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

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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

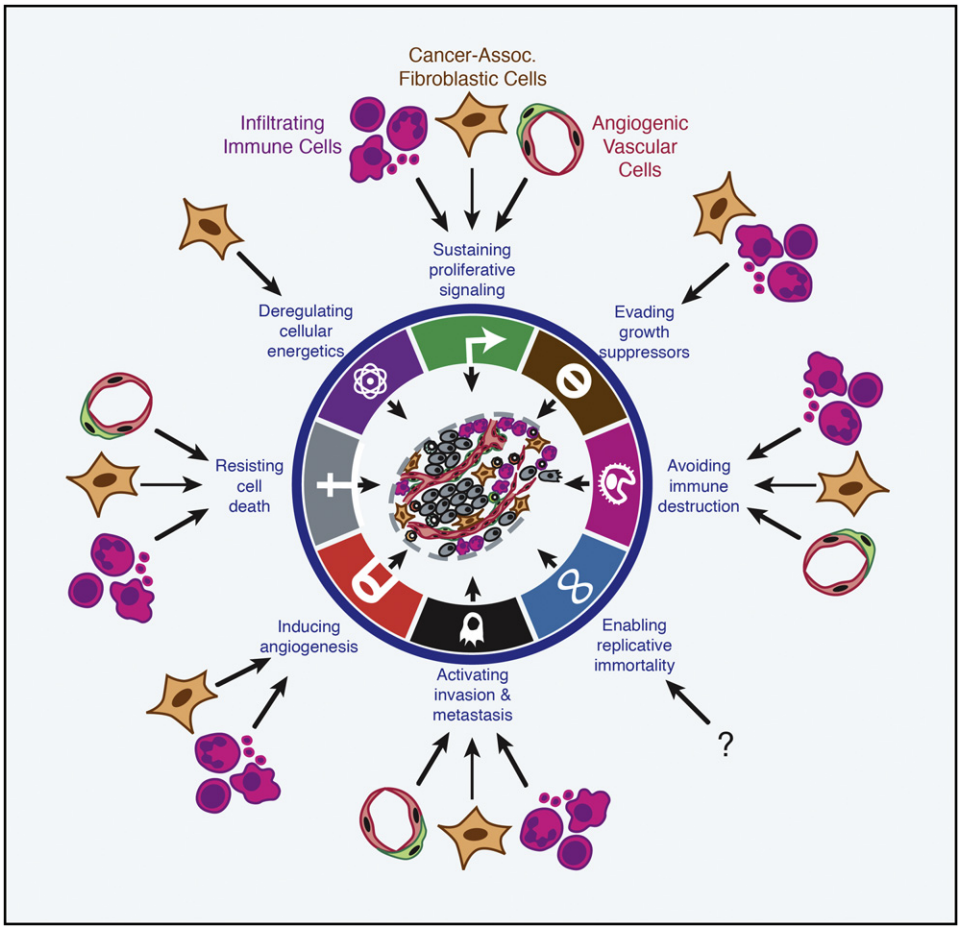

Figure 1. Multifactoral Contributions of Activated/Recruited Stromal Calls to the Hallmarks of Cancer
Of the eight acquired hallmark capabilities—six core and two emerging (Hanahan and Weinberg, 2011)—seven demonstrably involve contributions by stromal cells of the tumor microenvironment. The stromal 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, α4-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 α4-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-α, TGF-β, 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 β 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

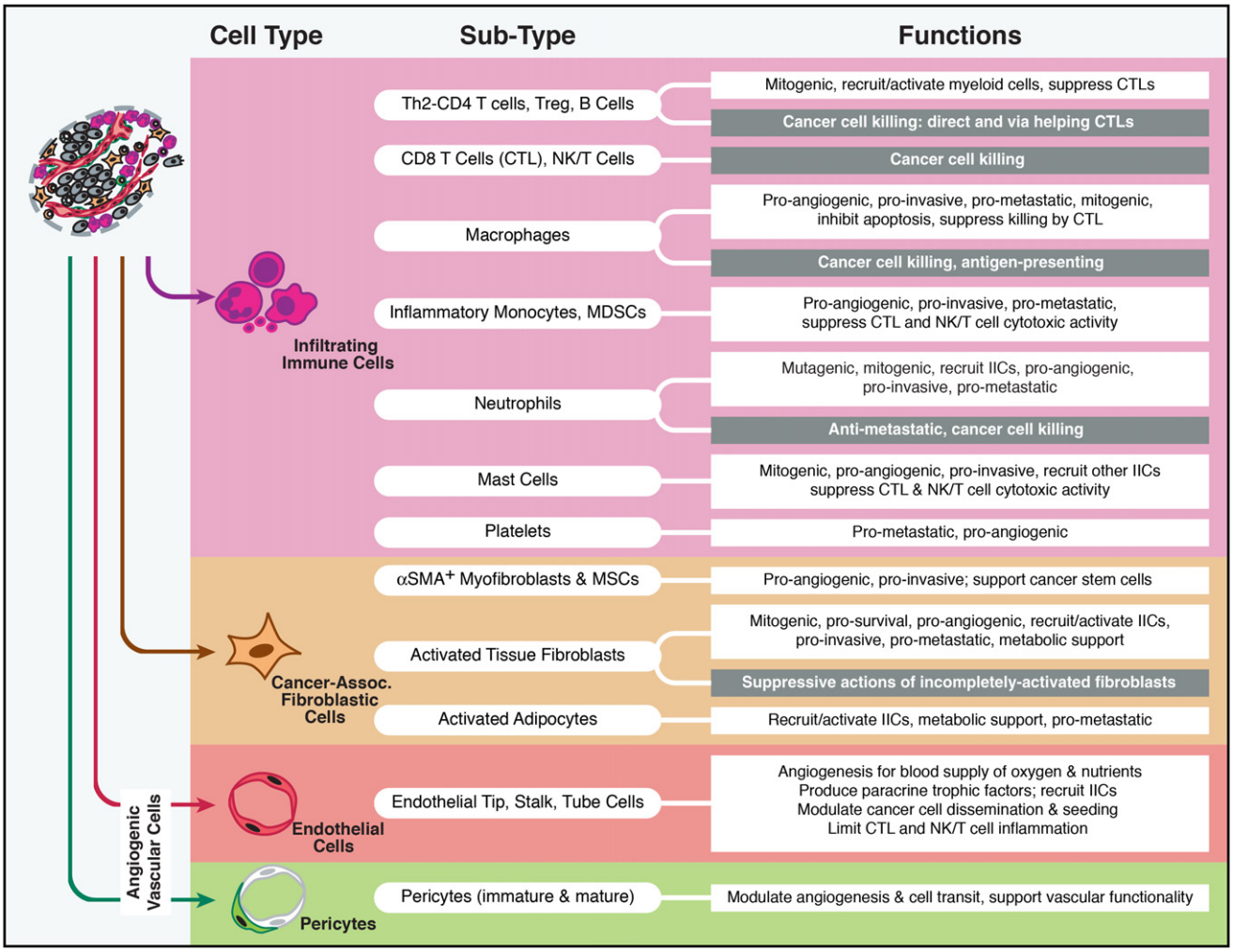

Figure 2. Multiple Stromal Cell Types and Subcell Types of the Tumor Microenvironment Can Variably Contribute to, or in Some Cases Oppose, Acquisition of the Seven Hallmark Functional Capabilities in Different Organ Sites, Tumor Types and Subtypes, and Stages of Progression

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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