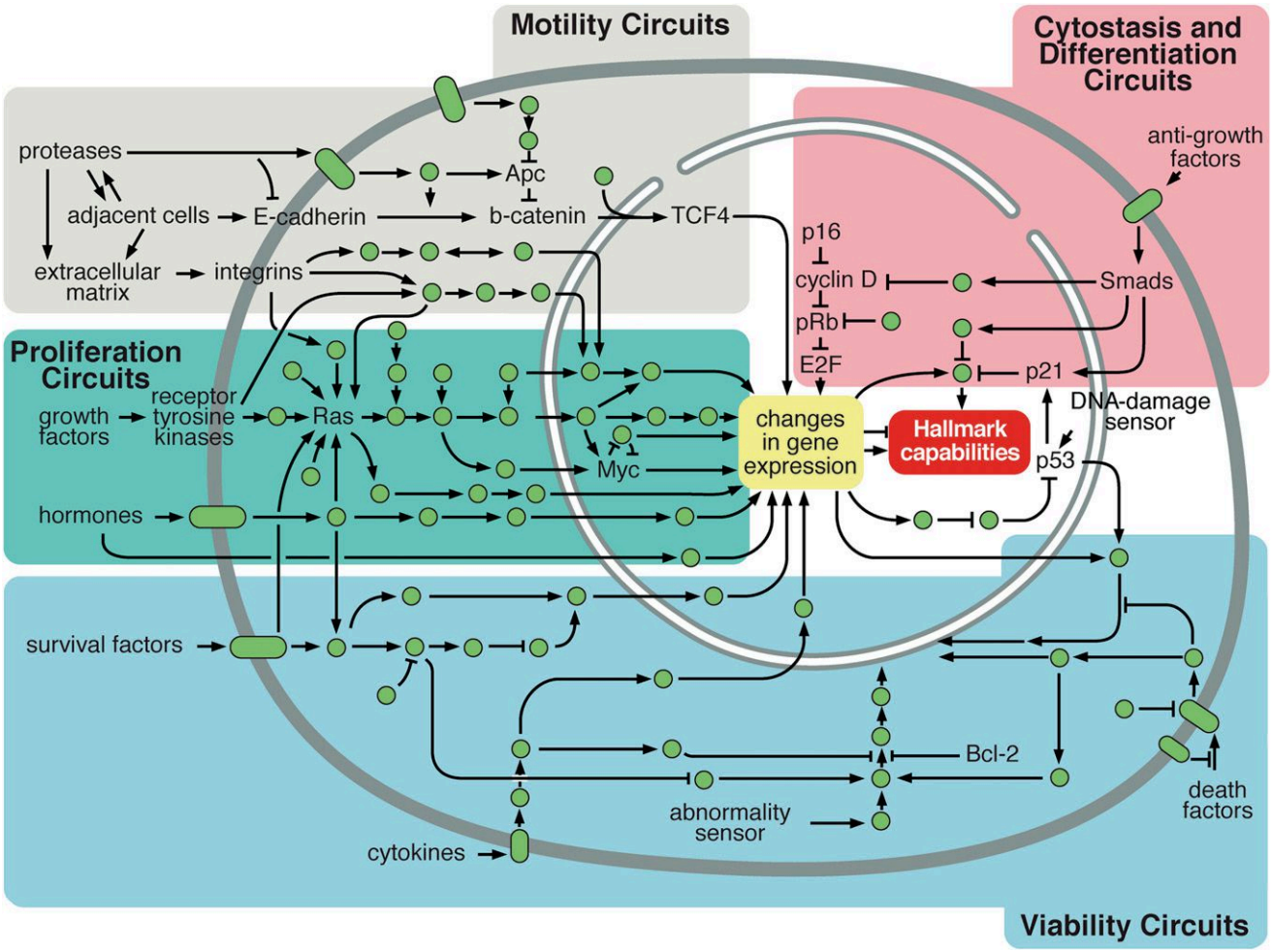


**Figure 2. Intracellular Signaling Networks Regulate the Operations of the Cancer Cell**
An elaborate integrated circuit operates within normal cells and is reprogrammed to regulate hallmark capabilities within cancer cells. Separate subcircuits, depicted here in differently colored fields, are specialized 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.

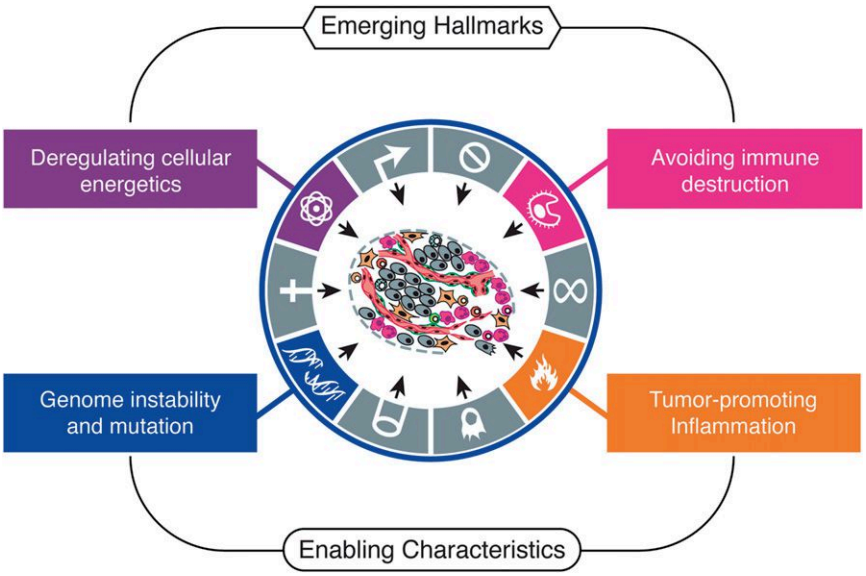


**Figure 3. Emerging Hallmarks and Enabling Characteristics**

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 HIF1$\alpha$ and HIF2$\alpha$ 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

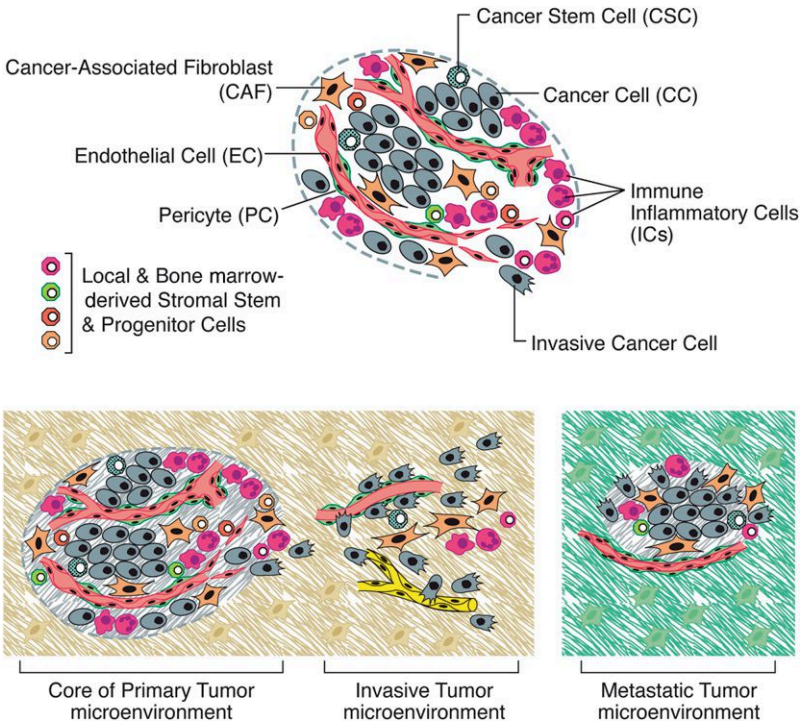


**Figure 4. The Cells of the Tumor Microenvironment**

(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

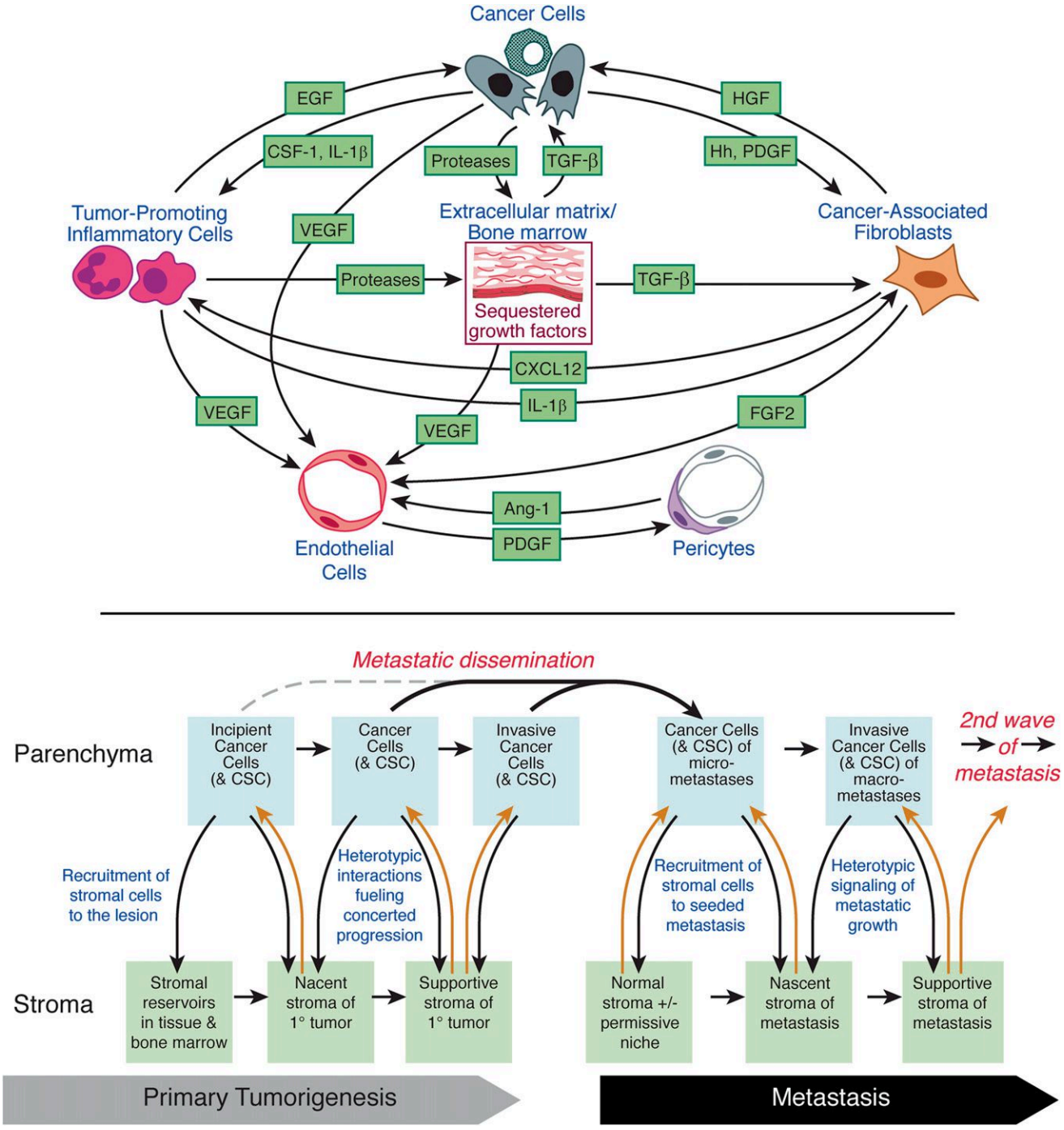


**Figure 5. Signaling Interactions in the Tumor Microenvironment during Malignant Progression**

(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

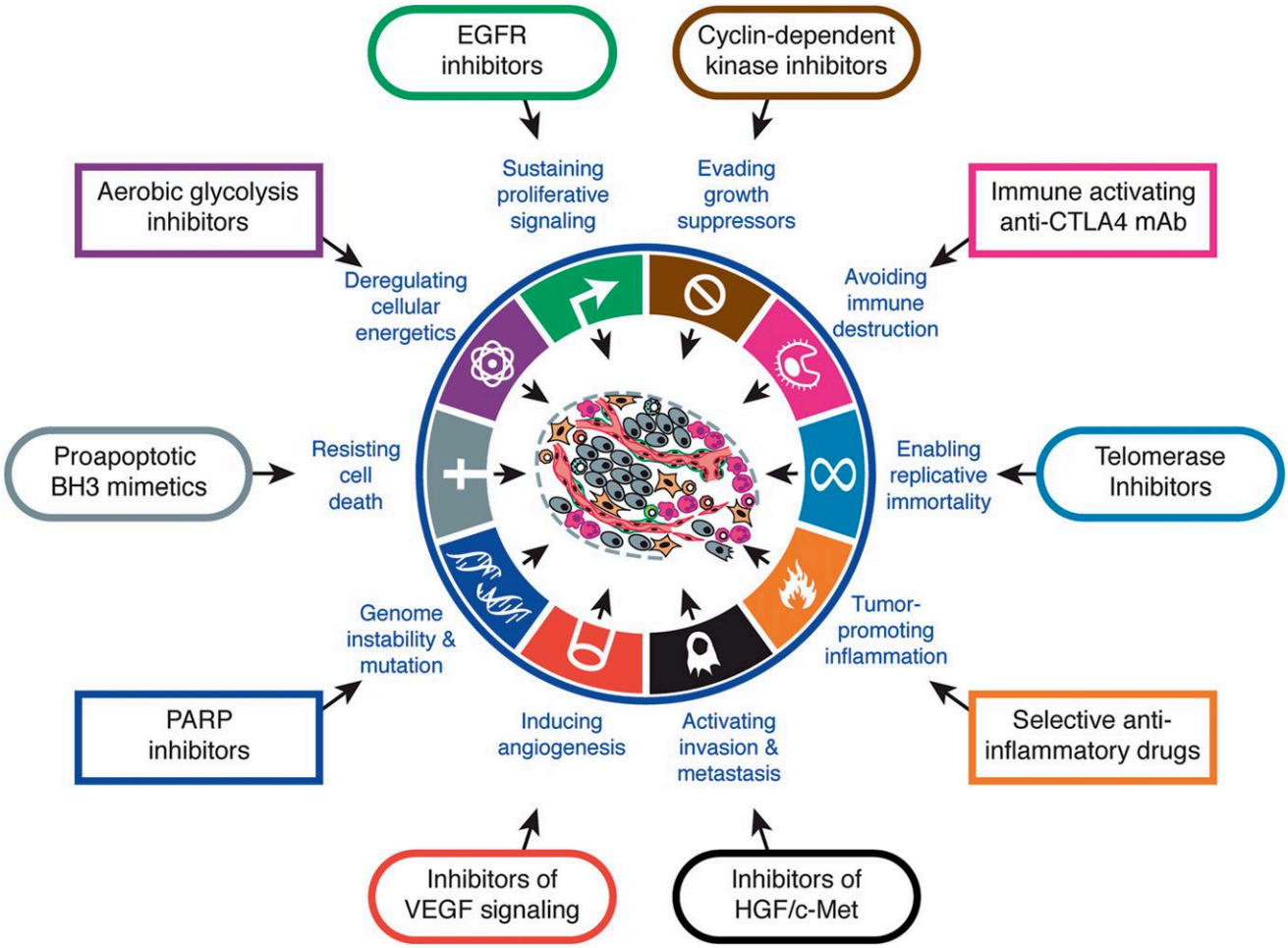


**Figure 6. Therapeutic Targeting of the Hallmarks of Cancer**

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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## REFERENCES

Adams, J.M., and Cory, S. (2007). The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene *26*, 1324–1337.

Aguirre-Ghiso, J.A. (2007). Models, mechanisms and clinical evidence for cancer dormancy. Nat. Rev. Cancer *7*, 834–846.

Ahmed, Z., and Bicknell, R. (2009). Angiogenic signalling pathways. Methods Mol. Biol. *467*, 3–24.

Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J., and Clarke, M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. USA *100*, 3983–3988.

Amaravadi, R.K., and Thompson, C.B. (2007). The roles of therapy-induced autophagy and necrosis in cancer treatment. Clin. Cancer Res. *13*, 7271–7279.

Amit, I., Citri, A., Shay, T., Lu, Y., Katz, M., Zhang, F., Tarcic, G., Siwak, D., Lahad, J., Jacob-Hirsch, J., et al. (2007). A module of negative feedback regulators defines growth factor signaling. Nat. Genet. *39*, 503–512.

Apel, A., Zentgraf, H., Büchler, M.W., and Herr, I. (2009). Autophagy-A double-edged sword in oncology. Int. J. Cancer *125*, 991–995.

Artandi, S.E., and DePinho, R.A. (2000). Mice without telomerase: what can they teach us about human cancer? Nat. Med. *6*, 852–855.

Artandi, S.E., and DePinho, R.A. (2010). Telomeres and telomerase in cancer. Carcinogenesis *31*, 9–18.

Azam, F., Mehta, S., and Harris, A.L. (2010). Mechanisms of resistance to antiangiogenesis therapy. Eur. J. Cancer *46*, 1323–1332.

Baeriswyl, V., and Christofori, G. (2009). The angiogenic switch in carcinogenesis. Semin. Cancer Biol. *19*, 329–337.

Baluk, P., Hashizume, H., and McDonald, D.M. (2005). Cellular abnormalities of blood vessels as targets in cancer. Curr. Opin. Genet. Dev. *15*, 102–111.

Barkan, D., Green, J.E., and Chambers, A.F. (2010). Extracellular matrix: a gatekeeper in the transition from dormancy to metastatic growth. Eur. J. Cancer *46*, 1181–1188.

Barnes, D.E., and Lindahl, T. (2004). Repair and genetic consequences of endogenous DNA base damage in mammalian cells. Annu. Rev. Genet. *38*, 445–476.

Barrallo-Gimeno, A., and Nieto, M.A. (2005). The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development *132*, 3151–3161.

Berdasco, M., and Esteller, M. (2010). Aberrant epigenetic landscape in cancer: How cellular identity goes awry. Dev. Cell *19*, 698–711.

Bergers, G., and Benjamin, L.E. (2003). Tumorigenesis and the angiogenic switch. Nat. Rev. Cancer *3*, 401–410.

Bergers, G., and Hanahan, D. (2008). Modes of resistance to anti-angiogenic therapy. Nat. Rev. Cancer *8*, 592–603.

Bergers, G., and Song, S. (2005). The role of pericytes in blood-vessel formation and maintenance. Neuro-oncol. *7*, 452–464.

Bergfeld, S.A., and DeClerck, Y.A. (2010). Bone marrow-derived mesenchymal stem cells and the tumor microenvironment. Cancer Metastasis Rev. *29*, 249–261.

Berx, G., and van Roy, F. (2009). Involvement of members of the cadherin superfamily in cancer. Cold Spring Harb. Perspect. Biol. *1*, a003129.

Bhowmick, N.A., Neilson, E.G., and Moses, H.L. (2004). Stromal fibroblasts in cancer initiation and progression. Nature *432*, 332–337.

Bierie, B., and Moses, H.L. (2006). Tumour microenvironment: TGFbeta: the molecular Jekyll and Hyde of cancer. Nat. Rev. Cancer *6*, 506–520.

Bindea, G., Mlecnik, B., Fridman, W.H., Pagès, F., and Galon, J. (2010). Natural immunity to cancer in humans. Curr. Opin. Immunol. *22*, 215–222.

Biswas, S.K., and Mantovani, A. (2010). Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat. Immunol. *11*, 889–896.

Blasco, M.A. (2005). Telomeres and human disease: ageing, cancer and beyond. Nat. Rev. Genet. *6*, 611–622.

Boiko, A.D., Razorenova, O.V., van de Rijn, M., Swetter, S.M., Johnson, D.L., Ly, D.P., Butler, P.D., Yang, G.P., Joshua, B., Kaplan, M.J., et al. (2010). Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. Nature *466*, 133–137.

Bonnet, D., and Dick, J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. *3*, 730–737.

Bos, P.D., Zhang, X.H., Nadal, C., Shu, W., Gomis, R.R., Nguyen, D.X., Minn, A.J., van de Vijver, M.J., Gerald, W.L., Foekens, J.A., and Massagué, J. (2009). Genes that mediate breast cancer metastasis to the brain. Nature *459*, 1005–1009.

Brabletz, T., Jung, A., Reu, S., Porzner, M., Hlubek, F., Kunz-Schughart, L.A., Knuechel, R., and Kirchner, T. (2001). Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. Proc. Natl. Acad. Sci. USA *98*, 10356–10361.

Brabletz, T., Jung, A., Spaderna, S., Hlubek, F., and Kirchner, T. (2005). Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. Nat. Rev. Cancer *5*, 744–749.

Buck, E., Eyzaguirre, A., Barr, S., Thompson, S., Sennello, R., Young, D., Iwata, K.K., Gibson, N.W., Cagnoni, P., and Haley, J.D. (2007). Loss of homotypic cell adhesion by epithelial-mesenchymal transition or mutation limits sensitivity to epidermal growth factor receptor inhibition. Mol. Cancer Ther. *6*, 532–541.

Burkhart, D.L., and Sage, J. (2008). Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nat. Rev. Cancer *8*, 671–682.

Cabrita, M.A., and Christofori, G. (2008). Sprouty proteins, masterminds of receptor tyrosine kinase signaling. Angiogenesis *11*, 53–62.

Campbell, P.J., Yachida, S., Mudie, L.J., Stephens, P.J., Pleasance, E.D., Stebbings, L.A., Morsberger, L.A., Latimer, C., McLaren, S., Lin, M.L., et al. (2010). The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature *467*, 1109–1113.

Cao, Y. (2010). Adipose tissue angiogenesis as a therapeutic target for obesity and metabolic diseases. Nat. Rev. Drug Discov. *9*, 107–115.

Carmeliet, P. (2005). VEGF as a key mediator of angiogenesis in cancer. Oncology *69* (*Suppl 3*), 4–10.

Carmeliet, P., and Jain, R.K. (2000). Angiogenesis in cancer and other diseases. Nature *407*, 249–257.

Cavallaro, U., and Christofori, G. (2004). Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. Nat. Rev. Cancer *4*, 118–132.

Cheng, N., Chytil, A., Shyr, Y., Joly, A., and Moses, H.L. (2008). Transforming growth factor-beta signaling-deficient fibroblasts enhance hepatocyte growth factor signaling in mammary carcinoma cells to promote scattering and invasion. Mol. Cancer Res. *6*, 1521–1533.

Chin, K., de Solorzano, C.O., Knowles, D., Jones, A., Chou, W., Rodriguez, E.G., Kuo, W.L., Ljung, B.M., Chew, K., Myambo, K., et al. (2004). In situ analyses of genome instability in breast cancer. Nat. Genet. *36*, 984–988.

Cho, R.W., and Clarke, M.F. (2008). Recent advances in cancer stem cells. Curr. Opin. Genet. Dev. *18*, 1–6.

Ciccia, A., and Elledge, S.J. (2010). The DNA damage response: making it safe to play with knives. Mol. Cell *40*, 179–204.

Coffelt, S.B., Lewis, C.E., Naldini, L., Brown, J.M., Ferrara, N., and De Palma, M. (2010). Elusive identities and overlapping phenotypes of proangiogenic myeloid cells in tumors. Am. J. Pathol. *176*, 1564–1576.

Coghlin, C., and Murray, G.I. (2010). Current and emerging concepts in tumour metastasis. J. Pathol. *222*, 1–15.

Collado, M., and Serrano, M. (2010). Senescence in tumours: evidence from mice and humans. Nat. Rev. Cancer *10*, 51–57.

Colotta, F., Allavena, P., Sica, A., Garlanda, C., and Mantovani, A. (2009). Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis *30*, 1073–1081.

Cong, Y., and Shay, J.W. (2008). Actions of human telomerase beyond telomeres. Cell Res. *18*, 725–732.

Creighton, C.J., Li, X., Landis, M., Dixon, J.M., Neumeister, V.M., Sjolund, A., Rimm, D.L., Wong, H., Rodriguez, A., Herschkowitz, J.I., et al. (2009). Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc. Natl. Acad. Sci. USA *106*, 13820–13825.

Curto, M., Cole, B.K., Lallemand, D., Liu, C.H., and McClatchey, A.I. (2007). Contact-dependent inhibition of EGFR signaling by Nf2/Merlin. J. Cell Biol. *177*, 893–903.

Davies, M.A., and Samuels, Y. (2010). Analysis of the genome to personalize therapy for melanoma. Oncogene *29*, 5545–5555.

DeBerardinis, R.J., Lum, J.J., Hatzivassiliou, G., and Thompson, C.B. (2008). The biology of cancer: Metabolic reprogramming fuels cell growth and proliferation. Cell Metab. *7*, 11–20.

Dejana, E., Orsenigo, F., Molendini, C., Baluk, P., and McDonald, D.M. (2009). Organization and signaling of endothelial cell-to-cell junctions in various regions of the blood and lymphatic vascular trees. Cell Tissue Res. *335*, 17–25.

Demicheli, R., Retsky, M.W., Hrushesky, W.J., Baum, M., and Gukas, I.D. (2008). The effects of surgery on tumor growth: a century of investigations. Ann. Oncol. *19*, 1821–1828.

DeNardo, D.G., Andreu, P., and Coussens, L.M. (2010). Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. Cancer Metastasis Rev. *29*, 309–316.

De Palma, M., Murdoch, C., Venneri, M.A., Naldini, L., and Lewis, C.E. (2007). Tie2-expressing monocytes: regulation of tumor angiogenesis and therapeutic implications. Trends Immunol. *28*, 519–524.

Deshpande, A., Sicinski, P., and Hinds, P.W. (2005). Cyclins and cdks in development and cancer: a perspective. Oncogene *24*, 2909–2915.

de Visser, K.E., Eichten, A., and Coussens, L.M. (2006). Paradoxical roles of the immune system during cancer development. Nat. Rev. Cancer *6*, 24–37.

Dirat, B., Bochet, L., Escourrou, G., Valet, P., and Muller, C. (2010). Unraveling the obesity and breast cancer links: a role for cancer-associated adipocytes? Endocr. Dev. *19*, 45–52.

Dvorak, H.F. (1986). Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N. Engl. J. Med. *315*, 1650–1659.

Ebos, J.M., Lee, C.R., and Kerbel, R.S. (2009). Tumor and host-mediated pathways of resistance and disease progression in response to antiangiogenic therapy. Clin. Cancer Res. *15*, 5020–5025.

Egeblad, M., Nakasone, E.S., and Werb, Z. (2010). Tumors as organs: complex tissues that interface with the entire organism. Dev. Cell *18*, 884–901.

El Hallani, S., Boisselier, B., Peglion, F., Rousseau, A., Colin, C., Idbaih, A., Marie, Y., Mokhtari, K., Thomas, J.L., Eichmann, A., et al. (2010). A new alternative mechanism in glioblastoma vascularization: tubular vasculogenic mimicry. Brain *133*, 973–982.

Ellis, L.M., and Reardon, D.A. (2009). Cancer: The nuances of therapy. Nature *458*, 290–292.

Esteller, M. (2007). Cancer epigenomics: DNA methylomes and histone-modification maps. Nat. Rev. Genet. *8*, 286–298.

Evan, G.I., and d'Adda di Fagagna, F. (2009). Cellular senescence: hot or what? Curr. Opin. Genet. Dev. *19*, 25–31.

Evan, G., and Littlewood, T. (1998). A matter of life and cell death. Science *281*, 1317–1322.

Fang, S., and Salven, P. (2011). Stem cells in tumor angiogenesis. J. Mol. Cell. Cardiol. *50*, 290–295.

Feron, O. (2009). Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells. Radiother. Oncol. *92*, 329–333.

Feldser, D.M., and Greider, C.W. (2007). Short telomeres limit tumor progression in vivo by inducing senescence. Cancer Cell *11*, 461–469.

Ferrara, N. (2009). Vascular endothelial growth factor. Arterioscler. Thromb. Vasc. Biol. *29*, 789–791.

Ferrara, N. (2010). Pathways mediating VEGF-independent tumor angiogenesis. Cytokine Growth Factor Rev. *21*, 21–26.

Ferrone, C., and Dranoff, G. (2010). Dual roles for immunity in gastrointestinal cancers. J. Clin. Oncol. *28*, 4045–4051.

Fidler, I.J. (2003). The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat. Rev. Cancer *3*, 453–458.

Folkman, J. (2002). Role of angiogenesis in tumor growth and metastasis. Semin. Oncol. *29*(6, Suppl 16), 15–18.

Folkman, J. (2006). Angiogenesis. Annu. Rev. Med. *57*, 1–18.

Folkman, J., and Kalluri, R. (2004). Cancer without disease. Nature *427*, 787.

Friedberg, E.C., Aguilera, A., Gellert, M., Hanawalt, P.C., Hays, J.B., Lehmann, A.R., Lindahl, T., Lowndes, N., Sarasin, A., and Wood, R.D. (2006). DNA repair: from molecular mechanism to human disease. DNA Repair (Amst.) *5*, 986–996.

Friedl, P., and Wolf, K. (2008). Tube travel: the role of proteases in individual and collective cancer cell invasion. Cancer Res. *68*, 7247–7249.

Friedl, P., and Wolf, K. (2010). Plasticity of cell migration: a multiscale tuning model. J. Cell Biol. *188*, 11–19.

Gaengel, K., Genové, G., Armulik, A., and Betsholtz, C. (2009). Endothelial-mural cell signaling in vascular development and angiogenesis. Arterioscler. Thromb. Vasc. Biol. *29*, 630–638.

Galluzzi, L., and Kroemer, G. (2008). Necroptosis: a specialized pathway of programmed necrosis. Cell *135*, 1161–1163.

Garzon, R., Marcucci, G., and Croce, C.M. (2010). Targeting microRNAs in cancer: rationale, strategies and challenges. Nat. Rev. Drug Discov. *9*, 775–789.

Gerhardt, H., and Semb, H. (2008). Pericytes: gatekeepers in tumour cell metastasis? J. Mol. Med. *86*, 135–144.

Ghebranious, N., and Donehower, L.A. (1998). Mouse models in tumor suppression. Oncogene *17*, 3385–3400.

Giaccia, A.J., and Schipani, E. (2010). Role of carcinoma-associated fibroblasts and hypoxia in tumor progression. Curr. Top. Microbiol. Immunol. *345*, 31–45.

Gilbertson, R.J., and Rich, J.N. (2007). Making a tumour's bed: glioblastoma stem cells and the vascular niche. Nat. Rev. Cancer *7*, 733–736.

Gocheva, V., Wang, H.W., Gadea, B.B., Shree, T., Hunter, K.E., Garfall, A.L., Berman, T., and Joyce, J.A. (2010). IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. Genes Dev. *24*, 241–255.

Grivennikov, S.I., Greten, F.R., and Karin, M. (2010). Immunity, inflammation, and cancer. Cell *140*, 883–899.

Gupta, G.P., Minn, A.J., Kang, Y., Siegel, P.M., Serganova, I., Cordón-Cardo, C., Olshen, A.B., Gerald, W.L., and Massagué, J. (2005). Identifying site-specific metastasis genes and functions. Cold Spring Harb. Symp. Quant. Biol. *70*, 149–158.

Gupta, P.B., Chaffer, C.L., and Weinberg, R.A. (2009). Cancer stem cells: mirage or reality? Nat. Med. *15*, 1010–1012.

Hanahan, D., and Folkman, J. (1996). Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell *86*, 353–364.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell *100*, 57–70.

Hansel, D.E., Meeker, A.K., Hicks, J., De Marzo, A.M., Lillemoe, K.D., Schulick, R., Hruban, R.H., Maitra, A., and Argani, P. (2006). Telomere length variation in biliary tract metaplasia, dysplasia, and carcinoma. Mod. Pathol. *19*, 772–779.

Hardee, M.E., Dewhirst, M.W., Agarwal, N., and Sorg, B.S. (2009). Novel imaging provides new insights into mechanisms of oxygen transport in tumors. Curr. Mol. Med. *9*, 435–441.

Harper, J.W., and Elledge, S.J. (2007). The DNA damage response: Ten years after. Mol. Cell *28*, 739–745.

Hezel, A.F., and Bardeesy, N. (2008). LKB1; linking cell structure and tumor suppression. Oncogene *27*, 6908–6919.

Hlubek, F., Brabletz, T., Budczies, J., Pfeiffer, S., Jung, A., and Kirchner, T. (2007). Heterogeneous expression of Wnt/beta-catenin target genes within colorectal cancer. Int. J. Cancer *121*, 1941–1948.

Hugo, H., Ackland, M.L., Blick, T., Lawrence, M.G., Clements, J.A., Williams, E.D., and Thompson, E.W. (2007). Epithelial—mesenchymal and mesenchymal—epithelial transitions in carcinoma progression. J. Cell. Physiol. *213*, 374–383.

Hsu, P.P., and Sabatini, D.M. (2008). Cancer cell metabolism: Warburg and beyond. Cell *134*, 703–707.

Hynes, N.E., and MacDonald, G. (2009). ErbB receptors and signaling pathways in cancer. Curr. Opin. Cell Biol. *21*, 177–184.

Ikushima, H., and Miyazono, K. (2010). TGFbeta signalling: a complex web in cancer progression. Nat. Rev. Cancer *10*, 415–424.

Ince, T.A., Richardson, A.L., Bell, G.W., Saitoh, M., Godar, S., Karnoub, A.E., Iglehart, J.D., and Weinberg, R.A. (2007). Transformation of different human breast epithelial cell types leads to distinct tumor phenotypes. Cancer Cell *12*, 160–170.

Jackson, S.P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. Nature *461*, 1071–1078.

Jiang, B.H., and Liu, L.Z. (2009). PI3K/PTEN signaling in angiogenesis and tumorigenesis. Adv. Cancer Res. *102*, 19–65.

Johansson, M., Denardo, D.G., and Coussens, L.M. (2008). Polarized immune responses differentially regulate cancer development. Immunol. Rev. *222*, 145–154.

Jones, P.A., and Baylin, S.B. (2007). The epigenomics of cancer. Cell *128*, 683–692.

Jones, R.G., and Thompson, C.B. (2009). Tumor suppressors and cell metabolism: a recipe for cancer growth. Genes Dev. *23*, 537–548.

Joyce, J.A., and Pollard, J.W. (2009). Microenvironmental regulation of metastasis. Nat. Rev. Cancer *9*, 239–252.

Junttila, M.R., and Evan, G.I. (2009). p53—a Jack of all trades but master of none. Nat. Rev. Cancer *9*, 821–829.

Kalluri, R., and Zeisberg, M. (2006). Fibroblasts in cancer. Nat. Rev. Cancer *6*, 392–401.

Kang, H.J., Choi, Y.S., Hong, S.B., Kim, K.W., Woo, R.S., Won, S.J., Kim, E.J., Jeon, H.K., Jo, S.Y., Kim, T.K., et al. (2004). Ectopic expression of the catalytic subunit of telomerase protects against brain injury resulting from ischemia and NMDA-induced neurotoxicity. J. Neurosci. *24*, 1280–1287.

Karin, M., Lawrence, T., and Nizet, V. (2006). Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. Cell *124*, 823–835.

Karnoub, A.E., and Weinberg, R.A. (2006–2007). Chemokine networks and breast cancer metastasis. Breast Dis. *26*, 75–85.

Karnoub, A.E., Dash, A.B., Vo, A.P., Sullivan, A., Brooks, M.W., Bell, G.W., Richardson, A.L., Polyak, K., Tubo, R., and Weinberg, R.A. (2007). Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature *449*, 557–563.

Kastan, M.B. (2008). DNA damage responses: mechanisms and roles in human disease: 2007 G.H.A. Clowes Memorial Award Lecture. Mol. Cancer Res. *6*, 517–524.

Kawai, T., Hiroi, S., Nakanishi, K., and Meeker, A.K. (2007). Telomere length and telomerase expression in atypical adenomatous hyperplasia and small bronchioloalveolar carcinoma of the lung. Am. J. Clin. Pathol. *127*, 254–262.

Kazerounian, S., Yee, K.O., and Lawler, J. (2008). Thrombospondins in cancer. Cell. Mol. Life Sci. *65*, 700–712.

Kenific, C.M., Thorburn, A., and Debnath, J. (2010). Autophagy and metastasis: another double-edged sword. Curr. Opin. Cell Biol. *22*, 241–245.

Kennedy, K.M., and Dewhirst, M.W. (2010). Tumor metabolism of lactate: the influence and therapeutic potential for MCT and CD147 regulation. Future Oncol. *6*, 127–148.

Kessenbrock, K., Plaks, V., and Werb, Z. (2010). Matrix metalloproteinases: Regulators of the tumor microenvironment. Cell *141*, 52–67.

Kim, M.Y., Oskarsson, T., Acharyya, S., Nguyen, D.X., Zhang, X.H., Norton, L., and Massagué, J. (2009). Tumor self-seeding by circulating cancer cells. Cell *139*, 1315–1326.

Kim, R., Emi, M., and Tanabe, K. (2007). Cancer immunoediting from immune surveillance to immune escape. Immunology *121*, 1–14.

Kinzler, K.W., and Vogelstein, B. (1997). Cancer-susceptibility genes. Gatekeepers and caretakers. Nature *386*, 761–763.

Klein, C.A. (2009). Parallel progression of primary tumours and metastases. Nat. Rev. Cancer *9*, 302–312.

Klymkowsky, M.W., and Savagner, P. (2009). Epithelial-mesenchymal transition: a cancer researcher's conceptual friend and foe. Am. J. Pathol. *174*, 1588–1593.

Korkola, J., and Gray, J.W. (2010). Breast cancer genomes—form and function. Curr. Opin. Genet. Dev. *20*, 4–14.

Kovacic, J.C., and Boehm, M. (2009). Resident vascular progenitor cells: an emerging role for non-terminally differentiated vessel-resident cells in vascular biology. Stem Cell Res. (Amst.) *2*, 2–15.

Kroemer, G., and Pouyssegur, J. (2008). Tumor cell metabolism: Cancer's Achilles' heel. Cancer Cell *13*, 472–482.

Lamagna, C., and Bergers, G. (2006). The bone marrow constitutes a reservoir of pericyte progenitors. J. Leukoc. Biol. *80*, 677–681.

Lane, D.P. (1992). Cancer. p53, guardian of the genome. Nature *358*, 15–16.

Lemmon, M.A., and Schlessinger, J. (2010). Cell signaling by receptor tyrosine kinases. Cell *141*, 1117–1134.

Levine, B., and Kroemer, G. (2008). Autophagy in the pathogenesis of disease. Cell *132*, 27–42.

Lipinski, M.M., and Jacks, T. (1999). The retinoblastoma gene family in differentiation and development. Oncogene *18*, 7873–7882.

Lobo, N.A., Shimono, Y., Qian, D., and Clarke, M.F. (2007). The biology of cancer stem cells. Annu. Rev. Cell Dev. Biol. *23*, 675–699.

Lowe, S.W., Cepero, E., and Evan, G. (2004). Intrinsic tumour suppression. Nature *432*, 307–315.

Luebeck, E.G. (2010). Cancer: Genomic evolution of metastasis. Nature *467*, 1053–1055.

Lu, Z., Luo, R.Z., Lu, Y., Zhang, X., Yu, Q., Khare, S., Kondo, S., Kondo, Y., Yu, Y., Mills, G.B., et al. (2008). The tumor suppressor gene ARHI regulates autophagy and tumor dormancy in human ovarian cancer cells. J. Clin. Invest. *118*, 3917–3929.

Luo, J., Solimini, N.L., and Elledge, S.J. (2009). Principles of cancer therapy: Oncogene and non-oncogene addiction. Cell *136*, 823–837.

Mac Gabhann, F., and Popel, A.S. (2008). Systems biology of vascular endothelial growth factors. Microcirculation *15*, 715–738.

Madsen, C.D., and Sahai, E. (2010). Cancer dissemination—Lessons from leukocytes. Dev. Cell *19*, 13–26.

Maida, Y., Yasukawa, M., Furuuchi, M., Lassmann, T., Possemato, R., Okamoto, N., Kasim, V., Hayashizaki, Y., Hahn, W.C., and Masutomi, K. (2009). An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA. Nature *461*, 230–235.

Mani, S.A., Guo, W., Liao, M.J., Eaton, E.N., Ayyanan, A., Zhou, A.Y., Brooks, M., Reinhard, F., Zhang, C.C., Shipitsin, M., et al. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell *133*, 704–715.

Mantovani, A. (2010). Molecular pathways linking inflammation and cancer. Curr. Mol. Med. *10*, 369–373.

Mantovani, A., Allavena, P., Sica, A., and Balkwill, F. (2008). Cancer-related inflammation. Nature *454*, 436–444.

Massagué, J. (2008). TGFβ in cancer. Cell *134*, 215–230.

Masutomi, K., Possemato, R., Wong, J.M., Currier, J.L., Tothova, Z., Manola, J.B., Ganesan, S., Lansdorp, P.M., Collins, K., and Hahn, W.C. (2005). The telomerase reverse transcriptase regulates chromatin state and DNA damage responses. Proc. Natl. Acad. Sci. USA *102*, 8222–8227.

Mathew, R., Karantza-Wadsworth, V., and White, E. (2007). Role of autophagy in cancer. Nat. Rev. Cancer *7*, 961–967.

McGowan, P.M., Kirstein, J.M., and Chambers, A.F. (2009). Micrometastatic disease and metastatic outgrowth: clinical issues and experimental approaches. Future Oncol. *5*, 1083–1098.

Micalizzi, D.S., Farabaugh, S.M., and Ford, H.L. (2010). Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression. J. Mammary Gland Biol. Neoplasia *15*, 117–134.

Mizushima, N. (2007). Autophagy: process and function. Genes Dev. *21*, 2861–2873.

Mohamed, M.M., and Sloane, B.F. (2006). Cysteine cathepsins: multifunctional enzymes in cancer. Nat. Rev. Cancer *6*, 764–775.

Mooi, W.J., and Peeper, D.S. (2006). Oncogene-induced cell senescence—halting on the road to cancer. N. Engl. J. Med. *355*, 1037–1046.

Morel, A.-P., Lièvre, M., Thomas, C., Hinkal, G., Ansieau, S., and Puisieux, A. (2008). Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS ONE *3*, e2888.

Mosesson, Y., Mills, G.B., and Yarden, Y. (2008). Derailed endocytosis: an emerging feature of cancer. Nat. Rev. Cancer *8*, 835–850.

Mougiakakos, D., Choudhury, A., Lladser, A., Kiessling, R., and Johansson, C.C. (2010). Regulatory T cells in cancer. Adv. Cancer Res. *107*, 57–117.

Murdoch, C., Muthana, M., Coffelt, S.B., and Lewis, C.E. (2008). The role of myeloid cells in the promotion of tumour angiogenesis. Nat. Rev. Cancer *8*, 618–631.

Nagy, J.A., Chang, S.H., Shih, S.C., Dvorak, A.M., and Dvorak, H.F. (2010). Heterogeneity of the tumor vasculature. Semin. Thromb. Hemost. *36*, 321–331.

Naumov, G.N., Folkman, J., Straume, O., and Akslen, L.A. (2008). Tumor-vascular interactions and tumor dormancy. APMIS *116*, 569–585.

Negrini, S., Gorgoulis, V.G., and Halazonetis, T.D. (2010). Genomic instability—an evolving hallmark of cancer. Nat. Rev. Mol. Cell Biol. *11*, 220–228.

Nelson, B.H. (2008). The impact of T-cell immunity on ovarian cancer outcomes. Immunol. Rev. *222*, 101–116.

Nguyen, D.X., Bos, P.D., and Massagué, J. (2009). Metastasis: from dissemination to organ-specific colonization. Nat. Rev. Cancer *9*, 274–284.

Norden, A.D., Drappatz, J., and Wen, P.Y. (2009). Antiangiogenic therapies for high-grade glioma. Nat. Rev. Neurol. *5*, 610–620.

Nyberg, P., Xie, L., and Kalluri, R. (2005). Endogenous inhibitors of angiogenesis. Cancer Res. *65*, 3967–3979.

Okada, T., Lopez-Lago, M., and Giancotti, F.G. (2005). Merlin/NF-2 mediates contact inhibition of growth by suppressing recruitment of Rac to the plasma membrane. J. Cell Biol. *171*, 361–371.

Olive, K.P., Jacobetz, M.A., Davidson, C.J., Gopinathan, A., McIntyre, D., Honess, D., Madhu, B., Goldgraben, M.A., Caldwell, M.E., Allard, D., et al. (2009). Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science *324*, 1457–1461.

Olson, P., Lu, J., Zhang, H., Shai, A., Chun, M.G., Wang, Y., Libutti, S.K., Nakakura, E.K., Golub, T.R., and Hanahan, D. (2009). MicroRNA dynamics in the stages of tumorigenesis correlate with hallmark capabilities of cancer. Genes Dev. *23*, 2152–2165.

O'Reilly, K.E., Rojo, F., She, Q.B., Solit, D., Mills, G.B., Smith, D., Lane, H., Hofmann, F., Hicklin, D.J., Ludwig, D.L., et al. (2006). mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res. *66*, 1500–1508.

Ostrand-Rosenberg, S., and Sinha, P. (2009). Myeloid-derived suppressor cells: linking inflammation and cancer. J. Immunol. *182*, 4499–4506.

Pagès, F., Galon, J., Dieu-Nosjean, M.C., Tartour, E., Sautès-Fridman, C., and Fridman, W.H. (2010). Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene *29*, 1093–1102.

Palermo, C., and Joyce, J.A. (2008). Cysteine cathepsin proteases as pharmacological targets in cancer. Trends Pharmacol. Sci. *29*, 22–28.

Park, J.I., Venteicher, A.S., Hong, J.Y., Choi, J., Jun, S., Shkreli, M., Chang, W., Meng, Z., Cheung, P., Ji, H., et al. (2009). Telomerase modulates Wnt signalling by association with target gene chromatin. Nature *460*, 66–72.

Partanen, J.I., Nieminen, A.I., and Klefstrom, J. (2009). 3D view to tumor suppression: Lkb1, polarity and the arrest of oncogenic c-Myc. Cell Cycle *8*, 716–724.

Pasquale, E.B. (2010). Eph receptors and ephrins in cancer: bidirectional signalling and beyond. Nat. Rev. Cancer *10*, 165–180.

Passos, J.F., Saretzki, G., and von Zglinicki, T. (2007). DNA damage in telomeres and mitochondria during cellular senescence: is there a connection? Nucleic Acids Res. *35*, 7505–7513.

Patenaude, A., Parker, J., and Karsan, A. (2010). Involvement of endothelial progenitor cells in tumor vascularization. Microvasc. Res. *79*, 217–223.

Peinado, H., Lavothskin, S., and Lyden, D. (2011). The secreted factors responsible for pre-metastatic niche formation: Old sayings and new thoughts. Semin. Cancer Biol. Published online January 18, 2011. 10.1016/j.semcancer.2011.01.002.

Peinado, H., Marin, F., Cubillo, E., Stark, H.J., Fusenig, N., Nieto, M.A., and Cano, A. (2004). Snail and E47 repressors of E-cadherin induce distinct invasive and angiogenic properties in vivo. J. Cell Sci. *117*, 2827–2839.

Perona, R. (2006). Cell signalling: growth factors and tyrosine kinase receptors. Clin. Transl. Oncol. *8*, 77–82.

Pietras, K., and Ostman, A. (2010). Hallmarks of cancer: interactions with the tumor stroma. Exp. Cell Res. *316*, 1324–1331.

Polyak, K., and Weinberg, R.A. (2009). Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat. Rev. Cancer *9*, 265–273.

Polyak, K., Haviv, I., and Campbell, I.G. (2009). Co-evolution of tumor cells and their microenvironment. Trends Genet. *25*, 30–38.

Potter, V.R. (1958). The biochemical approach to the cancer problem. Fed. Proc. *17*, 691–697.

Qian, B.Z., and Pollard, J.W. (2010). Macrophage diversity enhances tumor progression and metastasis. Cell *141*, 39–51.

Quintana, E., Shackleton, M., Sabel, M.S., Fullen, D.R., Johnson, T.M., and Morrison, S.J. (2008). Efficient tumour formation by single human melanoma cells. Nature *456*, 593–598.

Raica, M., Cimpean, A.M., and Ribatti, D. (2009). Angiogenesis in pre-malignant conditions. Eur. J. Cancer *45*, 1924–1934.

Räsänen, K., and Vaheri, A. (2010). Activation of fibroblasts in cancer stroma. Exp. Cell Res. *316*, 2713–2722.

Raynaud, C.M., Hernandez, J., Llorca, F.P., Nuciforo, P., Mathieu, M.C., Commo, F., Delaloge, S., Sabatier, L., André, F., and Soria, J.C. (2010). DNA damage repair and telomere length in normal breast, preneoplastic lesions, and invasive cancer. Am. J. Clin. Oncol. *33*, 341–345.

Raza, A., Franklin, M.J., and Dudek, A.Z. (2010). Pericytes and vessel maturation during tumor angiogenesis and metastasis. Am. J. Hematol. *85*, 593–598.

Reitman, Z.J., and Yan, H. (2010). Isocitrate dehydrogenase 1 and 2 mutations in cancer: alterations at a crossroads of cellular metabolism. J. Natl. Cancer Inst. *102*, 932–941.

Reya, T., Morrison, S.J., Clarke, M.F., and Weissman, I.L. (2001). Stem cells, cancer, and cancer stem cells. Nature *414*, 105–111.

Ribatti, D. (2009). Endogenous inhibitors of angiogenesis: a historical review. Leuk. Res. *33*, 638–644.

Ricci-Vitiani, L., Pallini, R., Biffoni, M., Todaro, M., Invernici, G., Cenci, T., Maira, G., Parati, E.A., Stassi, G., Larocca, L.M., and De Maria, R. (2010). Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. Nature *468*, 824–828.

Ruoslahti, E. (2002). Specialization of tumour vasculature. Nat. Rev. Cancer *2*, 83–90.

Ruoslahti, E., Bhatia, S.N., and Sailor, M.J. (2010). Targeting of drugs and nanoparticles to tumors. J. Cell Biol. *188*, 759–768.

Sabeh, F., Shimizu-Hirota, R., and Weiss, S.J. (2009). Protease-dependent versus -independent cancer cell invasion programs: three-dimensional amoeboid movement revisited. J. Cell Biol. *185*, 11–19.

Salk, J.J., Fox, E.J., and Loeb, L.A. (2010). Mutational heterogeneity in human cancers: origin and consequences. Ann. Rev. Pathol. *5*, 51–75.

Schäfer, M., and Werner, S. (2008). Cancer as an overhealing wound: an old hypothesis revisited. Nat. Rev. Mol. Cell Biol. *9*, 628–638.

Schmalhofer, O., Brabletz, S., and Brabletz, T. (2009). E-cadherin, beta-catenin, and ZEB1 in malignant progression of cancer. Cancer Metastasis Rev. *28*, 151–166.

Semenza, G.L. (2008). Tumor metabolism: cancer cells give and take lactate. J. Clin. Invest. *118*, 3835–3837.

Semenza, G.L. (2010a). HIF-1: upstream and downstream of cancer metabolism. Curr. Opin. Genet. Dev. *20*, 51–56.

Semenza, G.L. (2010b). Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene *29*, 625–634.

Seppinen, L., Sormunen, R., Soini, Y., Elamaa, H., Heljasvaara, R., and Pihlajaniemi, T. (2008). Lack of collagen XVIII accelerates cutaneous wound healing, while overexpression of its endostatin domain leads to delayed healing. Matrix Biol. *27*, 535–546.

Shaw, R.J. (2009). Tumor suppression by LKB1: SIK-ness prevents metastasis. Sci. Signal. *2*, pe55.

Shay, J.W., and Wright, W.E. (2000). Hayflick, his limit, and cellular ageing. Nat. Rev. Mol. Cell Biol. *1*, 72–76.

Sherr, C.J., and DePinho, R.A. (2000). Cellular senescence: Mitotic clock or culture shock? Cell *102*, 407–410.

Sherr, C.J., and McCormick, F. (2002). The RB and p53 pathways in cancer. Cancer Cell *2*, 103–112.

Shields, J.D., Kourtis, I.C., Tomei, A.A., Roberts, J.M., and Swartz, M.A. (2010). Induction of lymphoidlike stroma and immune escape by tumors that express the chemokine CCL21. Science *328*, 749–752.

Shimoda, M., Mellody, K.T., and Orimo, A. (2010). Carcinoma-associated fibroblasts are a rate-limiting determinant for tumour progression. Semin. Cell Dev. Biol. *21*, 19–25.

Sigal, A., and Rotter, V. (2000). Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. Cancer Res. *60*, 6788–6793.

Singh, A., and Settleman, J. (2010). EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene *29*, 4741–4751.

Sinha, S., and Levine, B. (2008). The autophagy effector Beclin 1: a novel BH3-only protein. Oncogene *27* (*Suppl 1*), S137–S148.

Smyth, M.J., Dunn, G.P., and Schreiber, R.D. (2006). Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv. Immunol. *90*, 1–50.

Soda, Y., Marumoto, T., Friedmann-Morvinski, D., Soda, M., Liu, F., Michiue, H., Pastorino, S., Yang, M., Hoffman, R.M., Kesari, S., and Verma, I.M. (2011). Feature Article: Transdifferentiation of glioblastoma cells into vascular endothelial cells. Proc. Natl. Acad. Sci. USA. Published online January 24, 2011.

Sudarsanam, S., and Johnson, D.E. (2010). Functional consequences of mTOR inhibition. Curr. Opin. Drug Discov. Devel. *13*, 31–40.

Strauss, D.C., and Thomas, J.M. (2010). Transmission of donor melanoma by organ transplantation. Lancet Oncol. *11*, 790–796.

Talmadge, J.E., and Fidler, I.J. (2010). AACR centennial series: the biology of cancer metastasis: historical perspective. Cancer Res. *70*, 5649–5669.

Tammela, T., and Alitalo, K. (2010). Lymphangiogenesis: Molecular mechanisms and future promise. Cell *140*, 460–476.

Taube, J.H., Herschkowitz, J.I., Komurov, K., Zhou, A.Y., Gupta, S., Yang, J., Hartwell, K., Onder, T.T., Gupta, P.B., Evans, K.W., et al. (2010). Core epithelial-to-mesenchymal transition interactome gene-expression signature is associated with claudin-low and metaplastic breast cancer subtypes. Proc. Natl. Acad. Sci. USA *107*, 15449–15454.

Teng, M.W.L., Swann, J.B., Koebel, C.M., Schreiber, R.D., and Smyth, M.J. (2008). Immune-mediated dormancy: an equilibrium with cancer. J. Leukoc. Biol. *84*, 988–993.

Thiery, J.P., and Sleeman, J.P. (2006). Complex networks orchestrate epithelial-mesenchymal transitions. Nat. Rev. Mol. Cell Biol. *7*, 131–142.

Thiery, J.P., Acloque, H., Huang, R.Y., and Nieto, M.A. (2009). Epithelial-mesenchymal transitions in development and disease. Cell *139*, 871–890.

Townson, J.L., and Chambers, A.F. (2006). Dormancy of solitary metastatic cells. Cell Cycle *5*, 1744–1750.

Turner, H.E., Harris, A.L., Melmed, S., and Wass, J.A. (2003). Angiogenesis in endocrine tumors. Endocr. Rev. *24*, 600–632.

Vajdic, C.M., and van Leeuwen, M.T. (2009). Cancer incidence and risk factors after solid organ transplantation. Int. J. Cancer *125*, 1747–1754.

Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science *324*, 1029–1033.

Verhoeff, J.J., van Tellingen, O., Claes, A., Stalpers, L.J., van Linde, M.E., Richel, D.J., Leenders, W.P., and van Furth, W.R. (2009). Concerns about anti-angiogenic treatment in patients with glioblastoma multiforme. BMC Cancer *9*, 444.

Wang, R., Chadalavada, K., Wilshire, J., Kowalik, U., Hovinga, K.E., Geber, A., Fligelman, B., Leversha, M., Brennan, C., and Tabar, V. (2010). Glioblastoma stem-like cells give rise to tumour endothelium. Nature *468*, 829–833.

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

Warburg, O. (1956b). On respiratory impairment in cancer cells. Science *124*, 269–270.

Warburg, O.H. (1930). The Metabolism of Tumours: Investigations from the Kaiser Wilhelm Institute for Biology, Berlin-Dahlem (London, UK: Arnold Constable).

Wertz, I.E., and Dixit, V.M. (2010). Regulation of death receptor signaling by the ubiquitin system. Cell Death Differ. *17*, 14–24.

White, E., Karp, C., Strohecker, A.M., Guo, Y., and Mathew, R. (2010). Role of autophagy in suppression of inflammation and cancer. Curr. Opin. Cell Biol. *22*, 212–217.

White, E., and DiPaola, R.S. (2009). The double-edged sword of autophagy modulation in cancer. Clin. Cancer Res. *15*, 5308–5316.

Willis, S.N., and Adams, J.M. (2005). Life in the balance: how BH3-only proteins induce apoptosis. Curr. Opin. Cell Biol. *17*, 617–625.

Witsch, E., Sela, M., and Yarden, Y. (2010). Roles for growth factors in cancer progression. Physiology (Bethesda) *25*, 85–101.

Wyckoff, J.B., Wang, Y., Lin, E.Y., Li, J.F., Goswami, S., Stanley, E.R., Segall, J.E., Pollard, J.W., and Condeelis, J. (2007). Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. Cancer Res. *67*, 2649–2656.

Yachida, S., Jones, S., Bozic, I., Antal, T., Leary, R., Fu, B., Kamiyama, M., Hruban, R.H., Eshleman, J.R., Nowak, M.A., et al. (2010). Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature *467*, 1114–1117.

Yang, J., and Weinberg, R.A. (2008). Epithelial-mesenchymal transition: At the crossroads of development and tumor metastasis. Dev. Cell *14*, 818–829.

Yang, L., Pang, Y., and Moses, H.L. (2010). TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. Trends Immunol. *31*, 220–227.

Yen, K.E., Bittinger, M.A., Su, S.M., and Fantin, V.R. (2010). Cancer-associated IDH mutations: biomarker and therapeutic opportunities. Oncogene *29*, 6409–6417.

Yilmaz, M., and Christofori, G. (2009). EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev. *28*, 15–33.

Yuan, T.L., and Cantley, L.C. (2008). PI3K pathway alterations in cancer: variations on a theme. Oncogene *27*, 5497–5510.

Zee, Y.K., O'Connor, J.P., Parker, G.J., Jackson, A., Clamp, A.R., Taylor, M.B., Clarke, N.W., and Jayson, G.C. (2010). Imaging angiogenesis of genitourinary tumors. Nat. Rev. Urol. *7*, 69–82.

Zhang, H., Herbert, B.S., Pan, K.H., Shay, J.W., and Cohen, S.N. (2004). Disparate effects of telomere attrition on gene expression during replicative senescence of human mammary epithelial cells cultured under different conditions. Oncogene *23*, 6193–6198.

Zong, W.X., and Thompson, C.B. (2006). Necrotic death as a cell fate. Genes Dev. *20*, 1–15.

Zumsteg, A., and Christofori, G. (2009). Corrupt policemen: inflammatory cells promote tumor angiogenesis. Curr. Opin. Oncol. *21*, 60–70.

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# Accessories to the Crime: Functions of Cells Recruited to the Tumor Microenvironment

Douglas Hanahan[1,*] and Lisa M. Coussens[2,*]
[1]The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Swiss Federal Institute of Technology Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[2]Department of Cell and Developmental Biology and Knight Cancer Institute, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239-3098, USA
*Correspondence: douglas.hanahan@epfl.ch (D.H.), coussenl@ohsu.edu (L.M.C.)
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**Mutationally corrupted cancer (stem) cells are the driving force of tumor development and progression. Yet, these transformed cells cannot do it alone. Assemblages of ostensibly normal tissue and bone marrow-derived (stromal) cells are recruited to constitute tumorigenic microenvironments. Most of the hallmarks of cancer are enabled and sustained to varying degrees through contributions from repertoires of stromal cell types and distinctive subcell types. Their contributory functions to hallmark capabilities are increasingly well understood, as are the reciprocal communications with neoplastic cancer cells that mediate their recruitment, activation, programming, and persistence. This enhanced understanding presents interesting new targets for anticancer therapy.**

The overarching focus of cancer research for the past four decades has been on the malignant cancer cell, seeking to understand the dominant oncogenes and tumor suppressor genes whose respective activation/upregulation or loss of function serve to impart aberrant properties on normal cells, thus contributing to their transformation into the cancerous cells that form the basis for malignancy. New tools and new data have continued to enrich our knowledge and insights into properties of malignant cells and the genetic aberrations that endow the proliferative foundation of cancer as a chronic disease. Whole-genome resequencing and genome-wide epigenetic and transcriptional profiling are presenting an avalanche of new data, with great expectations and concomitant challenges to distill it into a clarity of mechanism that can, in turn, be translated into more effective therapies. With rare exception, today's therapies for most forms of human cancer remain incompletely effective and transitory, despite knowledge of driving oncogenes and crucial oncogenic signaling pathways amenable to pharmacological intervention with targeted therapies. The challenge of distillation is, in fact, even more daunting if one incorporates the diversity of human cancers arising from distinctive cells of origin in different tissues and organs, with variable parameters of tumor development and progression, oncogenic mutation, prognosis, and response to therapy.

The hallmarks of cancer (Hanahan and Weinberg, 2000) were conceived to suggest a conceptual rationale—an underlying commonality—for this diversity and disparity in cancer cell genotypes and phenotypes, positing that the spectrum of cancers reflects different solutions to the same challenge to a prospective outlaw cell, being able to circumvent the intrinsic barriers and protective functions that have evolved in higher organisms to prevent unauthorized, chronic cell proliferation. A second premise was the now-increasingly accepted importance of the tumor microenvironment (TME), embodied in the concept that cancer cells do not manifest the disease alone, but rather conscript and corrupt resident and recruited normal cell types to serve as contributing members to the outlaw society of cells. Collaborative interactions between neoplastic cancer cells and their supporting stroma coalesce into the ectopic, chronically proliferative (and often disseminating) organ-like structures that typify most human cancers, in the form of tumors and local invasions, metastases, or vascular niches nurturing hematopoietic malignancies. Thus, in the past decade, the TME and its constituent "stromal" cells have collectively risen in prominence, now embracing a broad field of investigation. While some aspects of stroma have been long appreciated, in particular, the contributions of tumor angiogenesis and remodeled extracellular matrix (ECM) (Bissell et al., 1982; Dvorak, 1986; Folkman, 1974), the larger impact of the TME on tumor growth and progression, and on the resilience of most cancers in the face of therapy, is increasingly evident, but perhaps still not fully appreciated. This perspective, therefore, seeks to document the diverse functional contributions that stromal cell constituents of tumors can make toward cancer phenotypes, by illustrating how different stromal cell types demonstrably contribute to the core and emergent hallmarks of cancer, namely, sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming energy metabolism, and evading immune destruction. As will be seen below, stromal cells types are significantly influencing most of the hallmark capabilities, highlighting the realization that malignant cancer cells, despite all their mutational entitlement, do not act alone in elaborating the disease.

## Contributions of Stromal Cell Types to Hallmark Capabilities

While the contributions of certain stromal cell types to particular hallmarks is self-evident, in particular, that of endothelial cells to tumor angiogenesis, there are much broader contributions of stromal cells to the hallmarks of cancer (and hence to the nature of the disease). We present below illustrative but not

# Hallmarks of Cancer

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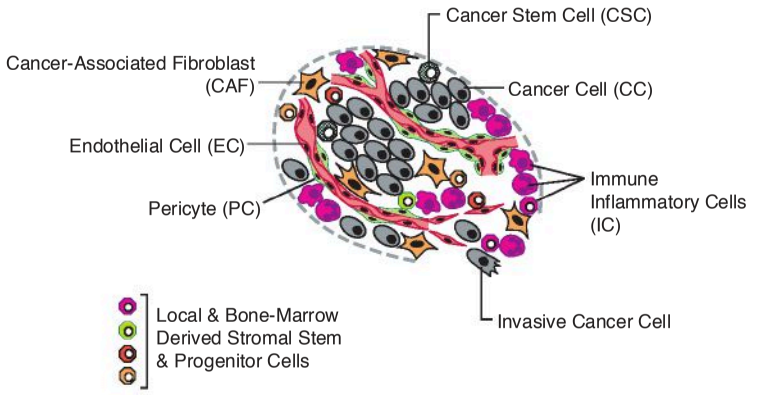
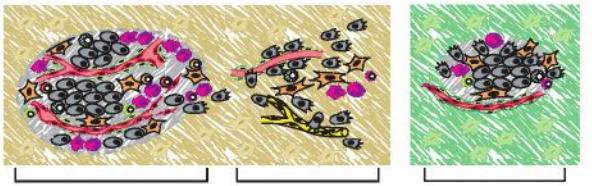
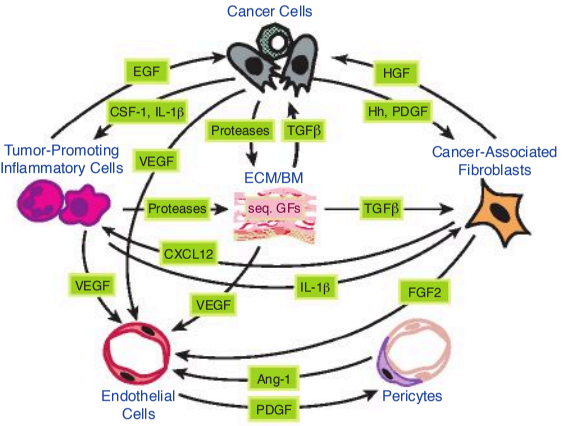


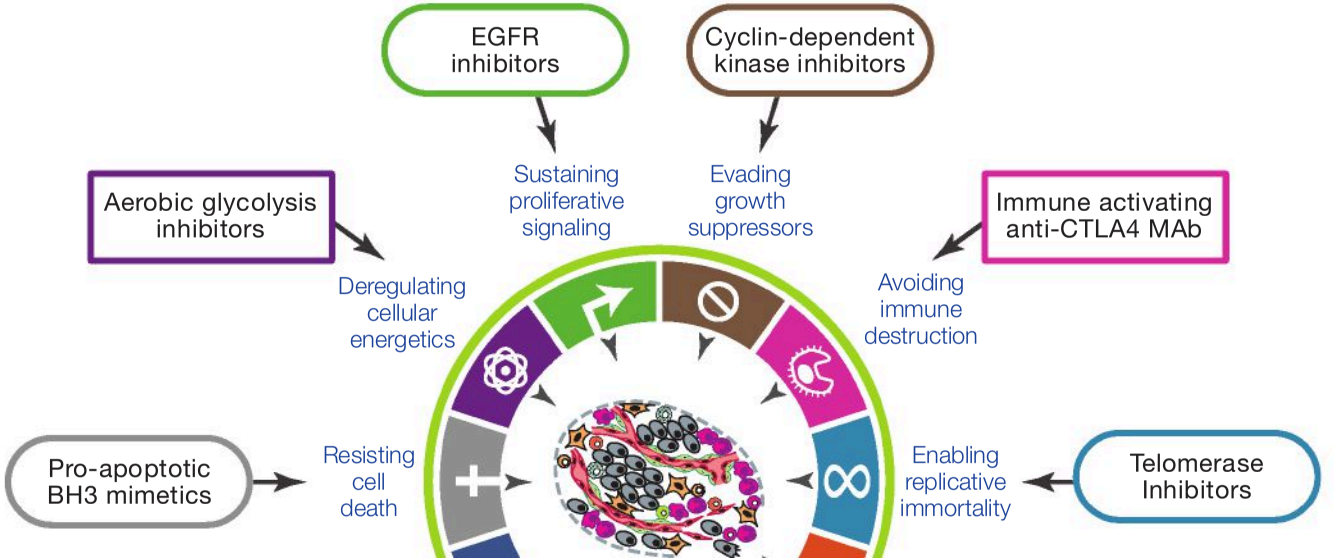




**The Cells of the Tumor Microenvironment**

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.

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



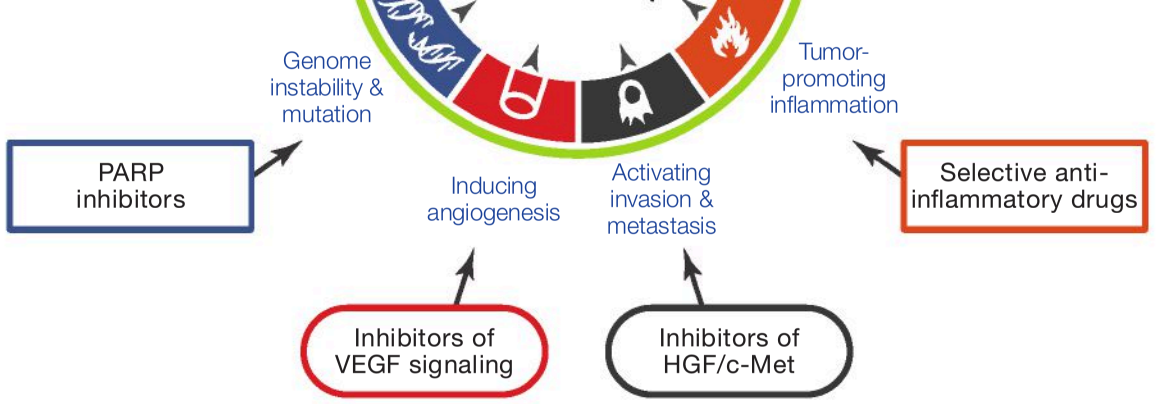

**Signaling interactions in the tumor microenvironment during malignant progression**

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.

**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 here, which also hold promise as cancer therapeutics. The drugs listed are but illustrative examples; there is a

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.)



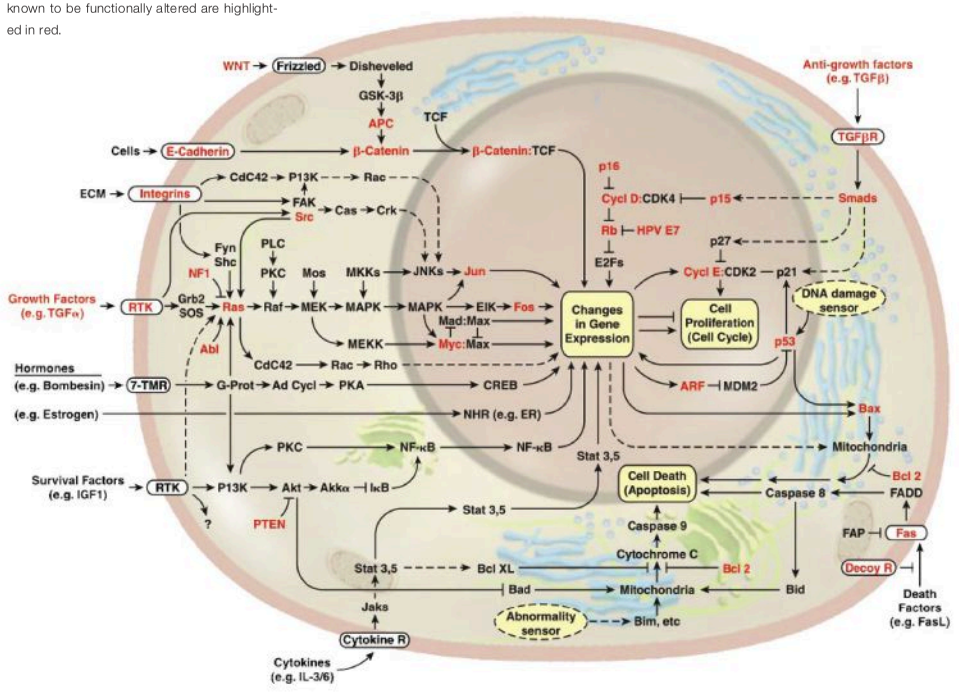

deep pipeline of candidate drugs with different molecular targets and modes of action in development for most of these hallmarks.

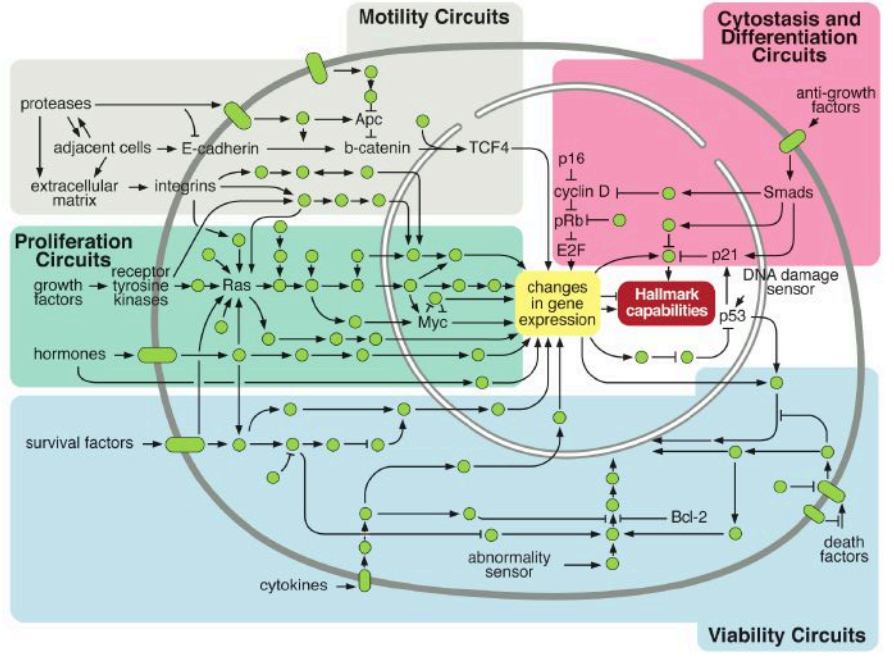
### The Emergent Integrated Circuit of the Cell

Progress in dissecting signaling pathways has begun to lay out a circuitry that will likely mimic electronic integrated circuits in complexity and finesse, where transistors are replaced by proteins (e.g., kinases and phosphatases) and the electrons by phosphates and lipids, among others. In addition to the prototypical growth signaling circuit centered around Ras and coupled to a spectrum of extracellular cues, other component circuits transmit antigrowth and differentiation signals or mediate commands to live or die by apoptosis. As for the genetic reprogramming of this integrated circuit in cancer cells, some of the genes known to be functionally altered are highlighted in red.

### 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.




Hanahan, D., and Weinberg, R.A. (2000). Cell 100, 57-70. Hanahan, D., and Weinberg, R.A. (2011). Cell 144, 646-674.

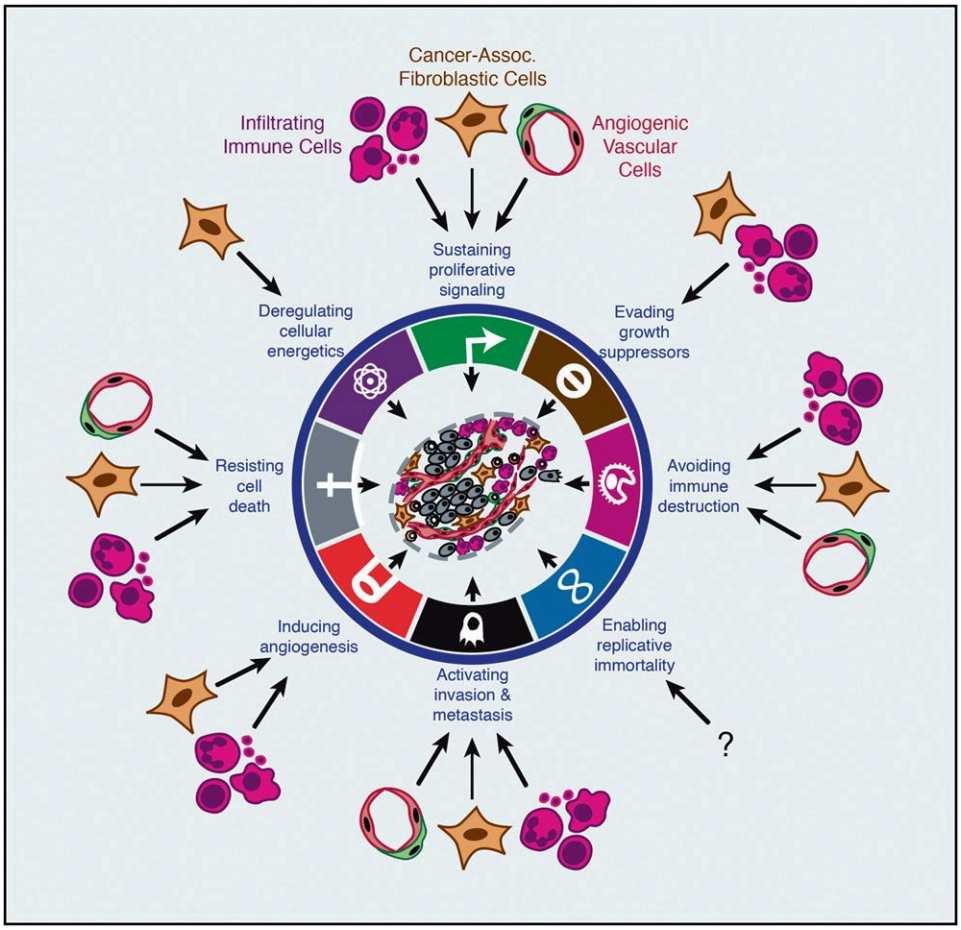



**Figure 1. Multifactoral Contributions of Activated/Recruited Stromal Calls to the Hallmarks of Cancer**

Of the eight acquired hallmark capabilities—six core and two emerging (Hanahan and Weinberg, 2011)—seven demonstrably involve contributions by stromal cells of the tumor microenvironment. The stromal cells can be divided into three general classes, depicted here by their involvement in particular hallmarks, illustrating the diversity of their functional contributions. Notably, the importance of each of these stromal cell classes varies with tumor type and organ, governed by parameters of the distinctive tumor microenvironments and underlying oncogenetic alterations in cancer cells and cancer stem cells that arise in primary tumors, and their invasive and metastatic colonizations. Moreover, distinctive cell types and subcell types within these classes can exert variable roles in enabling these capabilities, and in some cases by opposing them, as elaborated in the text and in Figure 2.

comprehensive examples of the functional roles that stromal cells play in enabling the various hallmark capabilities. Moreover, while we recognize that within each stromal subtype a spectrum of subpopulations exist, most notably in the case of cells in the innate immune system (myeloid-lineage cells), for simplicity, and to appeal to a general audience, we refer to these various subgroups within the general population as opposed to discussing activities of each, since comprehensive reviews describing these intricacies are available (Chow et al., 2011; Gabrilovich and Nagaraj, 2009; Mantovani et al., 2011; Porta et al., 2011). The breadth of stromal cell contributions to hallmark capabilities is illustrated in Figure 1, in which we have grouped the generic constituents of the stromal component of the TME into three general classes: angiogenic vascular cells (AVCs), infiltrating immune cells (IICs), and cancer-associated fibroblastic cells (CAFs).

### *Sustaining Proliferative Signaling*

Although driving oncogenic mutations conveying chronic proliferative stimuli in neoplastic cells are definitive for, and considered essential to, many forms of human cancer, virtually every stromal cell type has demonstrable ability to support hyperproliferation of cancer cells in one context or another. As such, paracrine and juxtacrine mitogenic signals supplied by stromal cell types may potentially be involved in different tumor types at virtually any stage of tumorigenesis and progression, ranging from the initiation of aberrant proliferation to the development of adaptive resistance to therapies targeting such driving oncogenic signals.

*Angiogenic Vascular Cells.* Certainly the most well-established extrinsic modulator of cancer cell (and thus tumor) growth is lesional neovascularization (Folkman, 1974), involving the tube-forming endothelial cells and their supporting pericytes that comprise the angiogenic vasculature (Armulik et al., 2005). It has long been evident in mouse models that the induction of angiogenesis, the "angiogenic switch" (Folkman et al., 1989), increases the rates of cancer cell proliferation in neoplasias and tumors (Bergers et al., 1999; Hanahan and Folkman, 1996), and that inhibition of angiogenesis can impair such

hyperproliferation (Bergers et al., 1999; Brem et al., 1993; Carmeliet and Jain, 2011; Ferrara and Alitalo, 1999; Parangi et al., 1996; Shaheen et al., 1999), presumably reflecting reduced bioavailability of blood-borne mitogenic growth factors, with or without concomitant antiapoptotic survival factors (see below). Notably, the (mitogenic) effects on cancer cells of angiogenic switching and its inhibition in human tumors remains only inferential, in large part due to a paucity of analyses involving serial biopsies of lesions during malignant progression, and throughout the course of therapeutic response and relapse/resistance to angiogenesis inhibitors.

Recently, AVCs have been implicated in local supply of growth-promoting trophic factors that are expressed and secreted—independent from blood-borne factors—by the endothelial cells, potentially acting to stimulate in a paracrine fashion multiple hallmark capabilities (Butler et al., 2010); the generality and importance of such "nonvascular" local support of cancer cell proliferation and other capabilities by tumor endothelial cells (and pericytes) is yet to be established.

*Infiltrating Immune Cells.* Although "inflammation and cancer" has become a rubric for the intersection of tumors with the immune system, many tumors show subtle infiltrations of immune cells that do not meet the classical definition of an inflammatory immune response, and yet are functionally instrumental in the tumor phenotypes discussed below; thus, we adopt the terminology of IICs to encompass both classic inflammation and more subtle involvement of immune cells in the TME. That said, virtually all adult solid tumors (carcinomas most notably) contain infiltrates of diverse leukocyte subsets including both myeloid- and lymphoid-lineage cells (Tlsty and Coussens, 2006), whose complexity and activation status vary depending on the tissue/organ locale, and stage of malignancy (Mantovani et al., 2008; Ruffell et al., 2011). IICs supply direct and indirect mitogenic growth mediators that stimulate proliferation of neoplastic cells, as well as other stromal cell types in their vicinity (Balkwill et al., 2005). Notable examples include epidermal growth factor (EGF), transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), fibroblast growth factors (FGFs), various interleukins (ILs), chemokines, histamine, and heparins (Balkwill et al., 2005). In addition, IICs express diverse classes of proteolytic enzymes (metallo, serine, and cysteine proteases) that can selectively cleave and thereby modify the structure and function of extracellular matrix (ECM), for example, uncaging bioactive mitogenic agents (Lu et al., 2011a). While such effects are reflective of typical leukocyte activities ascribed to repair of tissue damage (Dvorak et al., 2011; Tlsty and Coussens, 2006), the chronic presence of paracrine and juxtacrine mitogenic signaling molecules provided by IICs can supply evolving neoplastic cells with signals that help sustain their unchecked proliferation.

A recent study (Guerra et al., 2011) adds another intriguing contribution of IICs to the proliferative hallmark, demonstrating that inflammation of a pancreas harboring ductal epithelial cells with an activating mutation in the *K-ras* oncogene can obviate triggering of oncogene-induced cell senescence that otherwise limits hyperproliferation and malignant progression of nascent (initiated) pancreatic cancer cells; treatment of such cancer-predisposed mice with anti-inflammatory drugs restores oncogene-induced senescence, and impairs development of pancreatic cancer. The identity of the immune cell (sub)-type and of the paracrine signal(s) it supplies to inhibit oncogene-induced senescence remain to be elucidated, as does the potential involvement in other tumor types of this microenvironmental mechanism for circumventing senescence barriers to oncogene-driven hyperproliferation.

*Cancer-Associated Fibroblastic Cells.* Likely also reflecting corrupted wound healing and tissue repair mechanisms, a variety of fibroblastic cells can be recruited and/or activated to contribute to this and other hallmark capabilities (for recent reviews, see Cirri and Chiarugi, 2011; Franco et al., 2010; Pietras and Ostman, 2010; Räsänen and Vaheri, 2010). Thus, connective tissue fibroblasts proximal to neoplastic growths can be activated, and mesenchymal progenitors—in particular, mesenchymal stem cells (MSCs), both local and bone marrow derived—can be recruited and induced to differentiate into myofibroblasts defined in part by expression of alpha smooth muscle actin (αSMA) (Paunescu et al., 2011), or into adipocytes defined by expression of fatty acid binding protein-4 (FABP4) (Rosen and MacDougald, 2006). We group these similarly fibroblastic and yet distinctive cell types into a stromal cell class collectively referred to as CAFs (Hanahan and Weinberg, 2011, and references therein). Each of these CAF subtypes can contribute to a variety of tumor-promoting functions, with the potential to impact on multiple hallmark capabilities; their diversity in characteristics and in functional contributions in different organ-specific TMEs are increasingly well delineated, and appreciated. Thus, for example, CAFs can express and secrete signaling proteins that include mitogenic epithelial growth factors—hepatocyte growth factor (HGF), EGF family members, insulin-like growth factor-1 (IGF-1), stromal cell-derived factor-1 (SDF-1/CXCL12), and a variety of FGFs—with the capability to stimulate cancer cell proliferation (Cirri and Chiarugi, 2011; Erez et al., 2010; Franco et al., 2010; Kalluri and Zeisberg, 2006; Orimo et al., 2005; Räsänen and Vaheri, 2010; Rosen and MacDougald, 2006; Spaeth et al., 2009). CAFs can also orchestrate functional attributes associated with epithelial-to-mesenchymal transition (EMT) via secretion of TGF-β (Chaffer and Weinberg, 2011), which can also affect other hallmark traits noted below. In addition, both activated adipocytes and activated fibroblasts can express spectrums of "proinflammatory" mediators (Celis et al., 2005; Dirat et al., 2011; Erez et al., 2010), thereby recruiting and activating IICs that, in turn, provide mitogenic signals to cancer cells, as well as other cell types in the TME. The signals that activate, recruit, and "fine-tune" or "educate" CAFs are complex and variable between different tumor types, as are the particular roles they are implicated to play, in particular, TMEs, mirroring the complexity of IICs and of the oncogenic transformation events and mutational ontogeny of the cancer cells.

## Evading Growth Suppressors

Although suppression of unscheduled/chronic proliferation of incipient cancer cells is largely thought to involve cell intrinsic mechanisms, principally involving the p53 and pRb tumor suppressor pathways, there are intriguing examples of stromal cells in the TME helping cancer cells evade various forms of growth suppression, as illustrated by the following examples.

*Cancer-Associated, and Normal, Fibroblastic Cells.* The roster of induced gains of function that enable CAFs to support multiple

hallmark tumor phenotypes does not currently include paracrine factors that demonstrably short-circuit cancer cell-intrinsic growth suppressor pathways. There is, however, compelling evidence for causal loss of function elicited during the conversion of normal fibroblasts into CAFs. Experiments performed in coculture systems have clearly demonstrated that normal connective tissue fibroblasts (but not CAFs) from various organs can inhibit growth of cancer cells, in a process that requires contact of the ''normal'' fibroblasts with cancer cells, suggestive of roles (along with epithelial contact inhibition) in governing epithelial homeostasis and proliferative quiescence (Bissell and Hines, 2011; Flaberg et al., 2011). Thus, ''normal'' fibroblasts may serve as extrinsic epithelial growth suppressors, such that CAFs contribute to this particular hallmark capability by what they have lost from their cell of origin during the course of being reprogrammed (''educated'') as CAFs. An additional possibility, currently speculative, is that tissue fibroblasts activated into CAF-like states by other aberrant conditions (e.g., fibrosis, edema, or infection) might also produce proteases or other paracrine factors that disrupt normal epithelial architecture, thereby relieving the intrinsic growth suppression mediated by epithelial cell-cell adhesion, allowing initiation of neoplastic development.

*Infiltrating Immune Cells.* Epithelial cells are subject to an extrinsic form of growth suppression involving cell-cell and cell-ECM adhesion molecules that via their adhesive interactions transmit antigrowth signals to the cell cycle machinery; such antigrowth signals can, for example, overrule the proliferation-inducing signals of driving oncogenes such as c-Myc (Hezel and Bardeesy, 2008; Partanen et al., 2009). IICs express and secrete a variety of proteolytic enzymes (metallo, serine, and cysteine proteinases and heparanase) that, in addition to liberating mitogenic growth factors, can selectively cleave cell-cell and cell-ECM adhesion molecules, and/or ECM molecules (ligands for the latter), thereby disabling growth suppressing adhesion complexes maintaining homeostasis (Lu et al., 2011a; Mohamed and Sloane, 2006; Pontiggia et al., 2011; Xu et al., 2009).

## Resisting Cell Death

Tissues are endowed with embedded regulatory programs for controlling aberrant proliferation of resident cells, as well as for inhibiting ''invasion'' of foreign cell types, which act by inducing one form or another of cell death, of which apoptosis is the most prominent. Thus, in order to sustain their proliferative capacity and thrive ectopically, neoplastic cells must either develop intrinsic resistance to local cell death programs or instead coordinate development of cell extrinsic programs that safeguard their survival. Recent investigations have revealed the stromal/extrinsic capabilities for evading the tissue-protective mission of cell death programs that not only foster ectopic proliferation and survival of neoplastic cells, but also help to blunt effectiveness of cytotoxic and targeted therapy.

*Angiogenic Vascular Cells.* It is well established that vascularization of incipient neoplasias and tumors serves to attenuate cell death that would otherwise result from hypoxia and lack of serum-derived nutrients and survival factors. Indeed, the aforementioned studies from the 1990s report reduced apoptosis scaling hand-in-hand with increased proliferation of cancer cells following activation of the angiogenic switch, and conversely increased apoptosis resulting from pharmacological or genetic impairment of angiogenesis. Induction of both apoptosis and necrosis are almost invariable results of appreciable destruction of tumor vasculature, as contrasted to the alternative ''normalization'' of the tumor vasculature that results from weaker inhibitors of tumor angiogenesis and neovascularization (De Bock et al., 2011; Goel et al., 2011). The role of angiogenesis in limiting apoptosis is aptly illustrated by the effects of vascular disrupting agents that destroy the tumor vasculature, causing acute hypoxia and rampant cell death inside treated tumors, leaving behind hollow acellular cores enveloped by a rim of viable cells that survive by co-opting adjacent tissue vasculature (Daenen et al., 2009). Such studies establish vascularization, be it ''abnormal'' or ''normalized,'' as essential to the hallmark capability for limiting cancer cell death.

*Infiltrating Immune Cells.* Heterotypic and homotypic cell adhesion molecules provide various cell types—in their proper tissue microenvironments (e.g., organized epithelia) —with survival signals that help to maintain tissue integrity and homeostasis, such that cell detachment and loss of adhesion triggers apoptosis. One mechanism used by cancer cells to become independent of such dependence on homotypic survival signals involves IICs, which by binding to cancer cells take the place of their disconnected epithelial brethren, conveying on them the ability to survive in ectopic microenvironments by suppressing the triggering of cell death pathways. Thus, for example, $\alpha$4-integrin-expressing tumor-associated macrophages (TAMs) act in a juxtacrine manner to promote survival of metastatic breast cancer cells in lung by binding *vascular cell adhesion molecule-1 (VCAM-1*) expressed on breast cancer cells. The $\alpha$4-integrin/VCAM-1 interaction specifically activates Ezrin—a mediator of receptor tyrosine signaling—in breast carcinoma cells, which, in turn, induces PI3K/AKT signaling and suppression of apoptosis (Chen et al., 2011). A similar mechanism fosters expansion of macrometastatic breast cancer in bone (Lu et al., 2011c). In addition, TAMs also protect breast cancer cells from chemotherapy (taxol, etoposide, and doxorubicin)-induced cell death by a cathepsin protease-dependent mechanism (Shree et al., 2011). Collectively, these studies reveal the capability of macrophages (and monocytes) to provide survival signals to cancer cells that limits the impact on neoplastic progression of cancer cell death programs triggered by a variety of tissue-protective and therapy-induced mechanisms.

*Cancer-Associated Fibroblastic Cells.* A number of studies have implicated CAFs in the capability to limit the impact on tumor growth and progression of cancer cell apoptosis (Kalluri and Zeisberg, 2006; Loeffler et al., 2006; Pietras and Ostman, 2010). One modality involves the secretion of diffusible paracrine survival factors such as IGF-1 and IGF-2. A second relates to synthesis of ECM molecules and ECM-remodeling proteases that contribute to formation of a neoplastic ECM, distinctive from normal tissue stroma, that provides nondiffusible survival signals (e.g., ligands for antiapoptotic integrins); functional studies have implicated CAF-derived ECM in modulating cancer cell survival, among other traits (Lu et al., 2011a). Moreover, cancer-associated adipocytes, analogous to IICs, blunt the cytotoxic effects of radiation therapy and confer a radioresistant phenotype to breast cancer cells dependent on adipocyte-derived IL-6 (Bochet et al., 2011). While the generality (and relevance to human tumors) of these prosurvival effects has yet to be

established, it can be envisioned that such contributions by CAFs will prove to be operative in many forms of human cancer, and may also have differential clinical implications for individual patients with the same tumor type, such as obese patients whose cancers have been associated with more aggressive characteristics (Khandekar et al., 2011).

### *Enabling Replicative Immortality?*

Stabilizing telomere length and functionality to enable limitless replication of cancer cells is the essence of this hallmark, one that is seemingly independent of the TME, in that there is currently no substantive evidence for stromal contributions to telomere stabilization in cancer cells. While it could be argued that abrogation of senescence-inducing signals from normal stromal fibroblasts or antagonistic IICs is involved in enabling this hallmark, we consider that triggering such senescence is more likely involved in a first line of tissue defense focused on opposing (along with cell death and cell cycle arrest) inappropriate proliferation, long before replicative immortality becomes a factor, and thus stromal involvement in senescence and its circumvention is most logically associated with the proliferation and growth suppression hallmarks.

### *Inducing Angiogenesis*

In adult tissues, most blood vessels are quiescent, and angiogenesis (growth of new blood vessels from pre-existing ones) occurs only during the female reproductive cycle and under certain pathophysiological conditions, such as tissue remodeling associated with wound healing (Carmeliet and Jain, 2011). Whereas the cellular and molecular programs are common to both physiological and tumor angiogenesis, constitutively activated proangiogenic signaling in tumors make tumor-associated vessels distinctly irregular, chaotic, and inherently unstable (De Bock et al., 2011; McDonald and Choyke, 2003; Morikawa et al., 2002). Interestingly, tumors with reduced levels of such hyperactive angiogenic stimulation—resultant to limited abundance of vascular endothelial growth factor (VEGF) and other angiogenic regulatory factors in their TME, or to pharmacological suppression of VEGF—evidence so-called "vascular normalization," in which vessels are less torturous, with better pericyte coverage, and improved and less erratic blood flow (De Bock et al., 2011; Goel et al., 2011; Jain, 2005). Historically, tumor angiogenesis was envisioned to be principally regulated by cancer cells expressing proangiogenic factors, which is indeed one mechanism; there is, however, now abundant evidence that stromal cells in the TME are instrumental in switching on and sustaining chronic angiogenesis in many tumor types, as illustrated in the following examples.

*Infiltrating Immune Cells.* There is a tight interplay between IICs and vascular cells. Endothelial cells mediate leukocyte recruitment by expressing a repertoire of leukocyte adhesion molecules, while IICs produce a diverse assortment of soluble factors that influence endothelial cell behavior. Myeloid cells implicated in these interactions include subsets of granulocytes (neutrophils, basophils, and eosinophils), dendritic cells, TAMs, Tie2-expressing monocytes, immature myeloid cells (iMCs)/myeloid-derived suppressor cells (MDSCs), and mast cells. The soluble mediators produced by IICs implicated in regulating aspects of the angiogenic process include cytokines (VEGF, bFGF, TNF-$\alpha$, TGF-$\beta$, platelet-derived growth factor [PDGF], placental growth factor [PIGF]), Neuropilin-1, chemokines (CXCL12, IL-8/CXCL8), matrix metalloproteinases (MMPs, including MMP-2, -7, -9, -12, and -14), serine proteases (urokinase-type plasminogen activator), cysteine cathepsin proteases, DNA-damaging molecules (reactive oxygen species), histamine, and other bioactive mediators (nitric oxide). All of these effectors have demonstrated capabilities to regulate vascular cell survival, proliferation, and motility, along with tissue remodeling, culminating in new vessel formation (De Palma and Coussens, 2008).

TAMs regulate tumor angiogenesis largely through their production of VEGF-A; this connection is illustrated by restoration, via ectopic VEGF overexpression, of tumor angiogenesis otherwise impaired by macrophage depletion (Lin et al., 2007). Conversely, genetic deletion of the *VEGF-A* gene in macrophages attenuates tumor angiogenesis and results in a morphologically more normal vasculature, much as is seen with pharmacological inhibitors of VEGF signaling (Stockmann et al., 2008). In some mouse models of cancer, production of MMP-9 by TAMs increases bioavailability of otherwise limited (ECM sequestered) VEGF-A, thus providing an alternative, but still VEGF-dependent route for promoting angiogenesis (Bergers et al., 2000; Du et al., 2008; Giraudo et al., 2004). Similarly, TAM production of the VEGF family member PIGF stimulates angiogenesis in some tumors (Rolny et al., 2011) and thus TAMs may present a mechanism for acquiring resistance to anti-VEGF-A/VEGFR therapies (Fischer et al., 2007; Motzer et al., 2006; Willett et al., 2005).

The significance of TAMs as anticancer therapeutic targets has recently been emphasized by several studies reporting that reprogramming of tumor-promoting TAMs toward a phenotype embodied in conventional "antigen-presenting" macrophages can blunt tumor growth via processes that include impaired angiogenesis and vascular normalization. For example, histidine-rich glycoprotein HRG, a host-produced protein deposited in tumor stroma, can induce such a reprogramming of TAMs, resulting in vascular normalization and improved responses to chemotherapy (Rolny et al., 2011). Similar findings were reported by blockade of colony stimulating factor-1 (CSF-1) signaling, which resulted in macrophage depletion in mammary tumors, concomitant with reduced vascular density and improved responses to chemotherapy (Denardo et al., 2011). Common to both studies was enhanced anti-tumor immune responses by cytotoxic T lymphocytes (CTLs), thus indicating the complexity of dialogs by diverse stromal cell types in tumors, and the power of targeting one subtype to thereby subvert or alter bioactivities of other counterpart stromal cells.

While not as well studied, mast cells have long been recognized for their ability to foster tumor angiogenesis (Kessler et al., 1976). Recruitment of mast cells to human papilloma virus-induced squamous carcinomas (Coussens et al., 1999) or *Myc*-induced pancreatic $\beta$ cell tumors (Soucek et al., 2007) is required for macroscopic tumor expansion; treatment with mast cell inhibitors results in impaired induction and persistence of angiogenesis, thereby elevating hypoxia and cell death of both cancer cells and endothelial cells (Soucek et al., 2007). Mast cells are reservoirs of potent vascular mediators including VEGF, Angiopoietin-1, IL-8/CXCL8, histamine, and heparin; mast cells can also release proteases (e.g., MMP-9) that liberate ECM-sequestered proangiogenic growth factors (Bergers et al., 2000; Coussens et al., 1999), or indirectly regulate AVCs—in the

case of tryptase—via cleavage of protease-activated receptor-2 (PAR2) on CAFs, which activates proangiogenic signaling programs (Khazaie et al., 2011).

Other IICs associated with tumor angiogenesis include neutrophils and their myeloid progenitors, which produce MMP-9 and are demonstrably involved in angiogenic switching in some tumors (Nozawa et al., 2006; Pahler et al., 2008; Shojaei et al., 2007), and platelets, the enucleated minicells spun off from megakeryocytes whose principle role involves induction of blood clotting in response to bleeding. Platelets release distinctive granules containing either pro- or antiangiogenic regulatory molecules, and have been implicated in angiogenesis for decades (Sabrkhany et al., 2011); the precise roles and importance of platelets and the mechanisms of their regulated degranulation has been elusive. Recent studies however have reported that candidate effectors in platelets can be genetically manipulated (Labelle et al., 2011), thus enabling an avenue to clarify their roles in tumor angiogenesis.

*Cancer-Associated Fibroblastic Cells.* There is abundant evidence that CAFs are involved in orchestrating tumor angiogenesis in a variety of tumor types. First, CAFs in different TMEs can produce a number of proangiogenic signaling proteins, including VEGF, FGF2 plus other FGFs, and IL-8/CXCL8 and PDGF-C; of note, PDGF-C may rescue angiogenesis in some anti-VEGF resistant tumors (Crawford et al., 2009). In addition, CAFs as well as normal connective tissue fibroblasts are major biosynthetic sources of ECM proteins, in which angiogenic growth factors are sequestered. In contrast to typical normal fibroblasts, CAFs can also produce a variety of ECM-degrading enzymes that release such latent angiogenic factors (bFGF, VEGF, TGF-β), rendering them bioavailable to their receptors on endothelial cells (Kalluri and Zeisberg, 2006; Pietras and Ostman, 2010; Räsänen and Vaheri, 2010). Finally, CAFs can produce chemoattractants for proangiogenic macrophages, neutrophils, and other myeloid cells, thereby indirectly orchestrating tumor angiogenesis (Räsänen and Vaheri, 2010; Vong and Kalluri, 2011), as well as directly stimulating recruitment of endothelial precursor cells via secretion of CXCL12 (Orimo and Weinberg, 2007).

### Activating Invasion and Metastasis

All three classes of stromal cell are implicated as contributors in one context or another to the capability for invasion and metastasis, as the following examples illustrate.

*Angiogenic Vascular Cells.* The characteristics of chronically angiogenic (and morphologically abnormal) tumor vasculature have the added effect of contributing to cancer cell dissemination in the course of metastasis. Many tumors express high levels of the proangiogenic factor VEGF, also known and first identified as vascular permeability factor (Senger et al., 1983). VEGF signaling through VEGFR2 loosens tight junctions interconnecting endothelial tube cells, rendering vasculature permeable to leakage of blood into the interstitial TME, and concomitantly lowering barriers for intravasation of cancer cells into the circulation, particularly in tumors with high interstitial fluid pressure, which therefore counteracts pressure inside the vasculature. Tumor vasculature hyperstimulated by VEGF often has reduced pericyte coverage and looser association of such pericytes with endothelium, the significance of which has been revealed in studies where genetic or pharmacologic perturbation of pericyte coverage facilitates metastatic dissemination of cancer cells (Cooke et al., 2012; Xian et al., 2006). Hypoxia in and around tumor vessels also contributes to metastatic dissemination of cancer cells through the actions of genes regulated by hypoxia inducible (HIF) transcription factors, including VEGF and inducible nitric oxide synthase (iNOS), among many mediators. Notably, differential expression of HIFs by endothelial cells (and IICs) is particularly significant for metastasis (Branco-Price et al., 2012; Takeda et al., 2010), as they variably alter vascular tension and function, largely dependent on nitric oxide, which, in turn, loosens pericyte coverage (Kashiwagi et al., 2005), contributing thereby to metastatic success. Such studies establish the concept, still to be generalized, that impaired vascular integrity disables a significant barrier to blood-borne metastasis, and thus facilitates dissemination of cancer cells from primary human tumors.

The vasculature plays a similar role in metastatic seeding at distant sites, where an intact normal endothelium with intimate pericyte coverage can be envisaged to block cancer cell extravasation from the blood into normal parenchyma. Indeed, it is increasingly evident that metastatic primary tumors can precondition the vasculature in metastatic sites with factors such as VEGF, supplied systemically or produced locally by the disseminated cancer cells they spawn; the actions of VEGF on the endothelium at incipient metastatic sites facilitates both loosening of vessel walls for extravasation, and subsequent induction of angiogenesis to support metastatic tumor growth. Still to be clarified is the identification and possible roles of factors produced by endothelial cells and pericytes that contribute to metastatic processes.

*Infiltrating Immune Cells.* Functional studies spanning the last decade have unambiguously established and elaborated the roles of IICs in fostering metastasis. Mast cells and macrophages in primary tumor TMEs provide a wide range of proteases, including serine, cysteine, and metalloproteases (Kessenbrock et al., 2010; van Kempen et al., 2006) that foster ectopic tissue invasion by remodeling structural components of ECM (fibrillar collagens, elastins, or fibrin), which in turn provide conduits for malignant cell egress, as well as generating ECM fragments with proinvasive signaling activities. For example, the proteolytic activities of MMP-2 expressed by macrophages and other leukocytes effects the release of cryptic ECM fragments by cleaving laminin-5 γ2 chains that, in turn, mimic EGF receptor (EGFR) ligands and thus induce cell motility and invasion (Giannelli et al., 1997; Pirilä et al., 2003). Leukocyte-derived MMP-7 processes proheparin-bound-EGF (HB-EGF) into its bioactive form in pancreatic carcinoma cells (Cheng et al., 2007), resulting in repressed E-cadherin-mediated cell adhesion and potentiation of invasive growth (Wang et al., 2007a). Leukocyte-derived MMP-7 and cathepsin B further facilitate tumor cell motility and invasion by directly cleaving extracellular domains of E-cadherin (Gocheva et al., 2006; Vasiljeva et al., 2006). IIC-derived TNF-α enhances invasive/migratory phenotypes of breast, skin, and ovarian cancer cells through activation of downstream signaling cascades, including the Jun N-terminal kinase (JNK) and nuclear factor κB (NFκB) transcription factors, resulting in induced gene expression of proinvasive factors, e.g., EMMPRIN (extracellular matrix metalloprotease inducer) and MIF (migration inhibitory factor), whose expression enhances

MMP-2 and MMP-9 secretion and activity (Balkwill, 2009). Macrophage-derived TNF-α also potentiates Wnt/beta-catenin signaling during gastric carcinogenesis by activating Akt signaling and GSK3beta phosphorylation in initiated gastric epithelial cells independent of the NFκB pathway (Oguma et al., 2008).

IIC mediators also inhibit expression of known metastasis suppressor genes. T cells and macrophages infiltrating prostate cancers produce the TNF-α-related cytokine RANKL (Receptor Activator for NFκB Ligand). RANKL, through interaction with its receptor RANK, activates Inhibitor of NFκB Kinase α (IKKα), leading to transcriptional repression of the metastatic tumor suppressor gene *maspin* (Abraham et al., 2003; Sager et al., 1997); maspin inhibits metastasis by impairing cancer cell invasion, in part by altering expression of integrin adhesion molecules that anchor and thereby restrict cell mobility (Chen and Yates, 2006). Abrogation of IKKα activity restores *maspin* gene expression and significantly reduces lymphatic and pulmonary metastasis of prostatic tumor cells, further strengthening the causality link (Luo et al., 2007). Notably, in prostate cancer metastasis to bone, RANKL bioavailability, and hence suppression of mapsin in cancer cells, is regulated by osteoclast-supplied MMP-7, illustrating another means by which stromal cells in metastatic microenvironments can provide paracrine support for metastatic colonization (Gorden et al., 2007; Lynch et al., 2005).

Concentration gradients of growth factors established by leukocytes also coordinate tumor cell movement toward, and intravasation into, tumor-associated vasculature. For example, macrophages are the primary source of EGF in the developing mammary gland and in mouse models of breast cancer (Leek et al., 2000; Lewis and Pollard, 2006). EGF promotes invasion/chemotaxis and intravasation of breast carcinoma cells through a paracrine loop operative between tumor cells and macrophages that are required for mammary cancer cell migration (Wyckoff et al., 2004) via cofilin-dependent actin polymerization (Wang et al., 2007b). Transcriptome profiling has revealed that the TAMs participating in this paracrine interplay represent a unique subpopulation that associates intimately with tumor vessels (Ojalvo et al., 2010).

Long suspected but largely below the radar are platelets. A recent report solidified these suspicions (Labelle et al., 2011), revealing that platelets induce a transitory EMT by physically associating with blood-borne cancer cells, facilitating extravasation and seeding of metastases. Functional genetic studies demonstrated that the invasion- and metastasis-promoting activity of platelets involves platelet-derived TGF-ß ligand as well as an inducer of NFκB signaling that requires physical contact of platelets with cancer cells (suggestive perhaps of the membrane-bound Notch ligands). Thus, platelets can be added to the roster of tumor-promoting hematopoietic cells that facilitate invasion and metastasis. It is intriguing to consider the possibility that platelets might intravasate into premalignant tissues or primary tumors via leaky tumor vasculature, contributing therein to induction of EMT and locally invasive growth.

*Cancer-Associated Fibroblastic Cells.* There are increasing examples wherein CAFs modulate the capability of cancer cells to invade locally or establish secondary tumors at distant metastatic sites. One prominent CAF-derived effector of this capability is the c-Met ligand HGF, which stimulates via heightened c-Met signaling both invasiveness and proliferation. A second, CAF-derived effector, TGF-β, is demonstrably involved in activating EMT programs in certain cancer cells, thereby enabling their capability for invasion and metastasis (Chaffer and Weinberg, 2011); likely additional CAF mediators will prove to be involved in different contexts; thus, for example, CAF/MSC secretion of CCL5 stimulates breast cancer metastasis (Karnoub et al., 2007). Moreover, CAFs produce a distinctive (from normal fibroblasts) repertoire of ECM proteins as well as a variety of ECM remodeling enzymes that further modify the TME, rendering it more supportive of cancer cell invasion, both proximal to the CAFs as well in adjacent normal tissue (Chaffer and Weinberg, 2011; Cirri and Chiarugi, 2011; Kalluri and Zeisberg, 2006; Pietras and Ostman, 2010). CAFs are detected at the invasive fronts in some tumors, consistent with an active collaboration with cancer cells in invasion; such CAFs may reflect comigrating cells as well as normal tissue fibroblasts that have been reprogrammed by signals (e.g., PDGF and sonic hedgehog) released by cancer cells (or IICs). Such reprogramming is also evident in metastasis, where emigrating cancer cells induce expression of the ECM molecule periostin, necessary for efficient colonization in a mouse model of metastatic breast cancer (Malanchi et al., 2012). In another model system, cancer cells disseminate through the circulation in conjunction with primary tumor-derived CAFs (Duda et al., 2010), bringing the foundations of a TME to the metastatic site, a variation on the theme discussed above whereby cancer cells disseminate in association with macrophages or other myeloid cells.

Given the observations that fibrotic breast disease and increased breast density predispose to breast cancer, and that environmentally induced fibrotic disorders increase incidence of lung, skin, and pancreatic cancer, it is evident that the intensity of fibroblastic proliferation, accumulation and assembly may play other influential roles in tumor development and progression. Breast carcinogenesis is accompanied by lysyl oxidase-mediated crosslinking of collagen fibrils (largely produced by CAFs) that imparts a proinvasive phenotype on mammary cancer cells, which is dependent on enhanced PI3 kinase (PI3K) signaling, and associated with integrin clustering and increased presence of focal adhesions (Levental et al., 2009). Notably, genetic or pharmacological blockade of lysyl oxidase-mediated collagen crosslinking impedes late-stage cancer progression in mouse models of mammary carcinogenesis (Levental et al., 2009). Moreover, ablation of CAFs with an inhibitor of hedgehog signaling improves therapeutic delivery of cytotoxic drugs in a mouse model of pancreatic ductal adenocarcinoma, revealing that the desmoplastic stroma erected by CAFs represents a barrier to effective biodistribution of chemotherapy (Olive et al., 2009). The structural effects of CAFs on TMEs have been further revealed by studies perturbing other CAF-derived mediators. Notably, inhibiting either TGF-β, its type I receptor (Kano et al., 2007; Sounni et al., 2010), or the PDGF receptors (Pietras et al., 2001) similarly reduces interstitial fluid pressure in certain tumors, resulting in improved tumor hemodynamics and more favorable biodistribution of drugs, so too does reducing the abundance of the ECM component hyaluronic acid in the TME (Provenzano et al., 2012). Thus, in addition to producing soluble factors that modulate hallmark

phenotypes, CAFs can profoundly alter the physical parameters of the TME in some tumor types, consequently impacting delivery of therapeutics.

### Evading Immune Destruction

*Angiogenic Vascular Cells.* Although the aberrant morphology of the angiogenic tumor vasculature—loosened interconnections between endothelial cells and less intimate association and coverage by pericytes—evidently facilitates transit of cells across the vascular wall in both directions, there is abundant evidence that such routes of transit are in many cases insufficient for the massive influx of natural killer (NK) cells, CTLs, and NK T cells needed to achieve effective killing of cancer cells in tumors. As such, the tumor vasculature contributes to the hallmark capability of evading immune destruction by its inability to support intensive T cell inflammation. Numerous studies have documented this barrier to T cell influx, seen by the absence in tumors of high endothelial venules (HEVs) (Onrust et al., 1996), vascular structures serving as portals for mass transit of lymphocytes into and out of activated lymph nodes and heavily inflamed tissues. More recently, regulatory signals that render tumor vasculature nonpermissive for HEVs and such mass transit of CTLs have been identified, and their modulation was found to break down inflammatory barriers (Fisher et al., 2011; Manzur et al., 2008). Thus, an added benefit of ''anti-angiogenic'' strategies involving inhibition of VEGF signaling and of its consequent vascular abnormalities may be in enabling tumor immunity via HEV induction in the normalized vasculature (Goel et al., 2011; Manzur et al., 2008).

*Infiltrating Immune Cells.* IIC phenotypes in some tumors are similar to the resolution phase of wound healing, wherein the TME contains significant leukocytic infiltrations that convey immunosuppressive activity (ability to block antitumor CTL or NK/T cell-mediated killing of aberrant cells). These assemblages include regulatory T cells ($T_{reg}$), iMCs/MDSCs, TAMs programmed by Th2-type cytokines, and neutrophil and mast cell subtypes that collectively endow cancer cells with a mechanism to escape killing by T cells (Ruffell et al., 2010).

Macrophage progenitors exposed to a variety of immune-regulatory cytokines (IL-4, IL-13, etc.) and other factors (thymic stromal lymphopoietin, immune complexes, etc.) can differentiate to become alternatively activated TAMs with various tumor-promoting properties, as elaborated above. Among their distinctive phenotypes is absence of cytotoxic activity typified by conventional tissue macrophages (Qian and Pollard, 2010), instead manifesting an ability to block $CD8^+$ T cell proliferation or infiltration though release of factors with immunosuppressive potential (Denardo et al., 2011; Doedens et al., 2010; Kryczek et al., 2006; Movahedi et al., 2010). TAMs also indirectly foster immune suppression through recruitment of $T_{reg}$ cells via the chemokine CCL22 (Curiel et al., 2004). In murine tumor models, suppression of $CD8^+$ T cell proliferation by TAMs is at least partly dependent on metabolism of L-arginine via arginase-1 or iNOS (Doedens et al., 2010; Movahedi et al., 2010) resulting in production of oxygen radicals or nitrogen species (Lu et al., 2011b; Molon et al., 2011). In human TAMs, suppression of $CD8^+$ T cells can occur independent of L-arginine metabolism (Kryczek et al., 2006) and may instead rely on macrophage expression of ligands for T cell costimulatory receptors that mediate T cell inhibition (Topalian et al., 2012), as has been described for hepatocellular (Kuang et al., 2009) and ovarian (Kryczek et al., 2006) cancer. Data from human tumors indicate that the presence of TAMs expressing immune-suppressive markers correlates with reduced survival of patients with several types of solid tumors, and notably inversely correlates with $CD8^+$ T cell density in human breast cancer (Denardo et al., 2011).

iMCs encompass a diverse population of myeloid cells characterized in part by coexpression of surface markers CD11b and Gr1, and include monocytes variably referred to as MDSCs, inflammatory monocytes, and neutrophils (Ostrand-Rosenberg, 2008). MDSCs and iMCs are functionally characterized by their suppression of T cell proliferation via arginase I, inducible nitric oxide synthase expression, and perioxynitrite, and, at the same time, by their ability to promote generation of $T_{reg}$ cells (Ostrand-Rosenberg, 2008).

While the immunosuppressive activity of mast cells is not well described, it is clear that in addition to their prominent mitogenic and proangiogenic activities as discussed above, they also indirectly regulate immunosuppression (Wasiuk et al., 2009), by releasing cytokines that recruit CTL-suppressing MDSCs and $T_{regs}$. $T_{regs}$ are also recruited into neoplastic tissues by other cytokines, most notably CCL2 and TGF-β; their abundance (and hence their indictment as tumor promoting) correlates with poor outcome for several cancer types (van der Vliet et al., 2007). $T_{regs}$ typically play an important physiological role in suppressing responses to self-antigens, thereby preventing autoimmunity, and as such can be corrupted to dampen anti-tumor immunity. A related immunosuppressive strategy involves expression of the lymphatic chemokine CCL21 in tumors; CCL21 instructs lymphoid neogenesis and immune tolerization involving MDSCs and $T_{regs}$ so as to prevent autoimmunity; thus, when CCL-21 is ectopically expressed in tumors, it can contribute to suppression of antitumor immunity by altering the differentiation and function of IICs, biasing toward tumor-promoting subtypes (Shields et al., 2010).

*Cancer-Associated Fibroblastic Cells.* In addition to producing chemokines and other signals that recruit IICs, CAFs can demonstrably inhibit cytotoxic T cells and NK/T cells, in part by producing TGF-ß, thereby blunting destructive inflammatory responses that might otherwise disrupt tumor growth and progression (Stover et al., 2007).

### Reprogramming Energy Metabolism

There is now broad appreciation that cancer cells have altered metabolism to support chronic proliferation, in particular, flexible utilization of fuel sources and modes of consuming them to generate energy and biomaterials; most notable is the activation of aerobic glycolysis that complements the output of (sometimes reduced) oxidative phosphorylation for such purposes. While much of metabolic reprogramming is considered to be cell intrinsic to the cancer cells, there are both evident and emergent extrinsic modulators in the TME.

*Angiogenic Vascular Cells.* Variations in the density and functionality of the angiogenic tumor vasculature are well-established modulators of energy metabolism for the cancer cell; in particular, inadequate vascular function can result in hypoxia, activating the HIF response system, which among its myriad of effects can stimulate aerobic glycolysis, enabling cancer cells to survive and proliferate more effectively in conditions of vascular insufficiency, thereby concomitantly enhancing the

capability for invasive growth. It is of course arguable whether this effect on metabolism truly represents a functional contribution of tumor vasculature to the cancer cell and hence to malignant phenotypes, as opposed to a reaction to its impaired functionality, but the net result remains the same, that the nature of the aberrant vasculature of the TME impacts cancer cell metabolism.

*Infiltrating Immune Cells.* A specific role for IICs as regulators of altered energy metabolism in cancer cells is beginning to emerge (Trinchieri, 2011). While definitive genetic studies unambiguously linking IICs to tumor cell metabolism are still on the horizon, it has been reported that alternatively activated macrophages are implicated in the altered metabolism of tumors, as well as in the development of metabolic pathologies (Biswas and Mantovani, 2012).

*Cancer-Associated Fibroblastic Cells.* There is an intriguing line of evidence linking CAFs to an unconventional form of aerobic glycolysis, in which CAFs are induced by reactive oxygen species released by cancer cells to switch on aerobic glycolysis, secreting lactate and pyruvate that, in turn, can serve as fuel for cancer cell proliferation (Rattigan et al., 2012; Sotgia et al., 2012). A particular subclass of CAF, in which the intracellular scaffold protein Caveolin-1 is downregulated (by reactive oxygen species), displays this metabolic support phenotype, resulting in an activated TME that drives early tumor recurrence, metastasis, and poor clinical outcome in breast and prostate cancers (Sotgia et al., 2011). While yet to be generalized, the results (and the association of reduced Caveolin-1 in CAFs with poor prognosis) suggest that heterotypic supply of energy sources to cancer cells may prove to be yet another profound contribution made by CAFs to the TME, above and beyond the aforementioned roles in orchestrating cell proliferation (and survival), angiogenesis, invasion, and metastasis.

Recent data have also revealed that adipocytes similarly engage in ''metabolic coupling'' with cancer cells and thereby promote tumor progression (Martinez-Outschoorn et al., 2012) (Nieman et al., 2011). Metastatic ovarian carcinoma typically seeds into adipose tissue in peritoneum, resulting in reprogramming of proximal adipocytes toward a more catabolic state. In this state, ''activated'' adipocytes generate free fatty acids that are utilized by metastatic ovarian cancer cells to generate ATP via mitochondrial β-oxidation. Mitochondrial metabolism in metastatic ovarian cancer cells is fostered, thereby protecting them from apoptotic cell death, as well as improving chemoresistance, and enhancing their colonization into macrometastatic lesions (Nieman et al., 2011). Looking ahead, it will be interesting to determine if re-educated adipocytes are involved in tumor metabolism in other cancer types, perhaps in partnership with conventional fibroblast-derived CAFs, which as noted above are implicated in metabolic fueling of breast cancer cells (Sotgia et al., 2012).

Thus, a remarkable symbiotic relationship in energy metabolism is emerging between CAFs and cancer cells, in which CAFs in different TMEs can exchange energy sources with cancer cells to optimize metabolic efficiency and tumor growth, involving the alternative use of glucose and lactate, and other energy-rich molecules. The nature of the symbiosis can evidently vary depending on the TME: in some cases, the CAFs switch on aerobic glycolysis, utilizing glucose and secreting lactate that is taken up by cancer cells and used as fuel (Balliet et al., 2011; Ertel et al., 2012; Martinez-Outschoorn et al., 2012; Sotgia et al., 2012). In other cases, the symbiosis is opposite: cancer cells switch on aerobic glycolysis, utilizing glucose and exporting lactate, which the CAFs then take up and use as fuel to drive their tumor-promoting functional activities (Rattigan et al., 2012). No doubt further variations of the energy-sharing theme, and intricacies of mechanism, will be revealed as other TMEs are assessed for their metabolic phenotypes.

### *Beyond The Hallmarks: Supporting Cancer Stem Cells*

It has become evident in the past decade that most if not all malignancies contain a heterogeneous subpopulation of cancer cells with stem-like properties—cancer stem cells (CSCs)—that are instrumental in the pathologic manifestation of cancer, variably affecting initiation, persistence in the face of intrinsic barriers to expansive proliferation, metastatic progression, and the ability to rebound from ostensibly efficacious cancer therapies. Once again, this crucial dimension of the cancer cell is not strictly autonomous; rather, stromal cells demonstrably support CSCs. All three stromal cell classes have been implicated in functional support of CSCs in different neoplastic contexts, including, for example, (1) endothelial cells, pericytes, and perivascular IICs organized into specialized vascular niches in primary tumors (Calabrese et al., 2007) as well as metastatic sites (Kaplan et al., 2005; Lyden et al., 2001; Psaila and Lyden, 2009), and (2) the myofibroblastic/MSC subtype of CAFs, likely also present in metastatic vascular stem cell niches (Kidd et al., 2009; Korkaya et al., 2011; Liu et al., 2011; Spaeth et al., 2008, 2009); similar niche-forming cells may also nurture CSCs inside primary tumors.

In sum, there is compelling evidence for the insidious roles that normal cells play in cancer, having been recruited and/or activated to serve as members of corrupt TMEs, contributing to the functional capabilities embodied in most of the hallmarks of cancer (Figure 1). Their contributions are diverse and variable from one organ and oncogenic foundation in cancer (stem) cells to another. The three general classes of stromal cell contain multiple cell types and subcell types, of which major subtypes and their ascribed functions (in various neoplastic contexts) are summarized in Figure 2.

## Challenges in Charting Human Tumor Microenvironments

Much of the functional and correlative evidence presented above implicating stromal mechanisms has come from experiments performed in model systems, principally tumors growing and progressing in genetically engineered mouse models of cancer (GEMM) and human xenotransplant mice (increasingly now primary patient-derived xenotransplants [PDX]), as well as in cell and organ coculture assays. Moreover, the challenges in performing precise genetic manipulations of stromal cells in experimental tumors is considerable, and as such, some of the predicted contributions have not been definitively established in terms of their functional significance in relation to the driving forces embodied in the mutationally transformed cancer cells. And even then, a bigger question remains: do human cancers and their foundation in cancer cells (and cancer stem cells) develop, progress, metastasize, and acquire drug resistance with similar support by accessory cells recruited and redirected

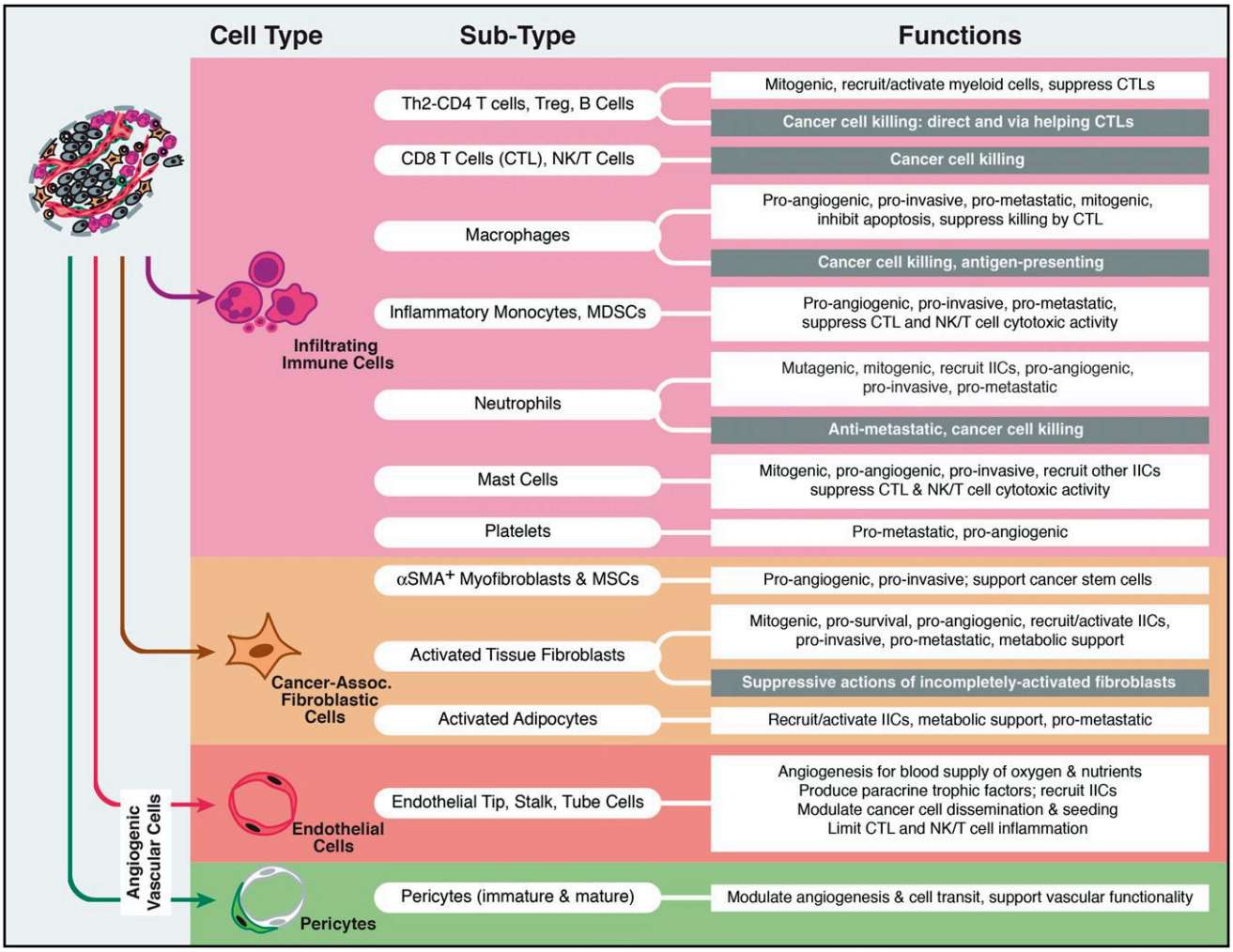



**Figure 2. Multiple Stromal Cell Types and Subcell Types of the Tumor Microenvironment Can Variably Contribute to, or in Some Cases Oppose, Acquisition of the Seven Hallmark Functional Capabilities in Different Organ Sites, Tumor Types and Subtypes, and Stages of Progression**

Major stromal cell subtypes are indicated, along with a synopsis of key functional contributions that such cell subtypes can make. The antagonistic functions of certain subcell types are highlighted in gray. The lists of subtypes and of their key functions are not comprehensive, but rather prominent examples. Not listed are molecular regulatory signals for, and effector agents of, the noted functions. Both lists will certainly be refined in coming years. Also not shown are the crucial cancer cells and cancer stem cells, with which these stromal cells dynamically interact to manifest cancer phenotypes (Hanahan and Weinberg, 2011). Th2, helper type 2; CD4 T cell, CD4-positive lymphocyte; Treg, regulatory T cell; CTL, cytotoxic T lymphocyte; NK/T, natural killer and natural killer T cell; MDSCs, myeloid-derived suppressor cells; αSMA, alpha smooth muscle actin; MSCs, mesenchymal stem cells.

to constitute an essential TME? And, how can their roles and functional importance be clarified across the broad spectrum of human malignancies, factoring in differences to the histologically distinct stages of tumor development and progression, the molecular genetic subtypes being recognized for many human cancers, and the individual patient to patient variations that are increasingly appreciated? Certainly, there is epidemiological evidence associating abundance of particular stromal cell types—density of neovascularization and abundance of tumor-promoting versus tumor antagonizing IICs—with prognosis in various human cancers (Balkwill and Mantovani, 2011). Beyond epidemiology, the path toward clarification is challenging. One possible approach may involve integration of representative mouse models of particular cancers (GEMM and PDX) with morphology-retaining biopsies and surgical resections from cancer patients: hypotheses and knowledge developed via functional studies in mouse models could be validated by analyzing the primary human samples for predicted determinants indicative of functional correlation. Among the analytic techniques that can be envisioned are (1) advanced histochemical methodology (multicolor immunostaining and in situ RNA hybridization); (2) precise laser capture microdissection of stromal cell types and subtypes populating lesions, facilitated by selective antibody capture, followed by bimolecular analysis, including deep sequencing of mRNA and miRNA; and (3) purification by flow cytometry—also using antibody and other cell surface identifiers—of viable stromal subcell types, followed by cell bioassays and molecular genetic analyses, again leveraging tools and knowledge from the model system(s) to ask if the human lesion manifests similar stromal cells and

functional effectors. Crosstalk and coordinated signaling pathways between neoplastic cells and stromal cell types identified in mouse models seem likely to prove indicative of similar (if not identical) interactions operating in cognate human tumors, but the challenge will be to establish the correlation. Initial glimpses into the power of evaluating human tumor stroma for risk prediction has provided tantalizing information indicating that aspects of the TME significantly correlate with overall survival, as well as response to therapy (Beck et al., 2011; Denardo et al., 2011; Finak et al., 2008). Advancements in noninvasive imaging and analysis of blood-borne tumor-derived material may also prove of value for profiling the constituents of the tumor stroma (Daldrup-Link et al., 2011; Weissleder, 2006). The future challenge is considerable, but the imperative to pursue it is clear, as there is little doubt that the TME and its conscripted stromal cells will prove to be instrumental factors in many human malignancies.

## Prospects and Obstacles for Therapeutic Targeting of Function-Enabling Stromal Cell Types

The demonstrable roles that stromal cells can in principle play in enabling or enhancing multiple hallmark capabilities (Figures 1 and 2) in different TMEs clearly motivates therapeutic targeting strategies aimed to abrogate their contributions. The task, however, will not be easy. A case in point involves antiangiogenic therapy, anticipated for decades as a paradigm-shifting approach to treating human cancer, by abrogating an essential hallmark capability. Potent angiogenic inhibitors have been developed, principally aimed at the VEGF and other proangiogenic signaling pathways. Several such drugs have successfully surpassed the efficacy bar in phase 3 clinical trials, and are consequently approved for use in particular cancer indications, representing a proof of principle that a hallmark-enabling stromal cell type is a valid therapeutic target. The reality check, however, is that clinical responses are typically transitory, and survival benefit limited in duration, indicative of the development of adaptive resistance; the explanation is likely multifactorial, based on preclinical studies in mouse models, which have revealed in some cases evasion of the signaling blockage (Casanovas et al., 2005), in others recruitment of additional or different subtypes of proangiogenic IICs or CAFs (Priceman et al., 2010; Shojaei et al., 2007), and in others shifting to heightened dependence on invasion and metastasis to co-opt normal tissue vasculature instead of producing a neovasculature (Ebos and Kerbel, 2011; Pàez-Ribes et al., 2009; Sennino et al., 2012). While sobering, such results nevertheless suggest solutions: if mechanisms of adaptive-evasive resistance to antiangiogenic therapy that are operative in particular cancer types can be identified and cotargeted, perhaps antiangiogenic therapy in such cancers can be rendered more enduring. There is similar promise, and likely pitfalls, in targeting CAFs and specific IIC subtypes, in regard to the goal of short-circuiting the multiple functional contributions they make to hallmark capabilities. One can anticipate both beneficial effects, and adaptive resistance. In regard to targeting tumor-promoting IICs, there are both encouraging examples (Denardo et al., 2011; Giraudo et al., 2004; Mazzieri et al., 2011; Pietras et al., 2008; Shree et al., 2011), and sobering cases of adaptive resistance, including substitution of a targeted subtype by another with redundant capabilities (Casanovas, 2011; Pahler et al., 2008). Here again, identification of resistance mechanisms may enable combinatorial strategies that counteract adaptive resistance when targeting CAFs and IICs and their functional contributions to hallmark capabilities, improving therapeutic efficacy. Such promise, however, may be qualified by yet another confounding complexity that will likely need to be addressed: individual patient heterogeneity. Thus, it may prove instrumental to factor into the equation individual variations in tumors from different patients. While ostensibly of the same type and histological and/or molecular genetic subtype, individual tumors may nevertheless have profound (and subtle) differences —in cancer cells and likely in the character or abundance of stromal cell (sub)-types that impact critical attributes of the TME, thereby consequently determining the extent of beneficial responses to mechanism-guided therapeutic (co)-targeting; this emerging realization is spawning the frontier of personalized cancer therapy (Haber et al., 2011; Martini et al., 2011). As alluded above, technology development and more routine protocols for informative tumor biopsy may allow the precise constitution of function-enhancing/enabling stromal cell types in a patient's (primary and/or metastatic) TME to be revealed, allowing fine tuning of therapeutic strategies with greater potential for beneficial impact on the disease.

## Conclusions

Cancer medicine is increasingly moving toward a new era of personalized diagnostics and therapeutics that aggressively embraces integrative approaches (De Palma and Hanahan, 2012). Looking forward, combinatorial strategies will target not only cancer cell-intrinsic pathways, but also cancer cell-extrinsic cells, pathways, and mediators at play in the TME. As the strategic goal of deciphering the roles of the TME in primary and metastatic tumor locales progresses, new discoveries can be envisioned to produce innovative multitargeting strategies that will be able to more thoroughly extinguish primary and metastatic disease, while circumventing elucidated adaptive resistance mechanisms to such therapies, profoundly altering the prognosis for many forms of human cancer (De Palma and Hanahan, 2012).

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### REFERENCES

Abraham, S., Zhang, W., Greenberg, N., and Zhang, M. (2003). J. Urol. *169*, 1157–1161.

Armulik, A., Abramsson, A., and Betsholtz, C. (2005). Circ. Res. *97*, 512–523.

Balkwill, F. (2009). Nat. Rev. Cancer *9*, 361–371.

Balkwill, F., Charles, K.A., and Mantovani, A. (2005). Cancer Cell *7*, 211–217.

Balkwill, F.R., and Mantovani, A. (2011). Semin. Cancer Biol.

Balliet, R.M., Capparelli, C., Guido, C., Pestell, T.G., Martinez-Outschoorn, U.E., Lin, Z., Whitaker-Menezes, D., Chiavarina, B., Pestell, R.G., Howell, A., et al. (2011). Cell Cycle *10*, 4065–4073.

Beck, A.H., Sangoi, A.R., Leung, S.M., Marinelli, R.J., Nielsen, T.O., van de Vijver, M.J., West, R.B., van de Rijn, M., and Koller, D. (2011). Sci. Trans. Med. *3*, 108ra113.

Bergers, G., Javaherian, K., Lo, K.M., Folkman, J., and Hanahan, D. (1999). Science *284*, 808–812.

Bergers, G., Brekken, R., McMahon, G., Vu, T.H., Itoh, T., Tamaki, K., Tanzawa, K., Thorpe, P., Itohara, S., Werb, Z., and Hanahan, D. (2000). Nat. Cell Biol. *2*, 737–744.

Bissell, M.J., and Hines, W.C. (2011). Nat. Med. *17*, 320–329.

Bissell, M.J., Hall, H.G., and Parry, G. (1982). J. Theor. Biol. *99*, 31–68.

Biswas, S.K., and Mantovani, A. (2012). Cell Metab., in press.

Bochet, L., Meulle, A., Imbert, S., Salles, B., Valet, P., and Muller, C. (2011). Biochem. Biophys. Res. Commun. *411*, 102–106.

Branco-Price, C., Zhang, N., Schnelle, M., Evans, C., Katschinski, D.M., Liao, D., Ellies, L., and Johnson, R.S. (2012). Cancer Cell *21*, 52–65.

Brem, H., Gresser, I., Grosfeld, J., and Folkman, J. (1993). J. Pediatr. Surg. *28*, 1253–1257.

Butler, J.M., Kobayashi, H., and Rafii, S. (2010). Nat. Rev. Cancer *10*, 138–146.

Calabrese, C., Poppleton, H., Kocak, M., Hogg, T.L., Fuller, C., Hamner, B., Oh, E.Y., Gaber, M.W., Finklestein, D., Allen, M., et al. (2007). Cancer Cell *11*, 69–82.

Carmeliet, P., and Jain, R.K. (2011). Nature *473*, 298–307.

Casanovas, O. (2011). J. Clin. Invest. *121*, 1244–1247.

Casanovas, O., Hicklin, D.J., Bergers, G., and Hanahan, D. (2005). Cancer Cell *8*, 299–309.

Celis, J.E., Moreira, J.M., Cabezón, T., Gromov, P., Friis, E., Rank, F., and Gromova, I. (2005). Mol. Cell. Proteomics *4*, 492–522.

Chaffer, C.L., and Weinberg, R.A. (2011). Science *331*, 1559–1564.

Chen, E.I., and Yates, J.R. (2006). IUBMB Life *58*, 25–29.

Chen, Q., Zhang, X.H., and Massagué, J. (2011). Cancer Cell *20*, 538–549.

Cheng, K., Xie, G., and Raufman, J.P. (2007). Biochem. Pharmacol. *73*, 1001–1012.

Chow, A., Brown, B.D., and Merad, M. (2011). Nat. Rev. Immunol. *11*, 788–798.

Cirri, P., and Chiarugi, P. (2011). Cancer Metastasis Rev. Published online November 11, 2011.

Cooke, V.G., LeBleu, V.S., Keskin, D., Khan, Z., O'Connell, J.T., Teng, Y., Duncan, M.B., Xie, L., Maeda, G., Vong, S., et al. (2012). Cancer Cell *21*, 66–81.

Coussens, L.M., Raymond, W.W., Bergers, G., Laig-Webster, M., Behrendtsen, O., Werb, Z., Caughey, G.H., and Hanahan, D. (1999). Genes Dev. *13*, 1382–1397.

Crawford, Y., Kasman, I., Yu, L., Zhong, C., Wu, X., Modrusan, Z., Kaminker, J., and Ferrara, N. (2009). Cancer Cell *15*, 21–34.

Curiel, T.J., Coukos, G., Zou, L., Alvarez, X., Cheng, P., Mottram, P., Evdemon-Hogan, M., Conejo-Garcia, J.R., Zhang, L., Burow, M., et al. (2004). Nat. Med. *10*, 942–949.

Daenen, L.G., Shaked, Y., Man, S., Xu, P., Voest, E.E., Hoffman, R.M., Chaplin, D.J., and Kerbel, R.S. (2009). Mol. Cancer Ther. *8*, 2872–2881.

Daldrup-Link, H.E., Golovko, D., Ruffell, B., Denardo, D.G., Castaneda, R., Ansari, C., Rao, J., Tikhomirov, G.A., Wendland, M.F., Corot, C., and Coussens, L.M. (2011). Clin. Cancer Res. *17*, 5695–5704.

De Bock, K., Cauwenberghs, S., and Carmeliet, P. (2011). Curr. Opin. Genet. Dev. *21*, 73–79.

De Palma, M., and Coussens, L.M. (2008). Immune cells and inflammatory mediators as regulators of tumor angiogenesis. In Angiogenesis: An Integrative Approach from Science to Medicine, W.D. Figg and J. Folkman, eds. (New York: Springer), pp. 225–238.

De Palma, M., and Hanahan, D. (2012). Mol. Oncol.. 10.1016/j.molonc.2012.01.011.

Denardo, D.G., Brennan, D.J., Rexhepaj, E., Ruffell, B., Shiao, S.L., Madden, S.F., Gallagher, W.M., Wadhwani, N., Keil, S.D., Junaid, S.A., et al. (2011). Cancer Discov *1*, 54–67.

Dirat, B., Bochet, L., Dabek, M., Daviaud, D., Dauvillier, S., Majed, B., Wang, Y.Y., Meulle, A., Salles, B., Le Gonidec, S., et al. (2011). Cancer Res. *71*, 2455–2465.

Doedens, A.L., Stockmann, C., Rubinstein, M.P., Liao, D., Zhang, N., DeNardo, D.G., Coussens, L.M., Karin, M., Goldrath, A.W., and Johnson, R.S. (2010). Cancer Res. *70*, 7465–7475.

Du, R., Lu, K.V., Petritsch, C., Liu, P., Ganss, R., Passegué, E., Song, H., Vandenberg, S., Johnson, R.S., Werb, Z., and Bergers, G. (2008). Cancer Cell *13*, 206–220.

Duda, D.G., Duyverman, A.M., Kohno, M., Snuderl, M., Steller, E.J., Fukumura, D., and Jain, R.K. (2010). Proc. Natl. Acad. Sci. USA *107*, 21677–21682.

Dvorak, H.F. (1986). N. Engl. J. Med. *315*, 1650–1659.

Dvorak, H.F., Weaver, V.M., Tlsty, T.D., and Bergers, G. (2011). J. Surg. Oncol. *103*, 468–474.

Ebos, J.M., and Kerbel, R.S. (2011). Nat Rev Clin Oncol *8*, 210–221.

Erez, N., Truitt, M., Olson, P., Arron, S.T., and Hanahan, D. (2010). Cancer Cell *17*, 135–147.

Ertel, A., Tsirigos, A., Whitaker-Menezes, D., Birbe, R.C., Pavlides, S., Martinez-Outschoorn, U.E., Pestell, R.G., Howell, A., Sotgia, F., and Lisanti, M.P. (2012). Cell Cycle *11*, 253–263.

Ferrara, N., and Alitalo, K. (1999). Nat. Med. *5*, 1359–1364.

Finak, G., Bertos, N., Pepin, F., Sadekova, S., Souleimanova, M., Zhao, H., Chen, H., Omeroglu, G., Meterissian, S., Omeroglu, A., et al. (2008). Nat. Med. *14*, 518–527.

Fischer, C., Jonckx, B., Mazzone, M., Zacchigna, S., Loges, S., Pattarini, L., Chorianopoulos, E., Liesenborghs, L., Koch, M., De Mol, M., et al. (2007). Cell *131*, 463–475.

Fisher, D.T., Chen, Q., Skitzki, J.J., Muhitch, J.B., Zhou, L., Appenheimer, M.M., Vardam, T.D., Weis, E.L., Passanese, J., Wang, W.C., et al. (2011). J. Clin. Invest. *121*, 3846–3859.

Flaberg, E., Markasz, L., Petranyi, G., Stuber, G., Dicso, F., Alchihabi, N., Oláh, E., Csízy, I., Józsa, T., Andrén, O., et al. (2011). Int. J. Cancer *128*, 2793–2802.

Folkman, J. (1974). Adv. Cancer Res. *19*, 331–358.

Folkman, J., Watson, K., Ingber, D., and Hanahan, D. (1989). Nature *339*, 58–61.

Franco, O.E., Shaw, A.K., Strand, D.W., and Hayward, S.W. (2010). Semin. Cell Dev. Biol. *21*, 33–39.

Gabrilovich, D.I., and Nagaraj, S. (2009). Nat. Rev. Immunol. *9*, 162–174.

Giannelli, G., Falk-Marzillier, J., Schiraldi, O., Stetler-Stevenson, W.G., and Quaranta, V. (1997). Science *277*, 225–228.

Giraudo, E., Inoue, M., and Hanahan, D. (2004). J. Clin. Invest. *114*, 623–633.

Gocheva, V., Zeng, W., Ke, D., Klimstra, D., Reinheckel, T., Peters, C., Hanahan, D., and Joyce, J.A. (2006). Genes Dev. *20*, 543–556.

Goel, S., Duda, D.G., Xu, L., Munn, L.L., Boucher, Y., Fukumura, D., and Jain, R.K. (2011). Physiol. Rev. *91*, 1071–1121.

Gorden, D.L., Fingleton, B., Crawford, H.C., Jansen, D.E., Lepage, M., and Matrisian, L.M. (2007). Int. J. Cancer *121*, 495–500.

Guerra, C., Collado, M., Navas, C., Schuhmacher, A.J., Hernández-Porras, I., Cañamero, M., Rodriguez-Justo, M., Serrano, M., and Barbacid, M. (2011). Cancer Cell *19*, 728–739.

Haber, D.A., Gray, N.S., and Baselga, J. (2011). Cell *145*, 19–24.

Hanahan, D., and Folkman, J. (1996). Cell *86*, 353–364.

Hanahan, D., and Weinberg, R.A. (2000). Cell *100*, 57–70.

Hanahan, D., and Weinberg, R.A. (2011). Cell *144*, 646–674.

Hezel, A.F., and Bardeesy, N. (2008). Oncogene *27*, 6908–6919.

Jain, R.K. (2005). Science *307*, 58–62.

Kalluri, R., and Zeisberg, M. (2006). Nat. Rev. Cancer *6*, 392–401.

Kano, M.R., Bae, Y., Iwata, C., Morishita, Y., Yashiro, M., Oka, M., Fujii, T., Komuro, A., Kiyono, K., Kaminishi, M., et al. (2007). Proc. Natl. Acad. Sci. USA *104*, 3460–3465.

Kaplan, R.N., Riba, R.D., Zacharoulis, S., Bramley, A.H., Vincent, L., Costa, C., MacDonald, D.D., Jin, D.K., Shido, K., Kerns, S.A., et al. (2005). Nature *438*, 820–827.

Karnoub, A.E., Dash, A.B., Vo, A.P., Sullivan, A., Brooks, M.W., Bell, G.W., Richardson, A.L., Polyak, K., Tubo, R., and Weinberg, R.A. (2007). Nature *449*, 557–563.

Kashiwagi, S., Izumi, Y., Gohongi, T., Demou, Z.N., Xu, L., Huang, P.L., Buerk, D.G., Munn, L.L., Jain, R.K., and Fukumura, D. (2005). J. Clin. Invest. *115*, 1816–1827.

Kessenbrock, K., Plaks, V., and Werb, Z. (2010). Cell *141*, 52–67.

Kessler, D.A., Langer, R.S., Pless, N.A., and Folkman, J. (1976). Int. J. Cancer *18*, 703–709.

Khandekar, M.J., Cohen, P., and Spiegelman, B.M. (2011). Nat. Rev. Cancer *11*, 886–895.

Khazaie, K., Blatner, N.R., Khan, M.W., Gounari, F., Gounaris, E., Dennis, K., Bonertz, A., Tsai, F.N., Strouch, M.J., Cheon, E., et al. (2011). Cancer Metastasis Rev. *30*, 45–60.

Kidd, S., Spaeth, E., Dembinski, J.L., Dietrich, M., Watson, K., Klopp, A., Battula, V.L., Weil, M., Andreeff, M., and Marini, F.C. (2009). Stem Cells *27*, 2614–2623.

Korkaya, H., Liu, S., and Wicha, M.S. (2011). J. Clin. Invest. *121*, 3804–3809.

Kryczek, I., Zou, L., Rodriguez, P., Zhu, G., Wei, S., Mottram, P., Brumlik, M., Cheng, P., Curiel, T., Myers, L., et al. (2006). J. Exp. Med. *203*, 871–881.

Kuang, D.M., Zhao, Q., Peng, C., Xu, J., Zhang, J.P., Wu, C., and Zheng, L. (2009). J. Exp. Med. *206*, 1327–1337.

Labelle, M., Begum, S., and Hynes, R.O. (2011). Cancer Cell *20*, 576–590.

Leek, R.D., Hunt, N.C., Landers, R.J., Lewis, C.E., Royds, J.A., and Harris, A.L. (2000). J. Pathol. *190*, 430–436.

Levental, K.R., Yu, H., Kass, L., Lakins, J.N., Egeblad, M., Erler, J.T., Fong, S.F., Csiszar, K., Giaccia, A., Weninger, W., et al. (2009). Cell *139*, 891–906.

Lewis, C.E., and Pollard, J.W. (2006). Cancer Res. *66*, 605–612.

Lin, E.Y., Li, J.F., Bricard, G., Wang, W., Deng, Y., Sellers, R., Porcelli, S.A., and Pollard, J.W. (2007). Mol. Oncol. *1*, 288–302.

Liu, S., Ginestier, C., Ou, S.J., Clouthier, S.G., Patel, S.H., Monville, F., Korkaya, H., Heath, A., Dutcher, J., Kleer, C.G., et al. (2011). Cancer Res. *71*, 614–624.

Loeffler, M., Krüger, J.A., Niethammer, A.G., and Reisfeld, R.A. (2006). J. Clin. Invest. *116*, 1955–1962.

Lu, P., Takai, K., Weaver, V.M., and Werb, Z. (2011a). Cold Spring Harb. Perspect. Biol. *3*. 10.1101/cshperspect.a005058.

Lu, T., Ramakrishnan, R., Altiok, S., Youn, J.I., Cheng, P., Celis, E., Pisarev, V., Sherman, S., Sporn, M.B., and Gabrilovich, D. (2011b). J. Clin. Invest. *121*, 4015–4029.

Lu, X., Mu, E., Wei, Y., Riethdorf, S., Yang, Q., Yuan, M., Yan, J., Hua, Y., Tiede, B.J., Lu, X., et al. (2011c). Cancer Cell *20*, 701–714.

Luo, J.L., Tan, W., Ricono, J.M., Korchynskyi, O., Zhang, M., Gonias, S.L., Cheresh, D.A., and Karin, M. (2007). Nature *446*, 690–694.

Lyden, D., Hattori, K., Dias, S., Costa, C., Blaikie, P., Butros, L., Chadburn, A., Heissig, B., Marks, W., Witte, L., et al. (2001). Nat. Med. *7*, 1194–1201.

Lynch, C.C., Hikosaka, A., Acuff, H.B., Martin, M.D., Kawai, N., Singh, R.K., Vargo-Gogola, T.C., Begtrup, J.L., Peterson, T.E., Fingleton, B., et al. (2005). Cancer Cell *7*, 485–496.

Malanchi, I., Santamaria-Martínez, A., Susanto, E., Peng, H., Lehr, H.A., Delaloye, J.F., and Huelsken, J. (2012). Nature *481*, 85–89.

Mantovani, A., Allavena, P., Sica, A., and Balkwill, F. (2008). Nature *454*, 436–444.

Mantovani, A., Cassatella, M.A., Costantini, C., and Jaillon, S. (2011). Nat. Rev. Immunol. *11*, 519–531.

Manzur, M., Hamzah, J., and Ganss, R. (2008). Cell Cycle *7*, 2452–2455.

Martinez-Outschoorn, U.E., Sotgia, F., and Lisanti, M.P. (2012). Cell Metab. *15*, 4–5.

Martini, M., Vecchione, L., Siena, S., Tejpar, S., and Bardelli, A. (2011). Nat. Rev. Clin. Oncol. *9*, 87–97.

Mazzieri, R., Pucci, F., Moi, D., Zonari, E., Ranghetti, A., Berti, A., Politi, L.S., Gentner, B., Brown, J.L., Naldini, L., and De Palma, M. (2011). Cancer Cell *19*, 512–526.

McDonald, D.M., and Choyke, P.L. (2003). Nat. Med. *9*, 713–725.

Mohamed, M.M., and Sloane, B.F. (2006). Nat. Rev. Cancer *6*, 764–775.

Molon, B., Ugel, S., Del Pozzo, F., Soldani, C., Zilio, S., Avella, D., De Palma, A., Mauri, P., Monegal, A., Rescigno, M., et al. (2011). J. Exp. Med. *208*, 1949–1962.

Morikawa, S., Baluk, P., Kaidoh, T., Haskell, A., Jain, R.K., and McDonald, D.M. (2002). Am. J. Pathol. *160*, 985–1000.

Motzer, R.J., Michaelson, M.D., Redman, B.G., Hudes, G.R., Wilding, G., Figlin, R.A., Ginsberg, M.S., Kim, S.T., Baum, C.M., DePrimo, S.E., et al. (2006). J. Clin. Oncol. *24*, 16–24.

Movahedi, K., Laoui, D., Gysemans, C., Baeten, M., Stangé, G., Van den Bossche, J., Mack, M., Pipeleers, D., In't Veld, P., De Baetselier, P., and Van Ginderachter, J.A. (2010). Cancer Res. *70*, 5728–5739.

Nieman, K.M., Kenny, H.A., Penicka, C.V., Ladanyi, A., Buell-Gutbrod, R., Zillhardt, M.R., Romero, I.L., Carey, M.S., Mills, G.B., Hotamisligil, G.S., et al. (2011). Nat. Med. *17*, 1498–1503.

Nozawa, H., Chiu, C., and Hanahan, D. (2006). Proc. Natl. Acad. Sci. USA *103*, 12493–12498.

Oguma, K., Oshima, H., Aoki, M., Uchio, R., Naka, K., Nakamura, S., Hirao, A., Saya, H., Taketo, M.M., and Oshima, M. (2008). EMBO J. *27*, 1671–1681.

Ojalvo, L.S., Whittaker, C.A., Condeelis, J.S., and Pollard, J.W. (2010). J. Immunol. *184*, 702–712.

Olive, K.P., Jacobetz, M.A., Davidson, C.J., Gopinathan, A., McIntyre, D., Honess, D., Madhu, B., Goldgraben, M.A., Caldwell, M.E., Allard, D., et al. (2009). Science *324*, 1457–1461.

Onrust, S.V., Hartl, P.M., Rosen, S.D., and Hanahan, D. (1996). J. Clin. Invest. *97*, 54–64.

Orimo, A., and Weinberg, R.A. (2007). Cancer Biol. Ther. *6*, 618–619.

Orimo, A., Gupta, P.B., Sgroi, D.C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., Carey, V.J., Richardson, A.L., and Weinberg, R.A. (2005). Cell *121*, 335–348.

Ostrand-Rosenberg, S. (2008). Curr. Opin. Genet. Dev. *18*, 11–18.

Pàez-Ribes, M., Allen, E., Hudock, J., Takeda, T., Okuyama, H., Viñals, F., Inoue, M., Bergers, G., Hanahan, D., and Casanovas, O. (2009). Cancer Cell *15*, 220–231.

Pahler, J.C., Tazzyman, S., Erez, N., Chen, Y.Y., Murdoch, C., Nozawa, H., Lewis, C.E., and Hanahan, D. (2008). Neoplasia *10*, 329–340.

Parangi, S., O'Reilly, M., Christofori, G., Holmgren, L., Grosfeld, J., Folkman, J., and Hanahan, D. (1996). Proc. Natl. Acad. Sci. USA *93*, 2002–2007.

Partanen, J.I., Nieminen, A.I., and Klefstrom, J. (2009). Cell Cycle *8*, 716–724.

Paunescu, V., Bojin, F.M., Tatu, C.A., Gavriliuc, O.I., Rosca, A., Gruia, A.T., Tanasie, G., Bunu, C., Crisnic, D., Gherghiceanu, M., et al. (2011). J. Cell. Mol. Med. *15*, 635–646.

Pietras, K., and Ostman, A. (2010). Exp. Cell Res. *316*, 1324–1331.

Pietras, K., Ostman, A., Sjöquist, M., Buchdunger, E., Reed, R.K., Heldin, C.H., and Rubin, K. (2001). Cancer Res. *61*, 2929–2934.

Pietras, K., Pahler, J., Bergers, G., and Hanahan, D. (2008). PLoS Med. *5*, e19.

Pirilä, E., Ramamurthy, N.S., Sorsa, T., Salo, T., Hietanen, J., and Maisi, P. (2003). Dig. Dis. Sci. *48*, 93–98.

Pontiggia, O., Sampayo, R., Raffo, D., Motter, A., Xu, R., Bissell, M.J., de Kier Joffé, E.B., and Simian, M. (2011). Breast Cancer Res. Treat. Published online September 21, 2011.

Porta, C., Riboldi, E., Totaro, M.G., Strauss, L., Sica, A., and Mantovani, A. (2011). Immunotherapy *3*, 1185–1202.

Priceman, S.J., Sung, J.L., Shaposhnik, Z., Burton, J.B., Torres-Collado, A.X., Moughon, D.L., Johnson, M., Lusis, A.J., Cohen, D.A., Iruela-Arispe, M.L., and Wu, L. (2010). Blood *115*, 1461–1471.

Provenzano, P.P., Cuevas, C., Chang, A.E., Goel, V.K., Von Hoff, D.D., and Hingorani, S.R. (2012). Cancer Cell, in press.

Psaila, B., and Lyden, D. (2009). Nat. Rev. Cancer *9*, 285–293.

Qian, B.Z., and Pollard, J.W. (2010). Cell *141*, 39–51.

Räsänen, K., and Vaheri, A. (2010). Exp. Cell Res. *316*, 2713–2722.

Rattigan, Y.I., Patel, B.B., Ackerstaff, E., Sukenick, G., Koutcher, J.A., Glod, J.W., and Banerjee, D. (2012). Exp. Cell Res. *318*, 326–335.

Rolny, C., Mazzone, M., Tugues, S., Laoui, D., Johansson, I., Coulon, C., Squadrito, M.L., Segura, I., Li, X., Knevels, E., et al. (2011). Cancer Cell *19*, 31–44.

Rosen, E.D., and MacDougald, O.A. (2006). Nat. Rev. Mol. Cell Biol. *7*, 885–896.

Ruffell, B., DeNardo, D.G., Affara, N.I., and Coussens, L.M. (2010). Cytokine Growth Factor Rev. *21*, 3–10.

Ruffell, B., Au, A., Rugo, H.S., Esserman, L.J., Hwang, E.S., and Coussens, L.M. (2011). Proc. Natl. Acad. Sci. USA. *109*, 2796–2801.

Sabrkhany, S., Griffioen, A.W., and Oude Egbrink, M.G. (2011). Biochim. Biophys. Acta *1815*, 189–196.

Sager, R., Sheng, S., Pemberton, P., and Hendrix, M.J. (1997). Adv. Exp. Med. Biol. *425*, 77–88.

Senger, D.R., Galli, S.J., Dvorak, A.M., Perruzzi, C.A., Harvey, V.S., and Dvorak, H.F. (1983). Science *219*, 983–985.

Sennino, B., Ishiguro-Oonuma, T., Wei, Y., Naylor, R.N., Williamson, C.W., Bhagwandin, V., Tabruyn, S.P., You, W.-K., Chapman, H.A., Christensen, J.G., et al. (2012). Cancer Discovery. Published online February 24, 2012. 10.1158/2159-8290.

Shaheen, R.M., Davis, D.W., Liu, W., Zebrowski, B.K., Wilson, M.R., Bucana, C.D., McConkey, D.J., McMahon, G., and Ellis, L.M. (1999). Cancer Res. *59*, 5412–5416.

Shields, J.D., Kourtis, I.C., Tomei, A.A., Roberts, J.M., and Swartz, M.A. (2010). Science *328*, 749–752.

Shojaei, F., Wu, X., Malik, A.K., Zhong, C., Baldwin, M.E., Schanz, S., Fuh, G., Gerber, H.P., and Ferrara, N. (2007). Nat. Biotechnol. *25*, 911–920.

Shree, T., Olson, O.C., Elie, B.T., Kester, J.C., Garfall, A.L., Simpson, K., Bell-McGuinn, K.M., Zabor, E.C., Brogi, E., and Joyce, J.A. (2011). Genes Dev. *25*, 2465–2479.

Sotgia, F., Martinez-Outschoorn, U.E., Pavlides, S., Howell, A., Pestell, R.G., and Lisanti, M.P. (2011). Breast Cancer Res. *13*, 213.

Sotgia, F., Martinez-Outschoorn, U.E., Howell, A., Pestell, R.G., Pavlides, S., and Lisanti, M.P. (2012). Annu. Rev. Pathol. *7*, 423–467.

Soucek, L., Lawlor, E.R., Soto, D., Shchors, K., Swigart, L.B., and Evan, G.I. (2007). Nat. Med. *13*, 1211–1218.

Sounni, N.E., Dehne, K., van Kempan, L.C.L., Egeblad, M., Affara, N.I., Cuevas, I., Wiesen, J., Junankar, S., Korets, L.V., Lee, J., et al. (2010). Dis. Model Mech. *3*, 317–332.

Spaeth, E., Klopp, A., Dembinski, J., Andreeff, M., and Marini, F. (2008). Gene Ther. *15*, 730–738.

Spaeth, E.L., Dembinski, J.L., Sasser, A.K., Watson, K., Klopp, A., Hall, B., Andreeff, M., and Marini, F. (2009). PLoS ONE *4*, e4992.

Stockmann, C., Doedens, A., Weidemann, A., Zhang, N., Takeda, N., Greenberg, J.I., Cheresh, D.A., and Johnson, R.S. (2008). Nature *456*, 814–818.

Stover, D.G., Bierie, B., and Moses, H.L. (2007). J. Cell. Biochem. *101*, 851–861.

Takeda, N., O'Dea, E.L., Doedens, A., Kim, J.W., Weidemann, A., Stockmann, C., Asagiri, M., Simon, M.C., Hoffmann, A., and Johnson, R.S. (2010). Genes Dev. *24*, 491–501.

Tlsty, T.D., and Coussens, L.M. (2006). Annu. Rev. Pathol. *1*, 119–150.

Topalian, S.L., Drake, C.G., and Pardoll, D.M. (2012). Curr. Opin. Immunol. Published online January 12, 2012.

Trinchieri, G. (2011). Annu. Rev. Immunol. Published online March 24, 2011.

van der Vliet, H.J., Koon, H.B., Atkins, M.B., Balk, S.P., and Exley, M.A. (2007). J. Immunother. *30*, 591–595.

van Kempen, L.C., de Visser, K.E., and Coussens, L.M. (2006). Eur. J. Cancer *42*, 728–734.

Vasiljeva, O., Papazoglou, A., Krüger, A., Brodoefel, H., Korovin, M., Deussing, J., Augustin, N., Nielsen, B.S., Almholt, K., Bogyo, M., et al. (2006). Cancer Res. *66*, 5242–5250.

Vong, S., and Kalluri, R. (2011). The role of stromal myofibroblast and extracellular matrix in tumor angiogenesis. Genes Cancer. Published online October 5, 2011.

Wang, F., Sloss, C., Zhang, X., Lee, S.W., and Cusack, J.C. (2007a). Cancer Res. *67*, 8486–8493.

Wang, W., Wyckoff, J.B., Goswami, S., Wang, Y., Sidani, M., Segall, J.E., and Condeelis, J.S. (2007b). Cancer Res. *67*, 3505–3511.

Wasiuk, A., de Vries, V.C., Hartmann, K., Roers, A., and Noelle, R.J. (2009). Clin. Exp. Immunol. *155*, 140–146.

Weissleder, R. (2006). Science *312*, 1168–1171.

Willett, C.G., Boucher, Y., Duda, D.G., di Tomaso, E., Munn, L.L., Tong, R.T., Kozin, S.V., Petit, L., Jain, R.K., Chung, D.C., et al. (2005). J. Clin. Oncol. *23*, 8136–8139.

Wyckoff, J., Wang, W., Lin, E.Y., Wang, Y., Pixley, F., Stanley, E.R., Graf, T., Pollard, J.W., Segall, J., and Condeelis, J. (2004). Cancer Res. *64*, 7022–7029.

Xian, X., Håkansson, J., Ståhlberg, A., Lindblom, P., Betsholtz, C., Gerhardt, H., and Semb, H. (2006). J. Clin. Invest. *116*, 642–651.

Xu, R., Boudreau, A., and Bissell, M.J. (2009). Cancer Metastasis Rev. *28*, 167–176.

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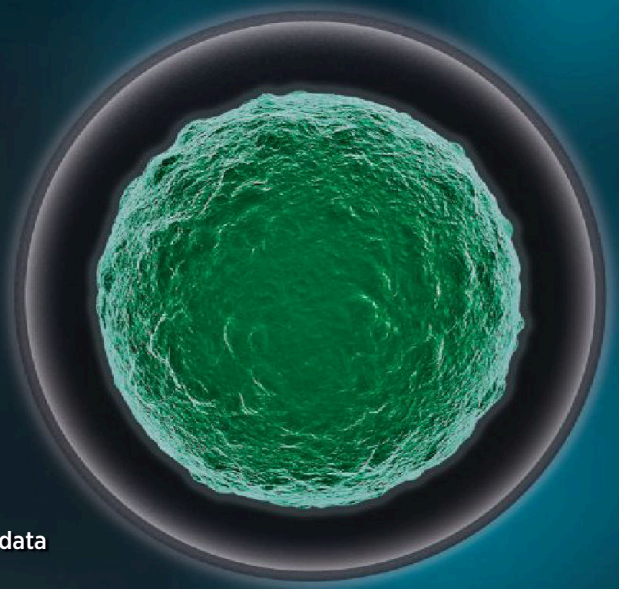

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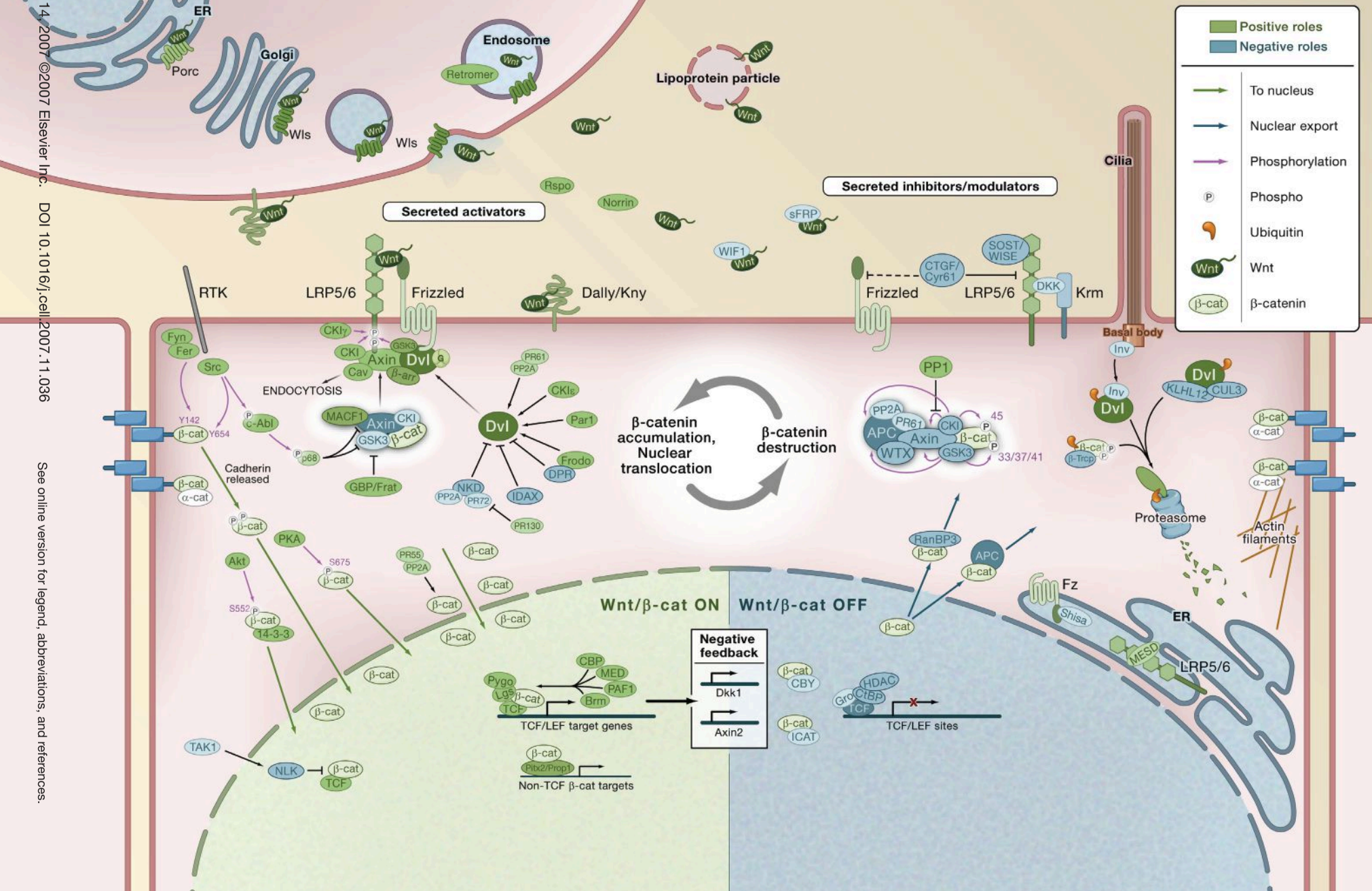
# SnapShot: Wnt/β-Catenin Signaling

Bryan T. MacDonald, Mikhail V. Semenov, and Xi He
The F.M. Kirby Neurobiology Center, Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA

Cell

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See online version for legend, abbreviations, and references.

# SnapShot: Wnt/β-Catenin Signaling

Cell

Bryan T. MacDonald, Mikhail V. Semenov, and Xi He
The F.M. Kirby Neurobiology Center, Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA

Molecules labeled in green and blue play positive and negative roles in Wnt/β-catenin signaling, respectively. Molecules that are labeled in both colors have dual roles.
(Top left) Wnt biogenesis. A lipid modification is added to Wnt ligands by Porc in the endoplasmic reticulum (ER). Wnt ligands are glycosylated in the ER and Golgi and require Wls (also known as Evi) to traffic through the Golgi to the plasma membrane. The retromer complex is also important for Wnt secretion, particularly for long-range signaling. Mature Wnt ligands may interact with lipoprotein particles. Proteoglycans Dally and Kny/Glypican may also facilitate Wnt distribution.
(Bottom right) Wnt receptor biogenesis. In cells that respond to Wnt the ER protein MESD is required for folding and trafficking of the Wnt receptor LRP5/6 to the plasma membrane, and the ER protein Shisa prevents folding and trafficking of the Fz protein to the plasma membrane.
(Right) Wnt/β-catenin signaling OFF. sFRP and WIF1 directly bind Wnt ligands and prevent Wnts from binding to receptors. SOST/WISE, CTGF/Cyr61 bind to LRP6 (CTGF may also bind to Fz) to prevent the formation of the Wnt-Fz-LRP5/6 receptor complex. DKK binds to and inhibits LRP5/6 in cooperation with the KRM receptor. In the absence of Wnt signaling, the scaffolding protein Axin and tumor suppressor APC form a β-catenin destruction complex that binds cytosolic β-catenin and facilitates sequential phosphorylation of β-catenin by CK1 (at S45) and GSK3 (at S33/S37/T41). The tumor suppressor WTX may also reside in this complex. Phosphorylated β-catenin is recognized by β-Trcp and ubiquitinated for degradation by the proteasome. In the nucleus, TCF assembles a transcriptional repressor complex to silence Wnt target genes via recruiting Gro, CtBP, and HDACs. Residual β-catenin is exported from the nucleus by RanBP3 and APC or bound by CBY or ICAT that prevents β-catenin association with TCF/LEF.
(Left) Wnt/β-catenin signaling ON. The Wnt ligand binds to Fz and LRP5/6 receptors to form a Fz-LRP5/6 complex. Dally and Kny can also bind Wnt and enrich Wnt concentration locally or help Wnt gradient distribution. Rspo proteins and Norrin are secreted agonists that bind to LRP5/6 and/or Fz to activate Wnt/β-catenin signaling. Formation of the Fz-LRP6 complex via Dvl promotes LRP6 phosphorylation by GSK3 and CK1γ and other CK1. Fz binds Dvl and phosphorylated LRP6 recruits Axin to the plasma membrane, resulting in inhibition of β-catenin phosphorylation/degradation by an as yet unknown mechanism. Fz-LRP6-Dvl aggregation may be involved. LRP6 association with caveolin may promote its endocytosis and signaling. Translocation of Axin is facilitated by MACF1. Trimeric G proteins may act between Fz and Dvl, and β-arrestin may associate with Dvl and Axin. Stabilized β-catenin is translocated to the nucleus where it binds TCF/LEF and recruits coactivators such as the Lgs/Pygo complex, CBP/p300, Brahma, MED12/Mediator, and the PAF1/Hyrax complex to initiate RNA transcription and elongation. TCF/β-catenin controls the expression of many genes that affect cell proliferation (i.e., *c-myc*, *cyclin D1*) and differentiation and also the expression of Wnt signaling inhibitory proteins (i.e., Dkk1, Axin2) that act as a negative feedback loop. In addition to TCF/LEF, β-catenin also binds to other transcription factors such as Prop1 and Pitx2 to coactivate non-TCF/LEF target genes.

### Other regulatory molecules

(1) Stimulatory roles: Dvl phosphorylation by CK1ε and Par1 isoforms; GSK3 bindng by GBP/FRAT; Axin dephosphorylation by PP1, which prevents Axin-GSK3 binding.
(2) Inhibitory roles: Dvl binding by NKD and IDAX; Dvl degradation by Inv and by the KLHL12-CUL3 complex. The primary cilia may suppress Wnt/β-catenin signaling via Inv-mediated Dvl degradation and other mechanisms. PP2A may have both stimulatory and inhibitory roles at different steps of the Wnt/β-catenin pathway depending on the regulatory subunits (PR55, PR61, PR72, and PR130). Frodo/DPR binds to Dsh/Dvl and may have stimulatory and inhibitory roles.

### Other signaling pathways that affect β-catenin signaling

PKA can phosphorylate β-catenin at S675 and inhibit β-catenin degradation; Akt/PKB can phosphorylate β-catenin at S552 and promote β-catenin nuclear localization. At the adherens junction, β-catenin binds to cadherin to coordinate cell adhesion with actin cytoskeleton through α-catenin. Plakoglobin/γ-catenin (not shown) is closely related to β-catenin although its primary function appears to be at the adherens junction. RTK signaling phosphorylates cadherin-bound β-catenin at Y142 and Y654, via RTK and/or c-Src/Fyn/Fer, to promote dissociation of β-catenin from cadherin, resulting in an increase in cytosolic β-catenin and TCF/β-catenin signaling. RTKs (such as the PDGF receptor) can directly regulate the Axin complex via c-Abl-mediated phosphorylation of p68 (an RNA helicase), which displaces β-catenin from Axin and promotes β-catenin nuclear accumulation/signaling. TCF may be phosphorylated by NLK, which is activated by TAK1, to prevent TCF/β-catenin from binding to DNA.

### Abbreviations

Akt/PKB, protein kinase B; APC, *adenomatous polyposis coli* gene product; α-cat, α-catenin/CTNNA; β-cat, β-catenin/CTNNB1; β-Trcp, β-tranducin repeat containing protein/FBXW1; Brm, Brahma/Brg-1; CBP, CREB-binding protein; CBY, chibby; CDH, cadherin; CK1, casein kinase 1; CtBP, C-terminal binding protein; CTGF, connective tissue growth factor; CUL3, Cullin3; Cyr61/CCN1; Dally, division abnormally delayed; DKK, dickkopf; DPR, dapper/Frodo; DVl, dishevelled; sFRP, secreted frizzled related protein; Evi, evenness interrupted; Fer, FPS/FES-related tyrosine kinase; Fyn, FYN tyrosine kinase; Fz/FZD, frizzled receptor; G, G protein αβγ subunits; GBP, GSK3-binding protein/Frat, frequently rearranged in advanced T cell lymphomas; Gro, groucho/TLE, transducin-like enhancer of split; GSK3, glycogen synthase kinase 3; HDAC, histone deacetylase; ICAT, inhibitor of β-catenin; IDAX/CXXC4, inhibition of Dvl/Axin complex; Inv, Inversin; KRM, kremen; LEF, lymphoid enhancer-binding factor; Lgs, Legless/Bcl9 (B cell lymphoma 9); LRP5/6, low-density lipoprotein-related receptor protein 5 or 6; MACF1, microtubule actin crosslinking factor 1; MED12, mediator subunit 12; MESD, *mesodermal development* gene product/Boca; NKD, naked cuticle; NLK, NEMO-like kinase; Norrin, Norrin disease protein; PAF1, parafibromin/hyrax; PAR1, par-1 kinase; PKA, protein kinase A; Porc, porcupine; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PR, protein phosphatase 2A regulatory unit; Pygo, pygopus; RanBP3, Ran-binding protein 3; Rspo, R-spondin; SOST, sclerostin; TAK1, TGF beta-activated kinase 1; TCF, T cell factor; WIF, Wnt inhibitory factor; Wls, Wntless.

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### REFERENCES

Brembeck, F.H., Rosario, M., and Birchmeier, W. (2006). Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. Curr. Opin. Genet. Dev. *16*, 51–59.

He, X., Semenov, M., Tamai, K., and Zeng, X. (2004). LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: Arrows point the way. Development *131*, 1663–1677.

Kimelman, D., and Xu, W. (2006). beta-catenin destruction complex: Insights and questions from a structural perspective. Oncogene *25*, 7482–7491.

Logan, C.Y., and Nusse, R. (2004). The Wnt signaling pathway in development and disease. Annu. Rev. Cell Dev. Biol. *20*, 781–810.

Luo, W., Peterson, A., Garcia, B.A., Coombs, G., Kofahl, B., Heinrich, R., Shabanowitz, J., Hunt, D.F., Yost, H.J., and Virshup, D.M. (2007). Protein phosphatase 1 regulates assembly and function of the beta-catenin degradation complex. EMBO J. *26*, 1511–1521.

Major, M.B., Camp, N.D., Berndt, J.D., Yi, X., Goldenberg, S.J., Hubbert, C., Biechele, T.L., Gingras, A.C., Zheng, N., Maccoss, M.J., et al. (2007). Wilms tumor suppressor WTX negatively regulates WNT/beta-catenin signaling. Science *316*, 1043–1046.

Romond, T., Metcalfe, C., and Bienz, M. (2007). Dynamic recruitment of Axin by Dishevelled protein assemblies. J. Cell Sci. *120*, 2402–2412.

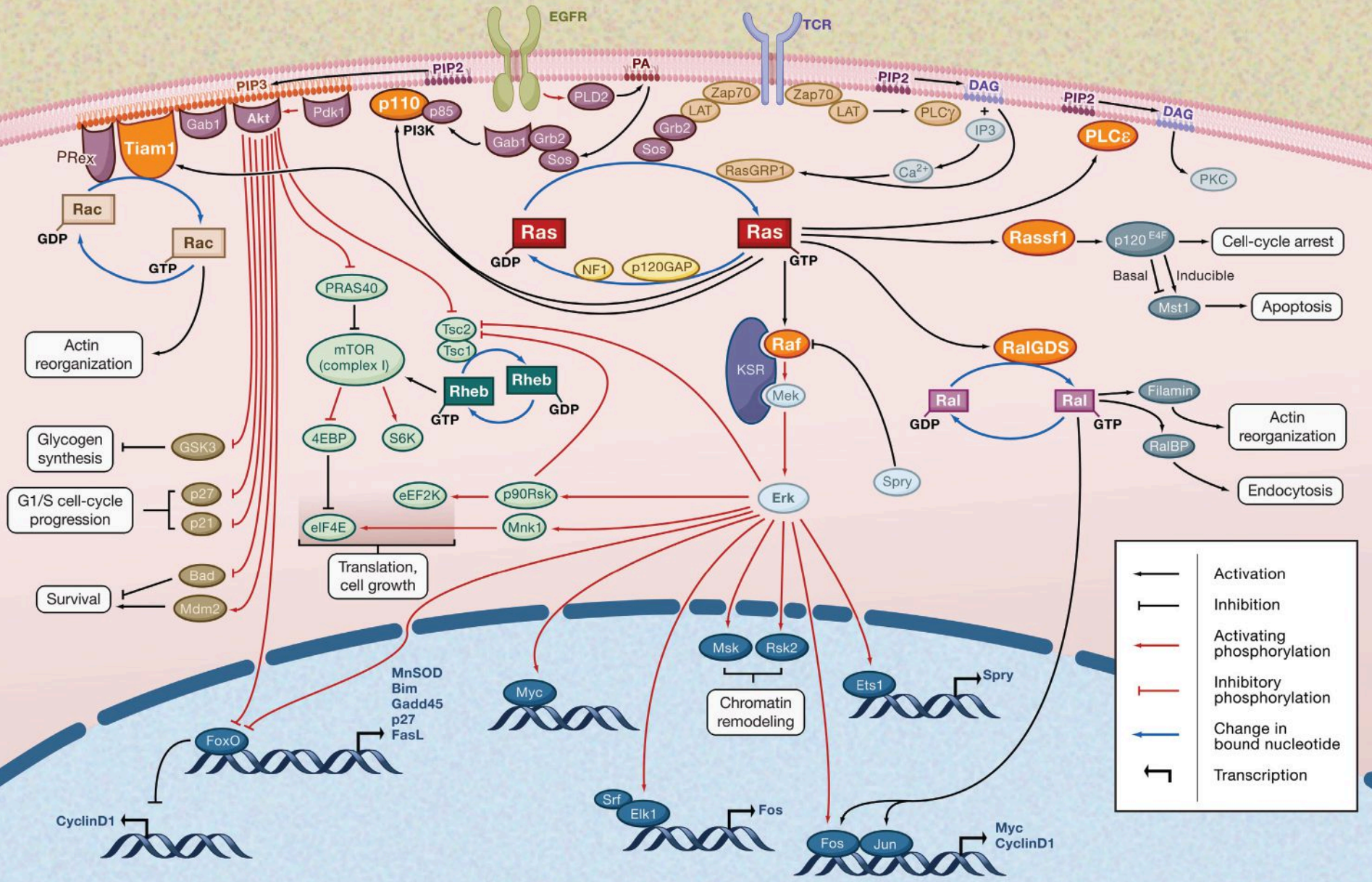
Simons, M., Gloy, J., Ganner, A., Bullerkotte, A., Bashkurov, M., Krönig, C., Schermer, B., Benzing, T., Cabello, O.A., Jenny, A., et al. (2005). Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. Nat. Genet. *37*, 537–543.

Stadeli, R., Hoffmans, R., and Basler, K. (2006). Transcription under the control of nuclear Arm/beta-catenin. Curr. Biol. *16*, R378–R385.

# SnapShot: Ras Signaling

Megan Cully and Julian Downward
Cancer Research UK London Research Institute, London WC2A 3PX, UK

Cell

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# SnapShot: Ras Signaling

Megan Cully and Julian Downward
Cancer Research UK London Research Institute, London WC2A 3PX, UK

Cell

Ras is a monomeric membrane-associated GTP-binding protein that regulates cell proliferation and survival in response to extracellular stimuli, such as activation of epidermal growth factor receptor (EGFR) or T cell receptor (TCR). Originally identified as an oncogene in murine sarcoma viruses, activating mutations in Ras have been found in about 30% of human tumors. Dysregulation of the Ras signaling pathway plays a key role in the progression of cancer. When bound to GTP, Ras is active and stimulates several downstream targets by direct interactions. Ras has intrinsic GTPase activity, which can be activated by GTPase-activating proteins (GAPs) such as the *NF1* tumor suppressor gene product and $p120^{GAP}$. Activation of Ras occurs largely through guanine nucleotide exchange factors (GEFs) such as Sos and RasGRP1, which catalyze the exchange of Ras-bound GDP with free GTP. Multiple downstream effector pathways mediate Ras signaling, including the Raf/MEK/ERK kinase cascade, the PI 3-kinase/Akt/mTor pathway, and the Ral GTPase pathway. Raf/MEK/ERK is the prototypical mitogen-activated protein kinase (MAPK) cascade, wherein each kinase phosphorylates and activates its downstream target, culminating in the activation of multiple targets including several transcription factors. Much of the recent work on the MAPK cascade has focused on negative feedback loops, such as the phosphorylation of Raf and Sos by ERK, as well as the transcription of negative regulators such as Sprouty and Spred. The second of the major downstream Ras targets, the PI 3-kinase/Akt/mTor pathway, contributes to cell growth, proliferation, and survival downstream of Ras. PI 3-kinase is a lipid kinase that phosphorylates phosphatidylinositol (4,5) bisphosphate ($PIP_2$) to generate the second messenger phosphatidylinositol (3,4,5) trisphosphate ($PIP_3$). $PIP_3$ activates Akt (PKB) and also GEFs for Rac GTPases, which regulate the actin cytoskeleton. Finally, Ras is able to activate another GTPase, Ral, through stimulation of the RalGDS family of guanine nucleotide exchange factors. Activation of Ral results in increased endocytosis and activation of the transcription factors Jun and Fos.

## REFERENCES

Buday, L., and Downward, J. (2008). Many faces of Ras activation. Biochim. Biophys. Acta Published online March 21, 2008. 10.1016/j.bbcan.2008.05.001.

Bunney, T.D., and Katan, M. (2006). Phospholipase C epsilon: linking second messengers and small GTPases. Trends Cell Biol. *16*, 640–648.

Kolch, W. (2005). Coordinating ERK/MAPK signalling through scaffolds and inhibitors. Nat. Rev. Mol. Cell Biol. *6*, 827–837.

Malumbres, M., and Barbacid, M. (2003). RAS oncogenes: the first 30 years. Nat. Rev. Cancer *3*, 459–465.

Mason, J.M., Morrison, D.J., Basson, M.A., and Licht, J.D. (2006). Sprouty proteins: multifaceted negative-feedback regulators of receptor tyrosine kinase signaling. Trends Cell Biol. *16*, 45–54.

Mitin, N., Rossman, K.L., and Der, C.J. (2005). Signaling interplay in Ras superfamily function. Curr. Biol. *15*, R563–R574.

Mor, A., Dustin, M.L., and Philips, M.R. (2007). Small GTPases and LFA-1 reciprocally modulate adhesion and signaling. Immunol. Rev. *218*, 114–125.

Murphy, L.O., and Blenis, J. (2006). MAPK signal specificity: the right place at the right time. Trends Biochem. Sci. *31*, 268–275.

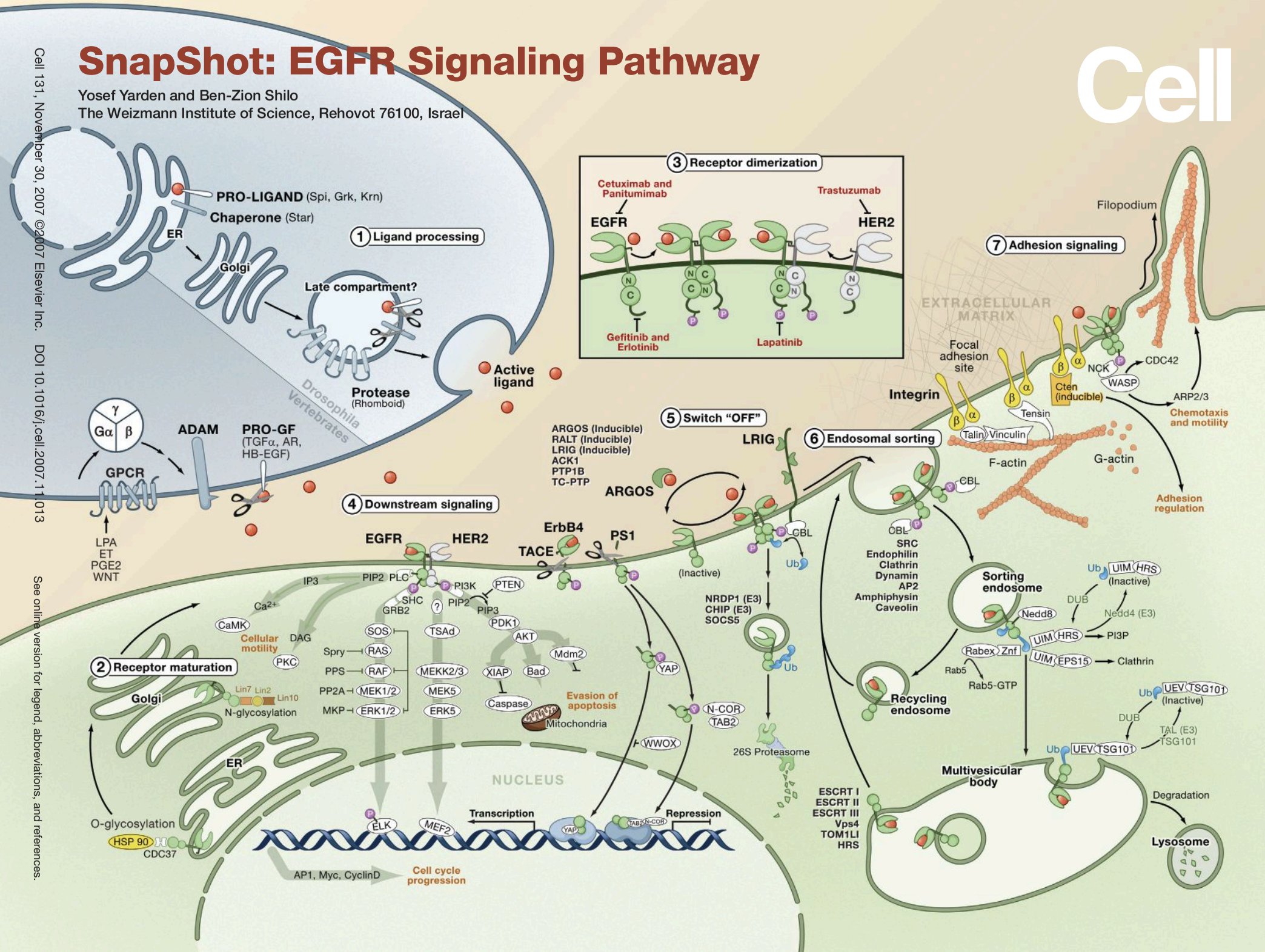
Sabatini, D.M. (2006). mTOR and cancer: insights into a complex relationship. Nat. Rev. Cancer *6*, 729–734.

Shaw, R.J., and Cantley, L.C. (2006). Ras, PI(3)K and mTOR signalling controls tumour cell growth. Nature *441*, 424–430.

# SnapShot: EGFR Signaling Pathway

Yosef Yarden and Ben-Zion Shilo
The Weizmann Institute of Science, Rehovot 76100, Israel

Cell

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See online version for legend, abbreviations, and references.

# SnapShot: EGFR Signaling Pathway

Cell

Yosef Yarden and Ben-Zion Shilo
The Weizmann Institute of Science, Rehovot 76100, Israel

The epidermal growth factor receptor (EGFR/ErbB) pathway plays pivotal roles in cell-cell communication in both vertebrate and invertebrates. In *Drosophila* and *C. elegans* the EGFR pathway participates in the determination of numerous cell fates, including the development of the compound eye and the vulva. The four EGFR orthologs in vertebrates form a layered signaling network that participates in specification of cell fate and coordinates cell proliferation. Mutations in components of the pathway are commonly involved in human cancer.

(1) Ligand processing: Different mechanisms are employed in vertebrates and invertebrates for processing the ligand in the signal-producing cell to generate the secreted, active form. In *Drosophila*, the ligand precursor for Spi, Grk, or Krn is retained in the ER. It associates with the chaperone Star and is trafficked to a late compartment where the intramembrane protease Rhomboid resides, cleaving the ligand to generate the secreted form. Rhomboid also cleaves the chaperone Star, thus attenuating the level of ligand that is trafficked. In vertebrates, the ligand precursors are trafficked to the plasma membrane, where they are cleaved by ADAM metalloproteases.

(2) Receptor maturation: The nascent forms of EGFR and its sibling, HER2, associate in the ER with a complex comprising the HSP90 chaperone and the kinase-dedicated adaptor, CDC37. Upon glycosylation and delivery to the plasma membrane, only the mature form of HER2, as well as some naturally occurring EGFR mutants, remain associated with the chaperone. In polarized tissues the PDZ domain proteins LIN-2, -7, and -10 associate with and stabilize the receptor in the basolateral surface.

(3) Receptor dimerization: In the absence of a ligand, EGFR exists in a conformation that suppresses kinase activity and restrains formation of receptor dimers. Ligand binding initiates a conformational alteration that unmasks a "dimerization loop," triggering receptor dimerization. These transitions are relayed across the plasma membrane to activate the bilobular kinase domain: the mostly beta-strand N-terminal lobe of one receptor is juxtaposed next to the C-lobe of the dimer partner, thereby forming a catalytically active asymmetric dimer. Variations on this activation scheme are found in the ErbB family. ErbB-3 is kinase dead but is able to transactivate dimer partners, whereas HER2/ErbB-2 is a ligand-less oncogenic receptor "locked" in the active conformation. Drugs in current clinical use include two EGFR tyrosine kinase inhibitors, as well as a dual EGFR and HER2 inhibitor. Also approved for clinical applications are a humanized monoclonal anti-HER2 antibody and two anti-EGFR antibodies.

(4) Downstream signaling: Receptor homo- or heterodimers undergo transphosphorylation on multiple tyrosine residues. This leads to the recruitment of a plethora of enzymes and adaptor proteins. For example, the SHC and GRB2 phosphotyrosine-binding adaptors link phosphorylated receptors, through a guanine nucleotide exchange protein (SOS) and a small GTP-binding protein (RAS), to a linear cascade culminating in ERK1 and ERK2, which translocate to the nucleus to stimulate various transcription factors. Nuclear translocation of active receptors, such as HER2 and ErbB-4, has also been described.

(5) Switch off: Delayed activation of a variety of suppressive mechanisms attenuates ligand-stimulated signaling or switches cells back to the resting state. These processes include receptor ubiquitinylation and dephosphorylation, kinase inactivation, ligand depletion, removal of active receptors from the cell surface, or proteasomal degradation. In addition, an inducible set of transcriptional repressors and RNA-binding proteins ensure signal desensitization.

(6) Endosomal sorting: Rapid clearance of active receptors and sorting for degradation in lysosomes involves receptor clustering over clathrin- or caveolin-coated regions and CBL-mediated conjugation of ubiquitin or NEDD8. Large ESCRT protein complexes sort ubiquitinylated receptors at the MVB. Independently, inflammatory cytokines and oxidative stress transregulate EGFR by phosphorylating and arresting the receptor in perinuclear vesicles.

(7) Adhesion signaling: In some cell types, cell migration is regulated by EGF-induced activation of NCK and PLC-gamma and subsequent activation of RHO family GTPases necessary for formation of filopodia and lamellipodia.

## Abbreviations

ADAM, a disintegrin and a metalloproteinase; CAMK, calmodulin-dependent protein kinase; DAG, diacylglycerol; DUB, deubiquitinating enzyme; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; ESCRT, endosomal sorting complex required for transport; GPCR, G protein-coupled receptor; HER2, human EGF receptor 2; IP3, inositol (1,4,5)-triphosphate; MAPK, mitogen-activated protein kinase; MKP, MAPK phosphatase; MVB, multivesicular body; NRDP1, neuregulin receptor degrading protein 1; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PLC, phospholipace C; PP5, phosphoprotein 5; PS-1, presenilin-1; TACE, tumor necrosis factor alpha converting enzyme; UBD, ubiquitin-binding domain

## REFERENCES

Amit, I., Citri, A., Shay, T., Lu, Y., Katz, M., Zhang, F., Tarcic, G., Siwak, D., Lahad, J., Jacob-Hirsch, J., et al. (2007). A module of negative feedback regulators defines growth factor signaling. Nat. Genet. *39*, 503–512.

Burgess, A.W., Cho, H.S., Eigenbrot, C., Ferguson, K.M., Garrett, T.P., Leahy, D.J., Lemmon, M.A., Sliwkowski, M.X., Ward, C.W., and Yokoyama, S. (2003). An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors. Mol. Cell *12*, 541–552.

Cao, Z., Wu, X., Yen, L., Sweeney, C., and Carraway, K.L., 3rd. (2007). Neuregulin-induced ErbB3 downregulation is mediated by a protein stability cascade involving the E3 ubiquitin ligase Nrdp1. Mol. Cell. Biol. *27*, 2180–2188.

Citri, A., and Yarden, Y. (2006). EGF-ERBB signalling: towards the systems level. Nat. Rev. Mol. Cell Biol. *7*, 505–516.

Freeman, M. (2004). Proteolysis within the membrane: rhomboids revealed. Nat. Rev. Mol. Cell Biol. *5*, 188–197.

Katz, M., Amit, I., Citri, A., Shay, T., Carvalho, S., Lavi, S., Milanezi, F., Lyass, L., Amariglio, N., Jacob-Hirsch, J., et al. (2007). A reciprocal tensin3-cten switch mediates EGF-driven mammary cell migration. Nat. Cell Biol. *9*, 961–969.

Khan, E.M., Heidinger, J.M., Levy, M., Lisanti, M.P., Ravid, T., and Goldkorn, T. (2006). Epidermal growth factor receptor exposed to oxidative stress undergoes Src- and caveolin-1-dependent perinuclear trafficking. J. Biol. Chem. *281*, 14486–14493.

Mattila, E., Pellinen, T., Nevo, J., Vuoriluoto, K., Arjonen, A., and Ivaska, J. (2005). Negative regulation of EGFR signalling through integrin-alpha1beta1-mediated activation of protein tyrosine phosphatase TCPTP. Nat. Cell Biol. *7*, 78–85.

Sardi, S.P., Murtie, J., Koirala, S., Patten, B.A., and Corfas, G. (2006). Presenilin-dependent ErbB4 nuclear signaling regulates the timing of astrogenesis in the developing brain. Cell *127*, 185–197.

Shilo, B.-Z. (2005). Regulating the dynamics of EGF receptor signaling in space and time. Development *132*, 4017–4027.

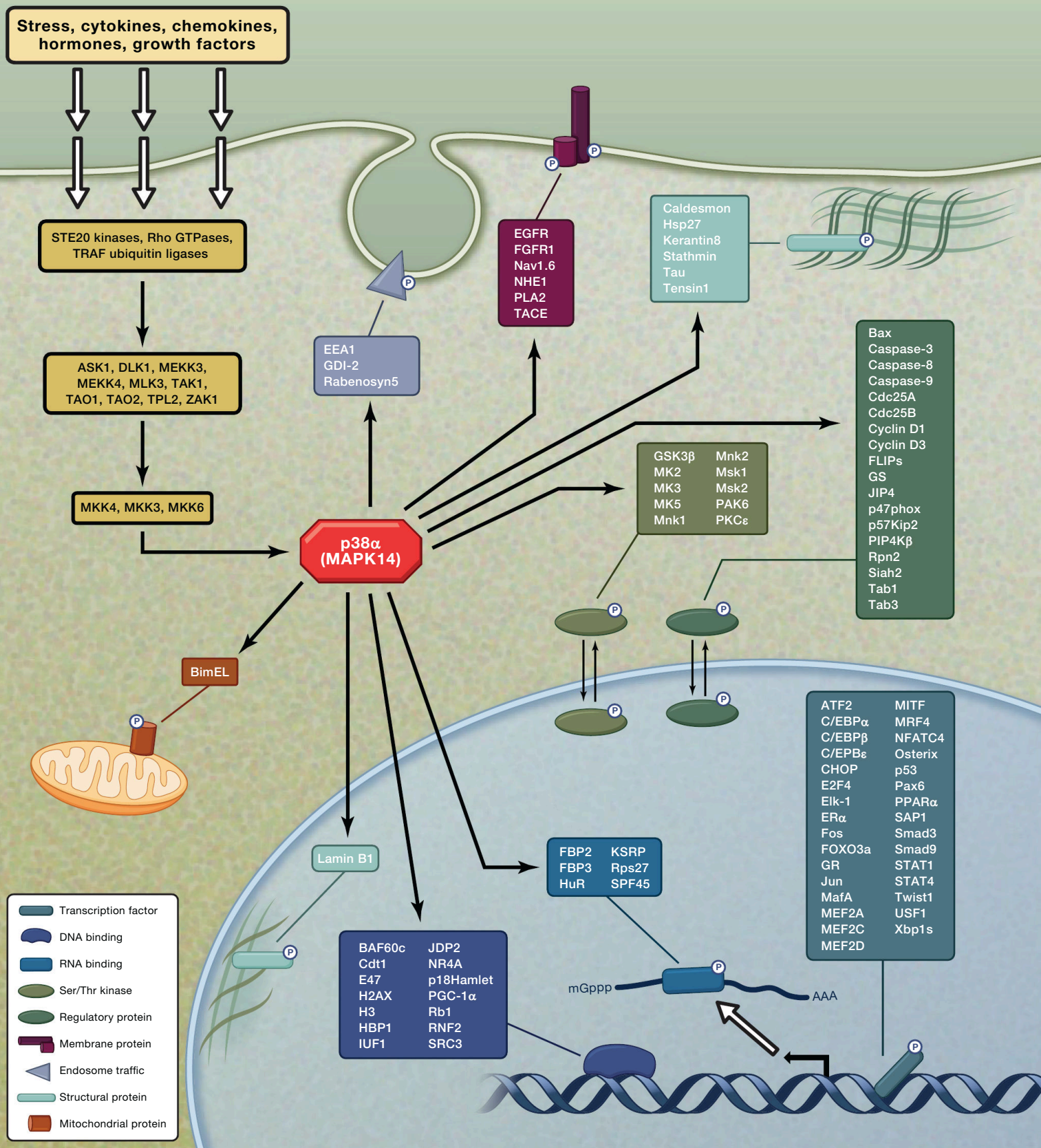
Tsruya, R., Wojtalla, A., Carmon, S., Yogev, S., Reich, A., Bibi, E., Merdes, G., Schejter, E., and Shilo, B.Z. (2007). Rhomboid cleaves Star to regulate the levels of secreted Spitz. EMBO J. *126*, 1211–1220.

Xu, W., Yuan, X., Xiang, Z., Mimnaugh, E., Marcu, M., and Neckers, L. (2005). Surface charge and hydrophobicity determine ErbB2 binding to the Hsp90 chaperone complex. Nat. Struct. Mol. Biol. *12*, 120–126.

Zhang, X., Gureasko, J., Shen, K., Cole, P.A., and Kuriyan, J. (2006). An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. Cell *125*, 1137–1149.

# SnapShot: p38 MAPK Signaling

Natalia Trempolec,[1] Natalia Dave-Coll,[1] and Angel R. Nebreda[1,2]
[1]Institute for Research in Biomedicine (IRB Barcelona), 08028 Barcelona, Spain
[2]Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain

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# SnapShot: p38 MAPK Signaling

Natalia Trempolec,[1] Natalia Dave-Coll,[1] and Angel R. Nebreda[1,2]
[1]Institute for Research in Biomedicine (IRB Barcelona), 08028 Barcelona, Spain
[2]Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain

Cell

## p38α: A Stress-Activated Protein Kinase with Multiple Functions

Mitogen-activated protein kinase (MAPK) pathways are important regulators of cellular responses to many extracellular stimuli. Typically, eukaryotic cells have several parallel MAPK pathways, which allow the integration of signals from different stimuli. One of these, the p38 MAPK pathway, has been conserved from yeast to mammals, in which there are four family members: p38α (MAPK14), p38β (MAPK11), p38δ (MAPK13), and p38γ (MAPK12). Most of what has been published on p38 MAPK signaling refers to p38α, which is ubiquitously expressed at high levels in most cell types. In contrast, p38β seems to be normally expressed at lower levels. The other two family members have more restricted tissue expression patterns. Activation of p38α is induced by most stress stimuli, including UV light, oxidative stress, and heat or osmotic shock, but also when cells are exposed to cytokines, chemokines, hormones, or growth factors. Taken together, it appears that p38α signaling helps cells to adequately respond to changing environmental conditions.

The extracellular stimuli usually lead to the activation of MAPKs via a cascade of phosphorylation events that involves at least two other kinases acting sequentially. MAP2Ks directly phosphorylate the activation loop of p38α on Thr and Tyr residues, leading to a conformational change that results in kinase activation. The three MAP2Ks that are known to activate p38α are, in turn, activated by phosphorylation on two conserved residues catalyzed by ten MAP3Ks. Upstream in the pathway, there is more diversity, and the activation of different MAP3Ks involves mechanisms like phosphorylation, ubiquitination, or protein-protein interaction, which facilitate integration of a wide range of signals. There is also evidence for the activation of p38α in particular cases by a noncanonical mechanism based on autophosphorylation, independently of MAP2Ks. Once p38α becomes activated, it can phosphorylate many substrates on Ser or Thr residues. This schematic depicts upstream regulators leading to p38α activation and the myriad downstream targets of p38α.

## p38α Substrates as a Source of Functional Diversity

A large number of publications have described the implications of the p38α-signaling pathway in multiple functions. However, comprehensive information about the p38α targets that are phosphorylated in response to different stimuli has not been compiled. We have found reports for 96 proteins that can be phosphorylated by p38α. A companion SnapShot that will be published in the February 14 issue will provide additional information about reported substrates, what residues are modified, and functional consequences.

About 55% of the known p38α substrates are located in the nucleus. These are mainly DNA- or RNA-binding proteins that are involved in the regulation of gene expression. There is evidence that 31 transcription factors can be directly phosphorylated by p38α, which in most cases results in the activation of transcription. Recent work has also connected p38α with chromatin remodeling via phosphorylation of BAF60c and p18$^{Hamlet}$, which are structural components of the SWI/SNF and SRCAP complexes, respectively. In addition, there are p38α substrates that can regulate mRNA processing (FBP2/3 and SPF45) or stability (HuR and KSRP).

Another important group of p38α substrates comprises proteins that are involved in signal transduction. These include two membrane receptors with Tyr kinase activity, EGFR and FGFR, and ten Ser/Thr kinases, which in turn can phosphorylate additional proteins and diversify the signal. Thus, MSK1 and MSK2 can regulate gene expression by direct phosphorylation of the transcription factors CREB and ATF1 and the chromatin protein histone H3, whereas MNK1 and MNK2 can regulate protein synthesis by phosphorylation of the initiation factor eIF4E. One of the first reported p38α substrates, MAPKAPK-2 (MK2), as well as the closely related MK3 can regulate mRNA stability by phosphorylation of ARE-binding proteins such as TTP or HuR. MK2 and MK3 also play important roles in actin filament remodeling by phosphorylation of Hsp27. Interestingly, some proteins can be potentially phosphorylated by both p38α and one of its downstream kinases, such as the MK2 substrates Cdc25B and HuR, or the MSK1/2 substrate histone H3. This double targeting of downstream substrates might function as a fail-safe mechanism to limit inappropriate effector activation.

A number of cytoplasmic proteins that are involved in different aspects of cell regulation can also be phosphorylated by p38α. This group includes proteins that mediate p38α antiproliferative functions, such as stress-induced cell-cycle arrest (p57$^{Kip2}$ and cyclin D1/3), and apoptosis (Bax and BimEL). However, p38α has also been reported to regulate cell survival through the phosphorylation of caspase-3 and caspase-8. Other cytoplasmic substrates of p38α may regulate proliferation and differentiation or specific processes such as cytoskeleton organization and intracellular membrane trafficking. Protein turnover can also be regulated by p38α at different levels either by phosphorylation-induced changes in the stability of the substrates or by phosphorylation of E3 ubiquitin ligases such as Siah2. In addition, p38α may inhibit proteasome activity by phosphorylation of the proteasome subunit Rpn2.

In summary, p38α plays key roles in the stress responses but is also implicated in multiple cellular functions not related to stress. Although many more p38α substrates likely remain to be discovered, the variety of known targets supports the notion that this signaling pathway connects many different stimuli to a broad spectrum of cell responses.

## ACKNOWLEDGMENTS

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## REFERENCES

Ashwell, J.D. (2006). The many paths to p38 mitogen-activated protein kinase activation in the immune system. Nat. Rev. Immunol. *6*, 532–540.

Coulthard, L.R., White, D.E., Jones, D.L., McDermott, M.F., and Burchill, S.A. (2009). p38(MAPK): stress responses from molecular mechanisms to therapeutics. Trends Mol. Med. *15*, 369–379.

Cuadrado, A., and Nebreda, A.R. (2010). Mechanisms and functions of p38 MAPK signalling. Biochem. J. *429*, 403–417.

Cuenda, A., and Rousseau, S. (2007). p38 MAP-kinases pathway regulation, function and role in human diseases. Biochim. Biophys. Acta *1773*, 1358–1375.

Gaestel, M. (2006). MAPKAP kinases - MKs - two's company, three's a crowd. Nat. Rev. Mol. Cell Biol. *7*, 120–130.

Nebreda, A.R., and Porras, A. (2000). p38 MAP kinases: beyond the stress response. Trends Biochem. Sci. *25*, 257–260.

Oeztuerk-Winder, F., and Ventura, J.J. (2012). The many faces of p38 mitogen-activated protein kinase in progenitor/stem cell differentiation. Biochem. J. *445*, 1–10.

Ono, K., and Han, J. (2000). The p38 signal transduction pathway: activation and function. Cell. Signal. *12*, 1–13.

Rincón, M., and Davis, R.J. (2009). Regulation of the immune response by stress-activated protein kinases. Immunol. Rev. *228*, 212–224.

Wagner, E.F., and Nebreda, A.R. (2009). Signal integration by JNK and p38 MAPK pathways in cancer development. Nat. Rev. Cancer *9*, 537–549.

# SnapShot: MicroRNAs in Cancer

Riccardo Spizzo, Milena S. Nicoloso, Carlo M. Croce, and George A. Calin
Experimental Therapeutics and Cancer Genetics, MD Anderson Cancer Center, Houston, TX 77030
and Comprehensive Cancer Center, Ohio State University, Columbus, OH 43210, USA

Cell

| Human MicroRNAs | Deregulation in Cancer | Molecular Mechanisms, Targets | Diagnostic, Prognostic Marker |
|---|---|---|---|
| ***let-7* family** (various) | Downregulated in lung, breast, gastric, ovary, prostate and colon cancers, CLL, leiomyomas; *miR-98* downregulated in head and neck cancer. Point mutation in *let-7e* transcript affects miRNA maturation. | Repress cell proliferation and growth. *let-7f* promotes angiogenesis. Targets: CCND1, CDC25a, CDC34, CDK6, CRD-BP, DICER, HMGA2, HOXA9, IMP-1, ITGB3, MYC, RAS, TLR4. | SNP in K-RAS 3′ UTR (*let-7a* binding site) increases NSCLC risk (cancer predisposition). Low expression of *let-7a-2* in lung and ovarian cancer and of *let-7b* in uveal melanoma (poor prognosis). *let-7i* affects chemotherapy potency. Intranasal delivery of *let-7a* reduces growth of Ras-induced mouse lung tumors. |
| | *let-7a-3* hypomethylated in lung adenocarcinoma; overexpressed in AML | *let-7a* represses NF2 and decreases chemotherapy-induced apoptosis in vitro. Target: CASP3. | |
| ***miR-10b*** (2q31.1, intergenic) | Downregulated in breast cancer. Overexpressed in metastatic breast cancer (does not predict metastasis in early stages). | Activates cell migration and extracellular matrix remodeling. Target: HOXD10. | |
| ***miR-15a, miR-16-1* cluster** (13q14.3, intron 4 noncoding RNA *DLEU2*) | Downregulated in CLL, DLBCL, multiple myeloma, pituitary adenoma, prostate and pancreatic cancer. Germline mutations in B-CLL patients and in NZB mice that develop CLL-like disorder. | Induce apoptosis in leukemia cells. *miR-16* regulates cell cycle by downregulating G0/G1 proteins. Targets: BCL2, CAPRIN1, CARD10, CCND1, CDK6, CDC27, CGI-38, CYCE, DMTF1, HMGA2, MCL1, MYB, NGN2, VEGF, WNT3A. | Low expression of *miR-15a*, *miR-16* in de novo CLL (good prognosis). *miR-16* modulates chemotherapy potency, sensitivity to vincristine in gastric cancer (predicts response). |
| | Upregulated in nasopharyngeal carcinoma | Target: BRCA1 | |
| ***miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-17-92* cluster** (13q31.3, intron 3 C13orf25) | Overexpression in lung and colon cancer, lymphoma, multiple myeloma, medulloblastoma | *miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1* increase tumor growth and tumor vascularization; *miR-20a* is antiapoptotic; transgenic *miR-17-92* mice develop lymphoproliferative disease and autoimmunity. Targets: AIB1, AML1, BIM1, CTGF, CDKN1A, E2F1, E2F2, E2F3, HIF-1A, PTEN, TGFBR2, TSP1, Rb2/P130. | High plasma levels of *miR-92* discriminate colorectal and gastric cancer from normal (diagnostic) |
| | LOH at *miR-17-92* locus in melanoma (20%), ovarian (16.5%) and breast (21.9%) cancer | *miR-17* reduces breast cancer cell proliferation. *miR-20* induces senescence via LRF. Targets: AIB1, CYCD1. | |
| ***miR-106b-93-25* cluster** (*7q22.1*) | Overexpression in gastric, colon, and prostate cancer, neuroblastoma, multiple myeloma | Reduces apoptotic response after TGFβ stimulation via BIM. Targets: CDKN1A, E2F1, BIM. | |
| ***miR-21*** (17q23.1, 3′UTR TMEM49) | Overexpression in glioblastoma, breast, lung, prostate, colon, stomach, esophageal, and cervical cancer, uterine leiomyosarcoma, DLBCL, head and neck cancer | *miR-21* knockdown induces apoptosis in glioblastoma. *miR-21* induces invasion, metastasis in colorectal cancer. Targets: BCL2, MASPIN, PDCD4, PTEN, TPM1, RECK, RASA1. | *miR-21* high expression in colon, breast, and pancreatic cancer (poor prognosis). *miR-21* high expression in de novo DLBCL (good prognosis). *miR-21* modulates chemosensitivity in NCI60 cells. |
| ***miR-29* family** (various) | Downregulation in CLL, colon, breast, and lung cancer, and cholangiocarcinomas | Induce aberrant methylation in lung cancer via DNMT3A,B; induce apoptosis via p53 and MCL1. Targets: CDC42, DNMT3A, B, MCL1, PIK3R1, TCL1. | *miR-29c* low expression correlates with short time from diagnosis to therapy in CLL (poor prognosis) |
| | Upregulation in breast cancer | Induces EMT transition, metastasis. Target: TTP metallopeptidase. | |
| ***miR-34* family** (1p36.23, 11q23.1, intergenic) | Downregulated in pancreatic cancer and Burkitt's lymphoma without MYC translocation. Hypermethylation of *miR-34b,c* in colon cancer. | *miR-34a* induces upregulation of p53, downregulation of E2F in colon cancer. Targets: BCL2, CCND1, CCNE2, CDK4,6, MYC, DLL1, E23, Notch1, MYCN, MET, HMGA2, SIRT1. | *miR-34a* low expression in CLL associated with p53 inactivation, chemotherapy-refractory disease (predicts response) |
| ***miR-101*** (1p31.3, 9p24.1) | Downregulation in prostate cancer, hepatocellular carcinoma, and bladder cancer | Alterations in global chromatin structure via repression of EZH2. Targets: COX2, EZH2, MCL1. | |
| ***miR-122a*** (18q21.31 intergenic) | Downregulation in hepatocellular carcinoma | Targets: CAT-1, CCNG1 | |
| ***miR-124a* family** (various) | Hypermethylation in colon, breast, gastric, and lung cancer, leukemia, lymphoma | Targets: CDK6, ITGB1, FOXA2, LAMC1, MTPN, MAPK14 | |
| ***miR-125a, miR-125b*** (various) | Downregulation in glioblastoma, breast, prostate and ovarian cancer | Targets: ERBB2, ERBB3, LIN28, LIN41, TNFSF4 | |
| | Upregulation in myelodysplastic syndrome and AML with t(2;11)(p21;q23), urothelial carcinoma | Target: p53 | |
| ***miR-127*** (14q32, RTL1 exon) | Hypermethylation in tumor cell lines | Targets: BCL6, RTL1 | |
| ***miR-143, miR-145* cluster** (intergenic, 5q32) | Downregulated in colon adenoma/carcinoma, in breast, lung, and cervical cancer, in B cell malignancies | *miR-143*, *miR-145* precursor sequences abnormally processed in colon cancer. Targets: MYC, ERK5, HOXA9, KRAS, PARP8. | |
| ***miR-155*** (21q21.3, exon 3 ncRNA BIC) | Overexpressed in pediatric Burkitt's lymphoma, Hodgkin's lymphoma, primary mediastinal lymphoma, DLBCL, breast, lung, colon, pancreatic cancer | Pre-B cell proliferation, lymphoblastic leukemia/high-grade lymphoma in *miR-155* transgenic mice. Targets: AGTR1, AID, IKBKE, TP53INP1. | High expression of *miR-155* in lung cancer, DLBCL, and aggressive CLL (poor prognosis) |
| ***miR-181* family** (various) | Overexpressed in breast, pancreas, prostate cancer | MYCN regulates transcription of *miR-181* cluster. Targets: HOXA11, TCL1. | Low expression of *miR-181* in aggressive CLL with 11q deletion (poor prognosis) |
| ***miR-221, miR-222* cluster** (Xp11.3, intergenic) | Overexpressed in CLL, thyroid papillary carcinoma, glioblastoma. Downregulated in AML. | Promotes cancer cell proliferation; *miR-221, miR-222* impair TRAIL-dependent response. Targets: c-KIT, P27, CDKN1B, P57, CDKN1C, ESR1. | |
| ***miR-200* family** (various) | Downregulated in clear-cell carcinoma, metastatic breast cancer | Promote invasion together with *miR-205*. Downregulation of *miR-200* family (and *miR-205*) directly involved in TGFβ-mediated EMT. Targets: ZEB1, 2; TGFβ. | |
| ***miR-372, miR-373* cluster** (19q13.41, intergenic) | Overexpression of *miR-373* in testicular cancer | Indirectly antagonize p53-mediated CDK inhibition during RAS-induced senescence. *miR-373* transactivates *CDH1* transcription by targeting the promoter region. Targets: LATS2, CD44. | High expression of *miR-372* in NSCLC (poor prognosis) |

Antitumorigenic
Oncogenic



See online version for legend and references.

# SnapShot: MicroRNAs in Cancer

Riccardo Spizzo, Milena S. Nicoloso, Carlo M. Croce, and George A. Calin
Experimental Therapeutics and Cancer Genetics, MD Anderson Cancer Center, Houston, TX 77030
and Comprehensive Cancer Center, Ohio State University, Columbus, OH 43210, USA

Cell

MicroRNAs (miRNAs) (http://microrna.sanger.ac.uk/sequences/) are 19 to 25 nucleotide-long noncoding RNA molecules that regulate gene expression both at the level of messenger RNA degradation and translation. MicroRNAs are typically excised from a 60 to 110 nucleotide-long hairpin precursor (fold-back) RNA structure (pre-miRNA) by the RNase III enzyme Dicer and are incorporated into the RNA-induced silencing complex (RISC). The pre-miRNA sequence is transcribed from a larger Pol II primary transcript (pri-miRNA) processed by Drosha and exported from the nucleus to the cytoplasm. Strongly conserved among distantly related organisms (including invertebrates, vertebrates, and plants), miRNAs are involved in a variety of biological processes including cell cycle regulation, differentiation, development, metabolism, neuronal patterning, and aging. Alterations in miRNA expression are involved in the initiation, progression, and metastasis of human tumors. Functional germline mutations in the *miR-15a* and *miR-16-1* cluster are associated with familial chronic lymphocytic leukemia (CLL) and breast cancer, whereas a common SNP in *pre-miR-146a* decreases mature miRNA expression and predisposes to papillary thyroid carcinoma. Furthermore, *miR-155* transgenic mice show proliferation of pre-B cells and develop lymphoblastic leukemia/high-grade lymphoma. Mice overexpressing *miR-17-92* in lymphocytes develop lymphoproliferative disease and autoimmunity and die prematurely. During cancer progression, dramatic overexpression or downregulation of mature and/or precursor miRNAs occurs in most tumors. The miRNAs *miR-10b*, *miR-373*, and *miR-520c* promote tumor invasion and metastasis, whereas *miR-335*, *miR-206*, and *miR-126* are suppressors of breast cancer metastasis. The differential expression of miRNA genes in malignant compared with normal tissue can be explained by three different mechanisms: location of miRNAs in cancer-associated genomic regions, epigenetic mechanisms, and alterations in the miRNA processing or transcription machinery. The *let-7* miRNA family and the *miR-15a*/*miR-16-1* clusters are located in loci deleted in lung cancer and CLL, respectively; the *miR-17-92* genomic locus is amplified in B cell lymphoma. The miRNA *miR-127* is re-expressed after DNA demethylation and histone deacetylase inhibition in cancer cells; *miR-124a* is transcriptionally inactivated by hypermethylation of CpG islands in different tumor cell lines. Impaired miRNA processing enhances cellular transformation and tumorigenesis, as shown by the conditional deletion of Dicer1, which increases tumor development in a K-Ras-induced mouse model of lung cancer. Moreover, reduced expression of Dicer and Drosha has been observed in various human cancers. Several transcription factors regulate the expression of miRNAs, e.g., the tumor suppressor protein p53 regulates the expression of *miR-34* family members, MYC is a negative regulator of miRNA expression, STAT3 regulates *miR-21* expression at the transcriptional level, and Twist transactivates *miR-10b* transcription.

The functional consequences of altered patterns of miRNA expression are just starting to be understood. Self-sustaining growth signals can be induced by *miR-21* overexpression, which is responsible for *PTEN* repression and loss of negative regulation by AKT kinase. Insensitivity to antigrowth signals is achieved by inhibition of E2F transcription factors caused by overexpression of the *miR-17-92* cluster on chromosome 13q31.1 and by overexpression of the *miR-106b-25* cluster on chromosome 7q22.1. Specifically, *miR-20a* inhibits expression of *E2F2* and *E2F3*, whereas *miR-17-5p*, *miR-20a*, *miR-106b*, and *miR-92* inhibit expression of *E2F1*. Modulation of apoptosis may occur by direct regulation of miRNAs by proapoptotic (p53 and *miR-34* family) or antiapoptotic (IL6/STAT3 and *miR-21*) pathways, or by direct targeting of antiapoptotic (BCL2, MCL1 proteins and the *miR-15/16* cluster) or proapoptotic (TP53BP1 and *miR-155*) proteins. Deregulation of telomerase activity can be achieved in tumors through reduced expression of *miR-138*, which represses translation of *TERT* mRNA and induces unrestricted proliferation. Blood vessel formation (angiogenesis) is regulated by miRNAs, and hypoxia is a proven regulator of miRNA expression. The oncomi*R-17-92* cluster that is regulated by MYC participates in the creation of a more aggressive, richly perfused tumor phenotype, whereas hypoxia contributes to the modulation of miRNA expression (including *miR-210*) partly by direct transcriptional activation of HIF-1 by specific miRNAs. Due to the specificity of targets and regulatory mechanisms, an miRNA may be an oncogene in one tumor type but a tumor suppressor in another.

MicroRNA expression profiling of human tumors has identified signatures that are associated with diagnosis, prognosis, and treatment efficacy. Distinct miRNA fingerprints characterize highly aggressive cancers, e.g., overexpression of *miR-155* and downregulation of *let-7* miRNAs in lung cancer cells indicates a poor prognosis. Furthermore, miRNA profiling can be used to successfully classify poorly differentiated tumors, including those with an unknown primary site. For example, high expression of *miR-21* is associated with poor survival and a poor therapeutic response to chemotherapy in colon cancer patients. The development of agents that block or mimic the expression and functions of miRNAs may represent a new therapeutic option for treating cancer patients.

**Abbreviations**

AID, activated induced cytidine deaminase; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B cell lymphoma; EMT, epithelial-mesenchymal transition; LOH, loss of heterozygosity; LRF, leukemia/lymphoma-related factor; NSCLC, non-small cell lung cancer; NZB, New Zealand Black; TTP, ADAM metallopeptidase with thrombospondin type I motif.

## REFERENCES

Ambros, V. (2003). MicroRNA pathways in flies and worms: Growth, death, fat, stress, and timing. Cell *113*, 673–676.

Bartel, D.P. (2004). MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell *116*, 281–297.

Berezikov, E., and Plasterk, R.H. (2005). Camels and zebrafish, viruses and cancer: A microRNA update. Hum. Mol. Genet. *14*, R183–R190.

Cummins, J.M., and Velculescu, V.E. (2006). Implications of microRNA profiling for cancer diagnosis. Oncogene *25*, 6220–6227.

Esquela-Kerscher, A., and Slack, F.J. (2006). OncomiRs—MicroRNAs with a role in cancer. Nat. Rev. Cancer *6*, 259–269.

Gregory, R.I., and Shiekhattar, R. (2005). MicroRNA biogenesis and cancer. Cancer Res. *65*, 3509–3512.

Hammond, S.M. (2006). MicroRNAs as Oncogenes. Curr. Opin. Genet. Dev. *16*, 4–9.

Mendell, J.T. (2008). miRiad roles for the *miR-17-92* cluster in development and disease. Cell *133*, 217–222.

Nicoloso, M.S., Spizzo, R., Shimizu, M., Rossi, S., and Calin, G.A. (2009). MicroRNAs—The micro steering wheel of tumor metastases. Nat. Rev. Cancer *9*, 293–302.

Stefani, G., and Slack, F.J. (2008). Small noncoding RNAs in animal development. Nat. Rev. Mol. Cell Biol. *9*, 219–230.

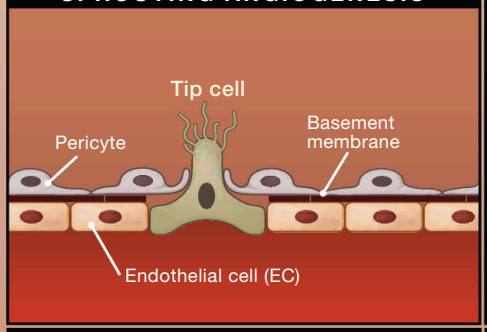
# SnapShot: Tumor Angiogenesis

Rakesh K. Jain[1] and Peter Carmeliet[2]
[1]Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA
[2]Vesalius Research Center, VIB and K.U. Leuven, B-3000 Leuven, Belgium

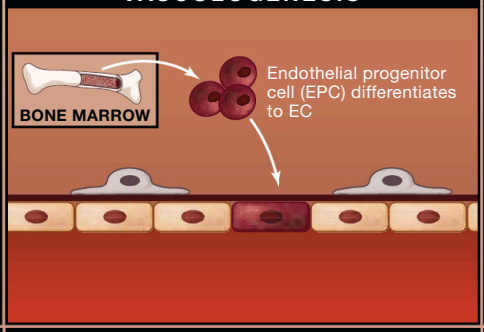
Cell

## How do tumors recruit blood vessels?

### SPROUTING ANGIOGENESIS




### VASCULOGENESIS

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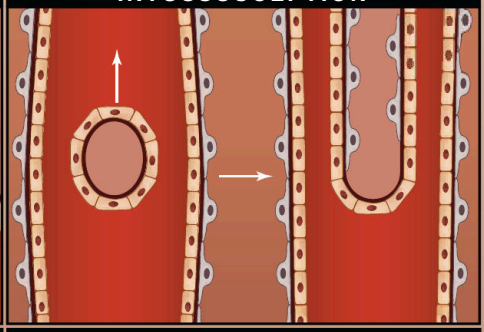
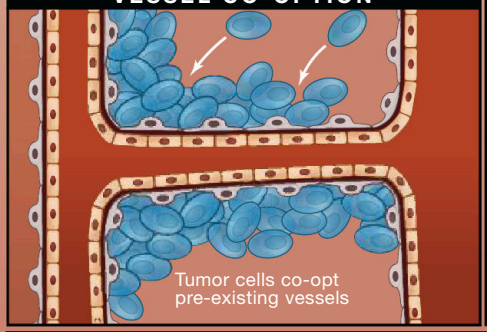
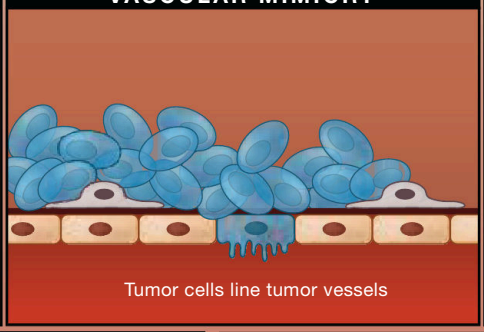


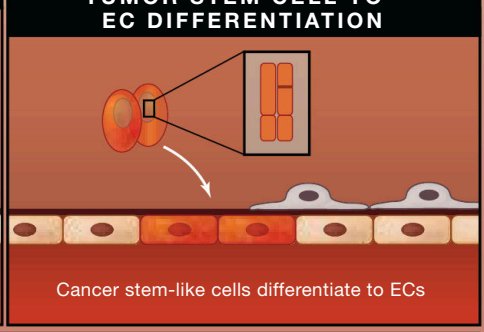
### VESSEL CO-OPTION




### VASCULAR MIMICRY




### TUMOR STEM CELL TO EC DIFFERENTIATION

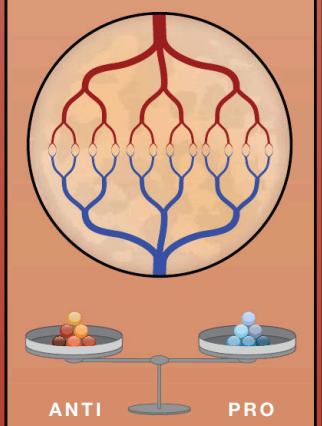



| Anti-VEGF drugs (2012) | |
|---|---|
| Bevacizumab | Metastatic colorectal cancer, metastatic nonsquamous non-small cell lung cancer, metastatic breast cancer, recurrent glioblastoma, metastatic renal cell carcinoma |
| Sunitinib | Metastatic renal cell carcinoma, gastrointestinal stromal tumors, pancreatic neuroendocrine tumors |
| Sorafenib | Metastatic renal cell carcinoma, unresectable hepatocellular carcinoma |
| Pazopanib | Metastatic renal cell carcinoma, advanced sarcoma |
| Vandetanib | Advanced medullary thyroid cancer |
| Axitinib | Advanced renal cell carcinoma |
| Regoranfenib | Metastatic colorectal cancer (approval pending) |
| Aflibercept | Metastatic colorectal cancer (approval pending) |

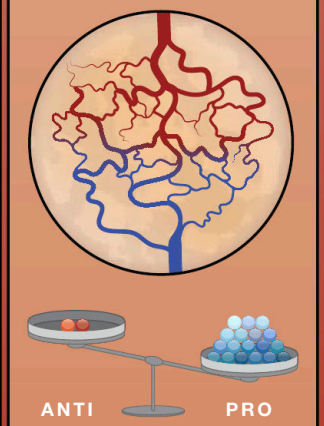
| Anti-angiopoietin/Tie2 pathway agents (in clinical trials) | |
|---|---|
| AMG 386 | Ovarian, breast, gastric, hepatocellular carcinoma, renal cell carcinoma, endometrial, colorectal carcinoma, glioblastoma |
| CVX-060 | Renal cell carcinoma |
| CVX-241 | Ovarian, primary peritoneal |
| MEDI-3617 | Advanced solid tumors |
| REGN910 | Advanced solid tumors |
| AMG-780 | Advanced solid tumors |
| CEP-1198 | Advanced solid tumors |
| ARRY-614 | Myelodysplastic syndromes |
| MGCD265 | Advanced malignancies, non-small cell lung carcinoma |
| Regorafenib | Gastrointestinal, renal cell carcinoma |

## How does anti-angiogenic therapy work?

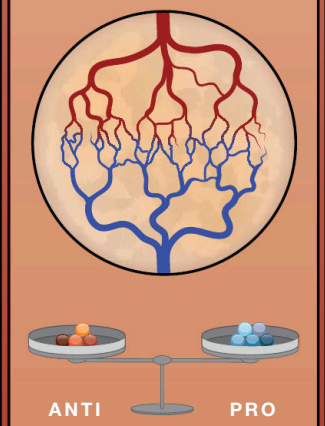
### NORMAL VASCULATURE




### ABNORMAL TUMOR VESSELS

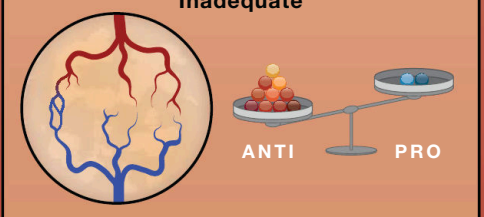



### NORMALIZED VESSELS

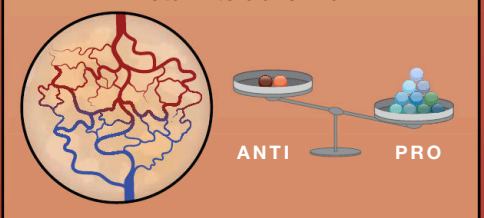



### NORMALIZATION WINDOW CLOSED

**Inadequate**




**Return to abnormal**



# SnapShot: Tumor Angiogenesis

Rakesh K. Jain[1] and Peter Carmeliet[2]
[1]Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA
[2]Vesalius Research Center, VIB and K.U. Leuven, B-3000 Leuven, Belgium

Cell

## How Do Tumors Recruit Blood Vessels?

Blood vessels are indispensible for tumor growth and metastasis. Hence, tumors exploit multiple avenues to recruit blood vessels. Angiogenesis—the sprouting of new blood vessels from the existing vasculature—is the most widely investigated mode of new vessel formation in tumors. There are five other mechanisms of new vessel recruitment (top panels; adapted from Carmeliet and Jain, 2011). However, their relevance in cancer is still being debated, and their molecular mechanisms are not well understood. Vasculogenesis involves vessel formation by endothelial progenitor cells (EPCs), which are recruited from the bone marrow and/or are resident in vascular walls. Intussusception is the splitting of pre-existing vessels to give rise to daughter vessels. Vessel co-option occurs when cancer cells grow around and co-opt the existing vasculature. Vascular mimicry is a process in which cancer cells get incorporated into the blood vessel wall. Tumor stem cell to EC differentiation occurs when cancer stem-like cells differentiate into endothelial cells (ECs). For historical reasons and, now, for convenience, the term "angiogenesis" is used to describe all of these methods of blood vessel recruitment by tumors.

## What Are the Approved and Emerging Anti-angiogenic Drugs?

### *Anti-VEGF Drugs*

Eight drugs that target the vascular endothelial growth factor (VEGF) pathway have already been approved or are pending approval for treatment of a variety of solid tumors (left table; updated from Carmeliet and Jain, 2011), and three others (not shown) have been approved for age-related wet macular degeneration that can lead to blindness. Among these, the anti-VEGF antibody bevacizumab (Avastin) is the most widely prescribed drug and has shown benefit in patients only when combined with chemotherapy or immunotherapy. An exception was its conditional approval for use as a monotherapy for recurrent glioblastoma, but confirmatory data from randomized phase III trials in these patients are pending. Although the US FDA has withdrawn approval of bevacizumab for metastatic breast cancer in the US, this drug is still given to these patients in Europe, except for in the United Kingdom. All other approved anti-VEGF agents are multitargeted receptor tyrosine kinase inhibitors and may have off-target effects.

### *Anti-angiopoietin/Tie2 Pathway Agents*

Similar to all targeted therapies, tumors develop resistance to anti-VEGF drugs. Therefore, considerable research and clinical effort is now directed toward finding new targets beyond VEGF (right table; adapted from Cascone and Heymach, 2012). One such target is the Angiopoietin/Tie2 pathway, which is involved in vessel stability. Eight agents that target this pathway (specifically or in combination with the VEGF pathway) are in clinical trials for multiple solid tumors.

## How Does Anti-angiogenic Therapy Work?

Anti-angiogenic therapy was originally developed to "starve" primary and metastatic tumors by blocking blood vessel recruitment. However, this concept has yet to be clinically validated because bevacizumab alone has failed to show survival benefits in randomized phase III trials to date. In fact, in several trials, bevacizumab monotherapy was discontinued for lack of efficacy. In contrast, bevacizumab added to chemotherapy or immunotherapy improved outcome in multiple phase III trials. Here, we offer one potential mechanism of this benefit (bottom; adapted from Jain, 2005).

In a normal tissue, the signals downstream of the pro-angiogenic molecules, such as VEGF and Ang2, are exquisitely counterbalanced by those from anti-angiogenic molecules such as sVEGFR1, thrombospondins, and semaphorins. Thus, the blood vessels exhibit normal structure and function (panel 1). In contrast, due to genetic and epigenetic changes, the balance is tipped in favor of new vessel formation in tumors. As a result, tumor vessels are highly abnormal both structurally and functionally (panel 2). This creates a hostile microenvironment in tumors—characterized by hypoxia, low pH, and elevated fluid pressure—which fuels tumor progression and treatment resistance via genetic instability, angiogenesis, immune suppression, inflammation, resistance to cell death, etc. If VEGF is neutralized using bevacizumab (or another anti-VEGF drug), this can cause pruning of some abnormal vessels and remodeling of the remaining vessel, resulting in a "normalized vasculature" (panel 3). In turn, this can reduce tumor hypoxia and fluid pressure that will improve the outcome of chemo-, radio-, and immune therapy. If the anti-angiogenic agent is too potent or the dose is too high, the balance can tip in the other direction and cause excessive vessel pruning. This can result in tumor regression and/or increased hypoxia (panel 4, top). However, increased hypoxia could decrease the efficacy of various therapies and may increase metastasis. Alternatively, tumors might switch to utilizing other angiogenic molecules and begin to make abnormal vessels again (panel 4, bottom). Hence, considerable effort is directed toward blocking these molecules.

Other mechanisms of benefit from combined anti-angiogenesis therapy and chemotherapy include: (1) anti-angiogenic agents directly killing cancer cells and sensitizing endothelial cells to cytotoxic drugs, (2) direct killing of endothelial drugs by cytotoxic drugs, and/or (3) decompression of tumor vessels through killing of tumor cells by cytotoxic and/or anti-angiogenic agents, leading to improved perfusion. Anti-angiogenic agents can also impair the recruitment of bone marrow-derived progenitors, which can differentiate to endothelial cells or release pro-angiogenic molecules.

Not every cancer patient responds to anti-VEGF-targeted agents. Among various evasion mechanisms, treated tumors can release additional pro-angiogenic molecules or recruit tumor vessels via sprouting-independent mechanisms, which may be less dependent on VEGF. Tumor endothelial cells can show signs of cytogenetic abnormalities, and cancer-like stem cells can differentiate into endothelial cells—processes that may reduce the sensitivity to anti-VEGF drugs. In addition, recruitment of angiocompetent myeloid cells, activation of cancer-associated fibroblasts, or coverage of tumor vessels by thick mural cell (pericyte) coats may also render tumors insensitive to VEGF-blockade.

Though we are still striving to better understand these mechanisms in preclinical studies, the emerging clinical data from two trials indicate that the patients whose brain tumor blood perfusion levels increase during anti-VEGF therapies survive longer than the patients whose blood flow does not increase. To our knowledge, there are no clinical data showing a correlation between decreased blood perfusion and increased survival in any tumor type.

## ACKNOWLEDGMENTS

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## REFERENCES

Carmeliet, P. (2005). Angiogenesis in life, disease and medicine. Nature *438*, 932–936.

Carmeliet, P., and Jain, R.K. (2011). Molecular mechanisms and clinical applications of angiogenesis. Nature *473*, 298–307.

Cascone, T., and Heymach, J.V. (2012). Targeting the angiopoietin/Tie2 pathway: cutting tumor vessels with a double-edged sword? J. Clin. Oncol. *30*, 441–444.

Chung, A.S., Lee, J., and Ferrara, N. (2010). Targeting the tumour vasculature: insights from physiological angiogenesis. Nat. Rev. Cancer *10*, 505–514.

Folkman, J. (2007). Angiogenesis: an organizing principle for drug discovery? Nat. Rev. Drug Discov. *6*, 273–286.

E. R. Gerstner, et al. Cediranib, a VEGF signaling inhibitor, in combination with chemoradiation in newly diagnosed glioblastoma leads to improved tumor blood flow and survival. *ASCO abstract* (2012).

Goel, S., Duda, D.G., Xu, L., Munn, L.L., Boucher, Y., Fukumura, D., and Jain, R.K. (2011). Normalization of the vasculature for treatment of cancer and other diseases. Physiol. Rev. *91*, 1071–1121.

Jain, R.K. (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science *307*, 58–62.

Padera, T.P., Stoll, B.R., Tooredman, J.B., Capen, D., di Tomaso, E., and Jain, R.K. (2004). Pathology: cancer cells compress intratumour vessels. Nature *427*, 695.

Sorensen, A.G., Emblem, K.E., Polaskova, P., Jennings, D., Kim, H., Ancukiewicz, M., Wang, M., Wen, P.Y., Ivy, P., Batchelor, T.T., and Jain, R.K. (2012). Increased survival of glioblastoma patients who respond to antiangiogenic therapy with elevated blood perfusion. Cancer Res. *72*, 402–407.

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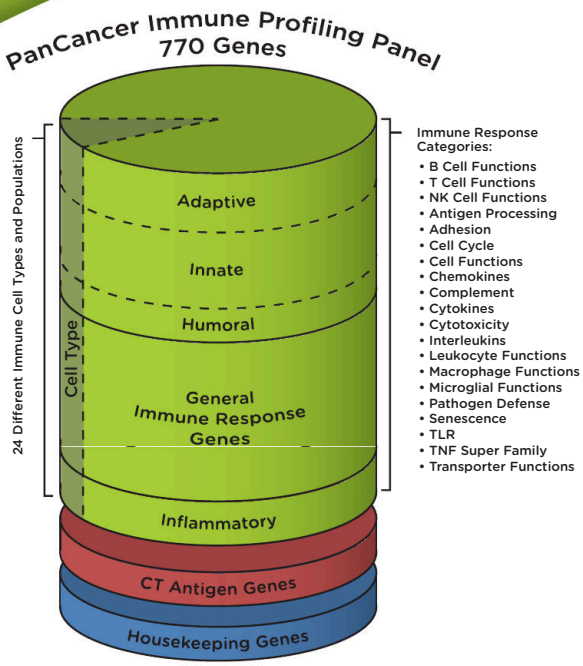


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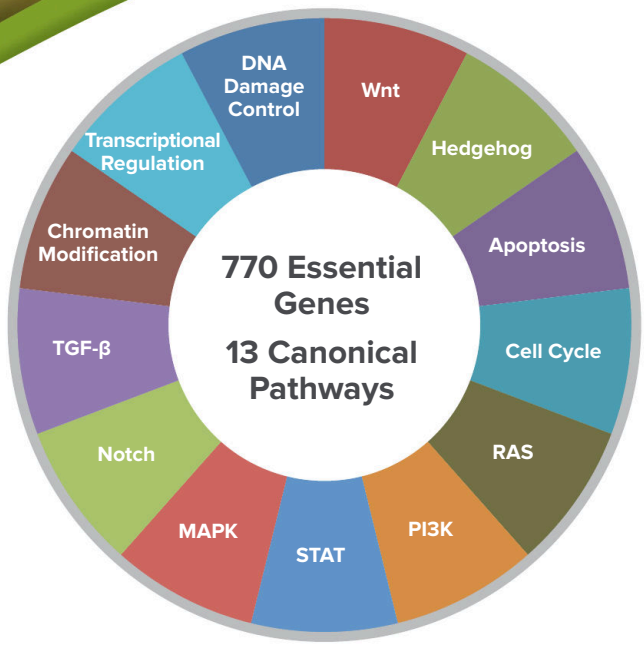


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