Journal of Cellular Biochemistry 101:805–815 (2007)

Tumor Microenvironment: The Role of the Tumor Stroma in Cancer

Hanchen Li, Xueli Fan, and JeanMarie Houghton*

Division of Gastroenterology, Department of Medicine and Cancer Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01605

Abstract The tumor microenvironment, composed of non-cancer cells and their stroma, has become recognized as a major factor influencing the growth of cancer. The microenvironment has been implicated in the regulation of cell growth, determining metastatic potential and possibly determining location of metastatic disease, and impacting the outcome of therapy. While the stromal cells are not malignant per se, their role in supporting cancer growth is so vital to the survival of the tumor that they have become an attractive target for chemotherapeutic agents. In this review, we will discuss the various cellular and molecular components of the stromal environment, their effects on cancer cell dynamics, and the rationale and implications of targeting this environment for control of cancer. Additionally, we will emphasize the role of the bone marrow-derived cell in providing cells for the stroma. J. Cell. Biochem. 101: 805–815, 2007. © 2007 Wiley-Liss, Inc.

Key words: tumor microenvironment; tumor stroma; cancer-associated fibroblasts; neovascularization; tumor-associated inflammation; bone marrow-derived stem cells; mesenchymal stem cells

Tumor cells and their stroma co-evolve. The logistics of which cell initiates and which cell responds to begin the process of cancer has not been worked out. Research in this area aimed at uncovering early events in carcinogenesis will undoubtedly broaden our understanding of cancer formation and provide novel targets for prevention and early eradication of lesions. What we do know about the tumor stromal environment comes mainly from the investigation of established tumors in humans, and in studies of genetically manipulated animal models. The stroma consists of a compilation of cells, including fibroblasts/myofibroblasts, glial, epithelial, fat, immune, vascular, smooth muscle, and immune cells along with the extracellular matrix (ECM) and extracellular molecules. While none of these cells are themselves malignant, due to their environment, their interactions with each other, and directly or indirectly with the cancer cells, they acquire an abnormal phenotype and altered function. This abnormal interplay consisting of cell–cell contact and active molecular crosstalk further drives the cancer stroma phenotype, and may result in permanent alterations in cell function. Growth factor and chemokine production by fibroblasts and immune cells is altered leading to direct stimulation of tumor cell growth and recruitment of precursor cells, which themselves respond with abnormal growth and proliferation. Malformed tumor vessels contribute to tumor hypoxia, acidosis, and increased interstitial fluid pressures. The tumor in turn responds with a unique repertoire of gene expression which in turn acts to alter cell growth, invasion, and ultimately metastasis. The unique interplay between the various aspects of the tumor and the microenviroment has been the recent target of molecular strategies for tumor treatment. In order to understand the complex interplay of the cells and the non-cellular stroma, we will discuss each major component separately prior to a global summary of the interactions between the components, followed by a brief discussion on targeting the stroma for therapy. Where

Grant sponsor: NIH/NCI; Grant number: 1 RO1 CA119061; Grant sponsor: Funderberg Gastric Cancer award.

*Correspondence to: JeanMarie Houghton, Division of Gastroenterology, Department of Medicine and Cancer Biology, LRB room 209, University of Massachusetts Medical School, Worcester, MA 01605.
E-mail: jeanmarie.houghton@umassmed.edu

Received 7 September 2006; Accepted 8 September 2006
DOI 10.1002/jcb.21159

appropriate, we will focus on the contribution of bone marrow-derived cells (BMDC) to the stroma.

CELLS OF THE STROMA

Cells within the stroma include fibroblasts, vascular, glial, smooth muscle, epithelial, and fat cells, and cells of the immune system. The most widely studied to date are the fibroblasts, immune cells, and the vascular cells, which we will focus on in this review.

Fibroblasts

Fibroblasts within normal tissue. In order to understand the role of fibroblasts in cancer, it is crucial to understand the role and the function of the normal fibroblast. In normal tissue, fibroblasts are the predominant cell type in the connective tissue stroma and are the primary producers of the non-cellular scaffolds—the extracellular matrix (ECM). Fibroblasts are responsible for the deposition of the fibrillar ECM—type I, type III, and type V collagen and fibronectin—and contribute to the formation of the basement membrane by secreting type IV collagen and laminin. The environment is not static; connective tissue and the ECM are continually remodeled through a dynamic process of ECM protein production and degradation by fibroblast-derived matrix metalloproteinases (MMPs). The turnover is, however, well regulated and restrained.

During wound repair, fibroblasts are responsible for orchestrating healing, and in order to do so become "activated," with increased proliferation and alterations in both phenotype and secretory capacity. Production of alpha-smooth muscle actin (α-SMA) allows cells to migrate into areas of damage and contract for tissue restitution. Fibroblasts serve as a scaffold and secrete increased levels of ECM proteins, growth factors, and chemotactic factors, thereby coordinating the influx of inflammatory cells and vascular progenitor cells as well as supplying the scaffold structure for cell growth and proliferation. Fibroblast activation during wound repair involves a dynamic crosstalk between the fibroblast and the injured epithelium. Direct contact with infiltrating immune cells via the adhesion molecules ICAM1 and VCAM1 [Clayton et al., 1998] and response to factors secreted directly from the injured mucosa including fibroblast growth factor 2 (FGF2), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and transforming growth factor-beta (TGF-β) [Zeisberg et al., 2000] are responsible for the conversion of a resting fibroblast to an activated fibroblast.

Where do these activated fibroblasts come from and where do they go? Activated fibroblasts are largely believed to originate from a pool of local fibroblasts recruited for tissue repair, however, the precise source of these cells has not been fully elucidated. After successful tissue repair, the number of activated fibroblasts which can be recovered in the area of healing dramatically decreases. It is unclear, however, if the activated fibroblasts revert to a non-activated status and remain within the tissue, or if the activated fibroblast is eliminated and replaced by resting fibroblasts from adjacent normal tissue. Data regarding the reversibility or permanency of the activated phenotype are inconclusive, but the answer to this question has substantial implications when extrapolating to tumor-associated fibroblasts and when targeting this population for anti-tumor therapy.

Fibroblasts within tumors. Fibroblasts are the main cellular component of tumor stroma comprising an integral component of the tumor. In some cancer types, fibroblasts constitute a larger proportion of cells within the tumor than do the cancer cells. Fibroblasts within tumors have an activated phenotype, and as such resemble fibroblasts in wound healing. These cancer-associated fibroblasts (CAFs) are functionally and phenotypically distinct from normal fibroblasts that are in the same tissue but not in the tumor environment. The distinction between these and physiologically activated fibroblasts is that they are perpetually activated, neither reverting to a normal phenotype nor undergoing apoptosis and elimination. CAFs are identified within tumor stroma by their spindyloid appearance and the expression of α-SMA; characteristics shared by activated fibroblasts in wounds.

There are several theories put forth on the origin of CAFs including activation of a tissue resident fibroblast, local cancer cells or epithelial cells undergoing epithelial-to-mesenchymal transition (EMT), or the migration and activation of a marrow-derived cell. The origin and the mechanism of activation may be different in

different tissues, and may include combination of cell types and signals. When reviewing the data supporting the activation of cells from the local fibroblast pool, the signals responsible for conversion of a normal fibroblast to a CAF are unclear. Experimentally, factors such as TGF-β can induce normal fibroblasts to express α-SMA [Ronnov-Jessen and Petersen, 1993], but it is not clear if these experimentally activated cells acquire other characteristics of CAFs, and if the phenotype is stable. These data suggest, but do not prove that factors secreted by tumor cells themselves may drive the phenotypic and functional alterations in the local fibroblast pool.

Another theory involves EMT—the notion that tumor cells detach, assume a more mesenchymal cell like phenotype, and acquire invasive and migratory ability. If EMT of cancer cells were the predominant source of CAFs, then the genetic alterations found in the cancer cells would be expected to be found within the stromal cells. Interestingly, studies evaluating the genetic makeup of both compartments do find genetic alterations present in both the cancer cells and the CAFs—however, these alterations are rarely identical, suggesting a few possibilities. A minority of cancer cells and stromal cells may share a common origin (the minority of cells demonstrating similar genetic alterations) supporting EMT of cancer cells for a subset of CAFs [Kurose et al., 2002; Petersen et al., 2003; Tuhkanen, 2004]. More likely, these genetic mutations represent an independent response of the CAF cell to the cancer environment. While EMT of cancer cells to CAFs is unlikely to account for the majority of cells within a tumor, an alternate suggestion is that EMT of surrounding normal epithelial cells may be an additional source of cells for CAF formation [Iwano et al., 2002].

Recent studies have shown surprising degrees of plasticity for BMDC and increasing evidence for BMDC participation in the formation of cancer. In a permissive environment, BMDC directly form gastric adenocarcinoma [Houghton et al., 2004], skin [Aractingi et al., 2005] and vascular tumors [Peters et al., 2005]. Additionally, there is mounting evidence that bone marrow-derived precursor cells invade tumors and function as CAFs [Iwano et al., 2002; Direkze et al., 2004] (Fig. 1). It is not clear if these cells become activated as a result of the tissue environment, or if they are a subset of

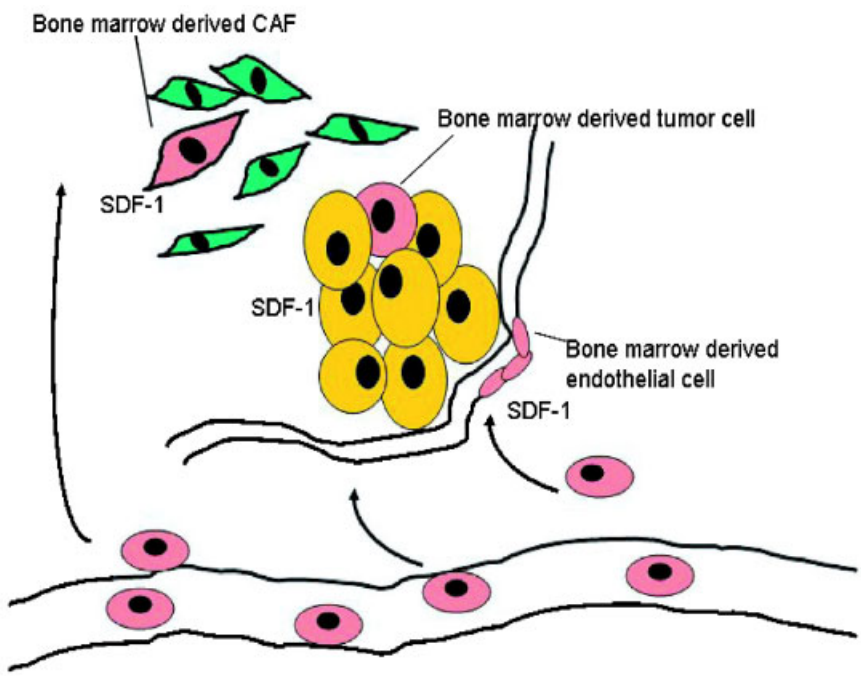

Fig. 1. BMDC contribute to tumor formation. Marrow-derived cells expressing CXCR4 (pink cells) chemotax to areas of hypoxia and inflammation in response to SDF-1 (secreted by CAFs and inflammatory cells), and incorporate into vessels as ECs, into the stroma as CAFs, and as tumor cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cells within the marrow with an already activated phenotype, preferentially recruited to the site of tumors. MSC have been reported to be inherently mutagenic, undergoing genetic alterations as a result of forced divisions [Serakinci et al., 2004; Rubio et al., 2005]. The trait of accumulating genetic mutations as a result of proliferation would explain the high degree of genetic alterations found in these cells, distinct from the alterations found in the surrounding tumor cells. An additional consideration is whether these marrow-derived cells fuse with peripheral cells or with cancer cells to acquire the CAF phenotype or if they function independent of fusion. Fusion followed by a reductive division could potentially explain some of the findings of EMT—with the simultaneous expression of epithelial and mesenchymal markers, and the acquisition of similar genetic damage, however, the phenomenon of fusion between BMDC and cancer cells has not been fully addressed.

How CAFs function to assist cancer growth. The role for CAFs in promoting cancer has been demonstrated in established tumors, carcinoma in situ, the initiation of cancer, and in orchestrating metastasis. Indirect data examining fibroblasts from peripheral sites in patients with a variety of cancers show these peripheral fibroblasts have more of an activated phenotype when compared with fibroblasts from patients without cancer, with an increase in proliferation and a reduction in growth requirements [Kopelovich, 1982; Schor, 1986]. These data suggest, but do not prove the association between activation of fibroblasts and an increased predilection for cancer. The use of mouse models of cancer employing fibroblasts engineered to overexpress hepatocyte growth factor (HGF) or TGF-β has shown that activated fibroblasts can initiate cancer at divergent sites including stomach and prostate [Bhowmick et al., 2004; Kuperwasser et al., 2004]. Additionally, once a lesion is initiated, CAFs have been shown to assist in proliferation and progression of cancer through the production of growth factors and chemotactic factors, angiogenesis factors, and MMPs (see below, non-cellular components of the stroma), allowing invasion and spread of cancer cells.

Creation of the metastatic niche. Perhaps the most intriguing data regarding the role of the stroma in cancer have been the discovery of the pre-metastatic niche. Factors elaborated by tumor cells attract hematopoietic bone marrow (HBM)-derived cells in a vascular endothelial growth factor-R1 (VEGF-R1) dependent fashion. The type and quantity of factors elaborated seem to determine the organ to which the HBM cells will be attracted to. The expression of VLA-4 allows adhesion of these BMDC and local cluster formation, a prerequisite for subsequent retention of malignant cells. Elaboration of MMP-9 from local fibroblasts and the BMDC alters the local environment, releases Kit-ligand and VEGF-A which in turn support the growth and development of c-kit expressing cancer cells [Kaplan et al., 2005]. In the model used, metastasis did not occur in the absence of BMDC recruitment, stressing the role for BMDC in orchestrating the growth and aggressive behavior of cancer.

Immune Cells of the Stroma

Monocytes/macrophages, neutrophils, and lymphocytes are recruited to and reside in the tumor stroma. Monocytes are actively recruited into tumors along defined chemotactic gradients. Once in the tumor, they differentite into tumor-associated macrohages (TAMs). TAMs appear to be preferentially attracted to and retained in areas of necrosis and hypoxia where they become phenotypically altered and upregulate hypoxia-induced transcription factors (see below). Macrophages also release a number of factors that influence endothelial cell behavior including VEGF, HGF, MMP2, IL-8. Neutrophils are recognized as stimulators of angiogenesis, via their release of VEGF, HGF, MMP2, and IL-8. Additional immune cell populations have a less well-documented role in carcinogenesis, and are not consistent residents of the stroma with their presence restricted to specific types of tumors. These include myeloid suppressor cells, which have the phenotypic characteristics of both macrophages and granulocytes and the diverse effects of immune suppression, production of MMP9 and VEGF, and the additional ability to directly incorporate into vessel walls [Serafini et al., 2004; Yang et al., 2004]. Mast cells are associated with angiogenesis and tumor induction in skin cancer models [Coussens et al., 2000; Crivellato and Ribatti, 2005], while eosinophils, associated with a host of solid tumors including colon, cervical, lung, breast, and ovary as well as some lymphomas, can influence angiogenesis via VEGF production.

Vascular Cells/Tumor Angiogenesis

Tumors require the formation of a complex vascular network to meet the metabolic and nutritional needs for growth. VEGF is the main factor involved in the formation of tumor vessels. It is secreted by the tumor cells directly and by fibroblasts and inflammatory cells in the stroma and is responsible for the "angiogenic switch" where new vasculature is formed to supply the tumor with nutrients. Tumor vessels formed as a result of VEGF are abnormal; they are non-uniformly distributed and irregularly shaped, inappropriately branched and tortuous, often ending blindly. They do not have the classic hierachical arrangement of arterioles, venules, and capillaries and often form arteriovenous shunts. These vessels are variably fenestrated and leaky leading to high interstitial pressures, further exacerbating tissue hypoxia and stimulating additional VEGF production [Carmeliet, 2005; Dvorak, 2005]. Under the influence of VEGF, tumor vessels are formed by one of several mechanisms including budding of existing vascular networks, recruitment of vascular progenitor cells to form new vascular channels, or "vascular mimicry"—a process by which tumor cell-lined channels contribute to the blood tributaries supplying the tumor [Ribatti et al., 2003].

Budding of existing vessels occurs in response to local growth signals emanating from tumor cells or from entrapped bone marrow-derived hematopoietic cells (HCs) within the tumors [Takura, 2006], CAFs, and inflammatory cells. Heparin sulfate proteoglycans on the tumor cell surface and in the extracellular environment [Stringer, 2006] and the SDF-1/CXCL12 axis [Genis et al., 2006] are critical signaling pathways for growth of new vessels from pre-existing vasculature (Fig. 2).

In addition to sprouting from pre-existing vessels, progenitor cells are recruited from more distant sites for neovascularization. HCs and endothelial cells (ECs) share a common ancestry during embryogenesis, the hemagioblast. Postnatally, CD133, CD34, and VEGFR-2 positive cell subsets in the bone marrow,

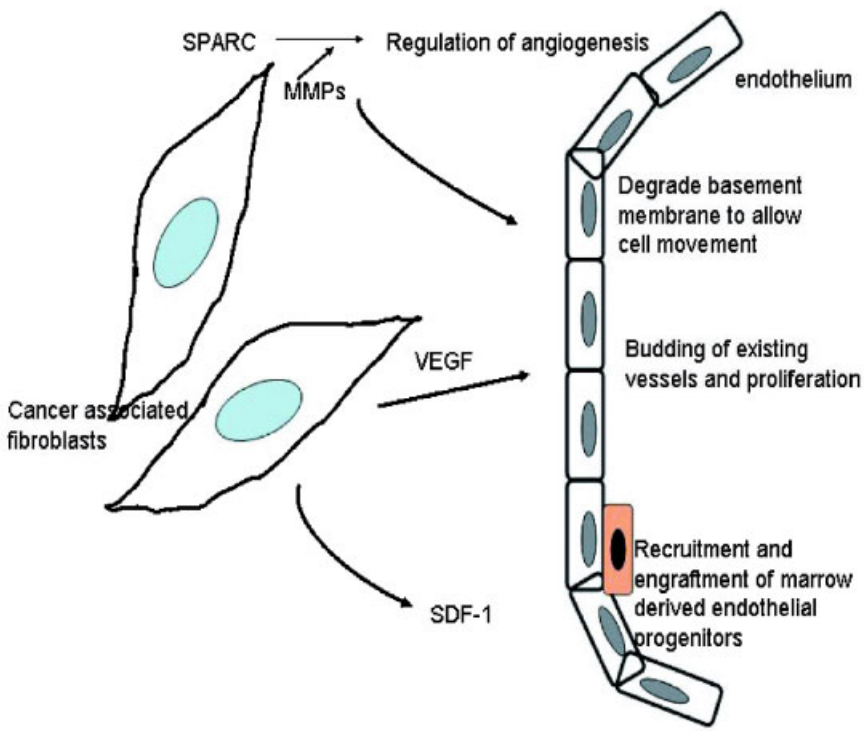

Fig. 2. CAFs drive tumor angiogenesis. CAFs secrete multiple factors which drive angiogenesis including VEGF, MMP, SPARC, SDF-1. VEGF is the primary growth factor driving the abnormal tumor vessel growth. MMPs have a direct effect on angiogenesis through degradation of basement membrane and ECM proteins as well as an indirect effect via proteolytic cleavage of angiogenic factors such as SPARC. SDF-1 secreted by fibroblasts as well as other cell types in the stroma is chemotactic for EPC. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

peripheral blood, and cord blood maintain the functional activity of the hemangioblasts in that they are able to differentiate into both HCs and ECs [Ribatti et al., 2002]. The existence of this adult hemangioblast-like cell along with additional in vivo data upholds the concept that endothelial progenitor cells (EPCs) of bone marrow origin exist, circulate, and contribute to adult angiogenesis. To date, the best marker of these vascular progenitor cells is the expression of CD133. VEGF in the circulation along with hypoxia mobilize marrow-derived EPCs [Takahashi et al., 1999] which are then recruited to tumor sites via a host of chemokines such as VEGF, angiopoietin, stromal cell-derived factor (SDF)-1α [Hattori et al., 2001; Iwaguro et al., 2002; Yamaguchi et al., 2003] secreted by the tumor cells and cells of the stroma. Once recruited, the EPC cells recruit additional EPCs through their own production of VEGF, HGF, G-CSF, and GM-CSF. The contribution of bone marrow-derived EPCs (BMD-EPC) to tumor vasculature is usually low. However, the actual percentage is dependant upon the tumor type, and in some instances, these cells can contribute a substantial portion of ECs.

EXTRACELLULAR MOLECULES

Cytokines and Growth Factors

Cytokines and growth factors are secreted by cells of the stroma as well as by cancer cells into the stroma. These factors are numerous, but for this review we will concentrate on the factors which have conclusively and consistently been shown to directly impact tumor behavior. These include TGF-β, SDF-1, secreted protein acidic rich in cysteine (SPARC), MMP, VEGF, and HIF-1-α.

TGF-β is best known as a growth inhibitor and a potent immunosuppressive factor. Under normal physiological conditions, TGF-β maintains tissue homeostasis through its effects on proliferation and apoptosis, effectively controlling the growth of epithelium, endothelium, neuronal tissue, and HCs. TGF-β also has strong inhibitory effects on the immune response, including reducing T-cell proliferation, inhibiting natural killer cell function, and interfering with antigen presentation. The net effect is to induce tolerance and prevent immune rejection of tissue. While proliferation of normal epithelium is strongly inhibited by TGF-β, cancer cells either lose this growth inhibitory response or usurp the pathway such that TGF-β stimulates proliferation of the cancer cell. Having lost or altered their own response, many tumors gain the ability to express TGF-β which then acts in an autocrine fashion to further stimulate growth, facilitate tumor invasion, and to protect the tumor from attack by the immune system. Effects on the vascular system and tumor angiogenesis include migration of vascular progenitor cells and growth control. CAFs are stimulated to proliferate, increase ECM production, and secrete cytokines. Additionally, TGF-β induces the EMT in cancer cells aiding local invasion as well as facilitating metastatic spread [Siegel and Massague, 2003].

Mesenchymal and marrow-derived stromal cells constitutively secrete SDF-1. Expression is upregulated in inflamed tissues [Houghton et al., 2004], in wounds and in cancer, where it acts to attract cells expressing the receptor, CXCR4. CXCR4 is expressed by an array of cancer cells, and the receptor ligand interaction functions as a mitogen for tumor cells and induces migration in a gradient-specific fashion resulting in local invasion as well as enabling cells to metastasize to distant sites expressing SDF-1 such as the bone marrow and peripheral organs. Marrow-derived EPC also chemotax to a SDF-1 gradient, and are recruited to tumor sites for neovascularization [reviewed in Berger and Kipps, 2006].

SPARC, known as osteonectin or BM-40, is secreted by fibroblasts and inflammatory cells of the stroma. SPARC is secreted in a bioactive form and can undergo further processing by the MMPs (see below) to release additional bioactive fragments. Functions of SPARC include cell–cell deadhesion, inhibition of proliferation, regulation of angiogenesis, and modulation of ECM production and composition [Sangeletti et al., 2003]. SPARC functions to recruit macrophages, leukocytes, and BMDC to tumors; these cells in turn contribute to the functioning of the stroma through direct cell–cell interactions and release of soluble factors.

Degradation and Remodeling Enzymes

In order for tumor cells to invade normal tissue they must lose their connection to each other, and invade and integrate into the surrounding normal structures. Normal tissue is designed to resist such breaches, making

invasion an active process on the part of the cancer cell. Tumor angiogenesis requires active remodeling and integration of new cells into existing structures. To facilitate restructuring, fibroblasts, macrophages, and ECs of the stroma express and secrete MMPs. Secretion of these molecules by the stromal cells is the result of a complex tumor-stroma crosstalk, involving multiple ligands and cellular signaling pathways [Stuelten et al., 2005]. As a family of compounds, the MMPs act to hydrolyze the extracellular proteins of the surrounding tissue which include collagen, laminin, elastin, fibrinogen, fibronectin, and vitronectin [Sternlicht and Werb, 2001; Boire et al., 2005]. From the earliest work addressing the role of MMPs and cancer, there has been a clear connection between certain members of the MMP group, degradation of the ECM, and local cancer cell invasion [Liotta et al., 1980], but the association with metastatic disease has been less clear. In addition to the ECM, MMPs have additional target proteins which include other proteinases, proteinase inhibitors, clotting factors, chemokines and chemotactic factors, growth factors, a variety of cell surface receptors, and cell matrix adhesion molecules [Coussens et al., 2002]. MMPs have also been implicated in initiating the EMT and in promoting genomic instability [Radisky et al., 2005], affording them a prominent role in both tumor progression and prevention. In light of the diverse functions of the MMP molecules, mouse models of cancer examining knockout or knockdown of individual members of the MMP family give varying phenotypes, dependent upon which MMP is altered and what cancer model is used. We briefly describe the prominent members of this family and their association with cancer.

Membrane type matrix metalloprotineases (MT-MMPs) are a subclass of the MMPs which can be expressed on cells of the stroma, and by cancer cells themselves. While most of the MMPs are secreted proteins, the MT-MMPs are membrane bound with a cytoplasmic domain important for cell signaling. MT1-MMP is the best characterized of this group and has been shown to activate MMP2, display its own proteolytic activity against the ECM, and to play a role in bone degradation/formation. During angiogenesis, quiescent ECs become activated and migrate to areas of neovascularization. These cells release MMPs, specifically MT1-MMP, in order to degrade basement membranes and allow cell movement [Genis et al., 2006]. Increased expression of MT1-MMP is seen in glioma, papillary thyroid, breast cancer, and gastric cancer suggesting a role, however, as the absence of MT1-MMP causes severe skeletal defects and early death, in vivo cancer models are lacking. MMP-1, derived from stromal fibroblasts, functions to cleave and activate PAR1 creating PAR1-dependent Ca^{2+} signaling for growth, invasion, and migration [Pei, 2005]. MMP-2, also fibroblast derived, inhibits bFGF-induced angiogenesis with resulting alterations in tumor growth. Mice deficient in MMP2 have markedly reduced tumor angiogenesis and a delay in tumor progression. Additional targets for MMP2 include connective tissue growth factor (CTGF) galectin-1, osteopontin, death receptor, heat shock protein 90 (HSP90), procollagen C proteinase enhancer protein (PCPE), and the membrane bound chemokine, fructalkine. Fractalkine is released from its membrane location by MMP2 as a fully functional domain. Additionally, MMP-2 cleaves fractalkine at positions 4–5, releasing an antagonist form [Overall and Dean, 2006] stressing the sometimes opposing actions of the MMPs. Macrophage-derived MMP9 is responsible for VEGF mobilization from matrix stores—the primary signal for neovascularization. MMP9 is also critical for the recruitment and engraftment of BMDC into tumor vasculature [Jodele et al., 2005]. The contribution of BMDC to the vasculature differs greatly between tumor types, and this contribution will help determine the impact that MMP9 inhibition has on tumor angiogenesis. Macrophage elastase (MMP12) has been less studied, but appears to inhibit the growth of cancer. In a Lewis lung cancer model a deficiency of MMP12 suppressed the growth of lung metastasis, but not the number of metastatic foci [Houghton et al., 2006]. Less is known about the function of MMP12 in other models. As a group, MMP expression is prominent in tumor stroma, and plays a vital role in the local growth, invasion, and migration of cancer cells.

Tissue inhibitors of metalloproteinases (TIMPs) also play a role in local growth and spread of tumors. TIMP-1 has been shown to have an anti-apoptotic effect on bone marrow stromal cells in culture mediated through the PI3-kinase and JNK signaling pathways, and is independent of the effects of TIMP on MMP

activity [Guo et al., 2006]. These findings suggest an effect of the TIMPs on cells within the tumor environment which are of marrow origin and include bone marrow-derived tumor fibroblasts, bone marrow-derived ECs within tumor vasculature, and bone marrow-derived tumor cells.

VEGF is secreted by CAFs and is implicated in many aspects of cancer growth including angiogenesis, ECM remodeling, generation of inflammatory cytokines, and hematopoietic stem cell development. It is a potent vascular growth factor and the main stimulus for tumor angiogenesis (see section on Tumor Angiogenesis). VEGF acts in the generation of inflammatory cytokines and is responsible for the upregulation of several other pro-angiogenic molecules and prometastatic molecules. VEGF plays a crucial role in recruiting VEGF-R1 positive HBM progenitor cells to peripheral sites to initiate the pre-metastatic niche [Kaplan et al., 2005]. The main stimulus for production in tumor stroma is hypoxia, resulting in the recruitment of endothelial progenitor and mesenchymal stem cells to sites of ischemia [Okuyama et al., 2006], which in turn secrete additional VEGF.

IMMUNE REGULATION BY THE STROMA

Through the secretion of cytokines, chemokines, and other factors, **s**tromal cells are instrumental in creating the unique environment of chronic inflammation and immune tolerance, allowing cancer cells exposure to growth factors while avoiding immune-mediated elimination. The individual components of the environment may differ between tumor types and models studied, with immune enhancing and immune suppressing pathways simultaneously activated. However, in order for the tumor to survive, the net result needs to be suppression of any immune response directed toward the tumor cells. Stromal cells are the main source of thrombospondin-1 (TSP-1) which has both positive and negative effects on angiogenesis, and effects on tumor-tissue mediated immune suppression via activation of TGF-β and direct interaction with immune cells [reviewed in Silzle and Randolph, 2004]. MMP cleavage products of several proteins such as fibronectin and collagen are chemotactic for leukocytes, modulate their proliferation and cytokine release [Barilla and Crsons, 2000]. MMPs also cleave cytokines with variable effects. For example, cleavage of IL-1β leads to both activation and inactivation depending upon the cleavage site, and processing of monocyte chemotactic protein (MCP) by the MMPs leads to its downregulation. Direct release of HGF, IGF, and fibroblast activation protein (FAP) drives tumor cell growth while also functioning as immune modulators [reviewed in Silzle and Randolph, 2004].

HYPOXIA AND THE TUMOR MICROENVIRONMENT

Hypoxia in solid tumors is the result of structurally and functionally inadequate vessels and high metabolic demand, and is further aggravated by cancer-induced anemia. Hypoxia correlates with aggressive behavior of tumors, and resistance to therapy. Hypoxia-inducible factors (HIFs) are cellular transcription factors involved in the response to environmental stress. Important targets of the HIF system which are relevant to cancer biology include MDR-1 [Comerford et al., 2002], IGF-2 [Feldser et al., 1999], telomerase [Nishi et al., 2004], and CXCR4/SDF-1 [Ceradini et al., 2004]. The HIFs orchestrate neovasularization, glucose metabolism, survival, and tumor spread in response to hypoxia and are major regulators of tumor cell adaptation to hyoxic stress. In addition, cells with genomic and proteomic changes favoring survival under hypoxic conditions will proliferate, thereby increasing metabolism and demand for nutrients, exacerbating hypoxic conditions which in turn will lead to the selection and expansion of more aggressive clones. VEGF production, induced by hypoxia, leads to inadequate neovascularization, inadequate tissue oxygenation, and a cycle of additional VEGF production (see sections on Tumor Angiogenesis and Extracellular Molecules).

CONTRIBUTIONS OF THE MICROENVIRONMENT TO GENETIC INSTABILITY AND EPIGENETIC CHANGES

As tumors progress, the cells display increased genetic instability. The number of mutations found in tumor cells cannot be accounted for by the rate of mutations occurring in somatic cells, leading to the notion that cancer cells develop a mutator phenotype.

Conditions within the tumor microenvironment including oxidative stress, hypoxia, nutrient deprivation, and low pH contribute to genetic instability through the induction of increased DNA damage enhanced mutagenesis and impaired DNA damage pathways [for a full review of this topic see Bindra and Glazer, 2005].

TARGETING THE MICROENVIRONMENT FOR CANCER CHEMOTHERAPY

The tumor stroma, consisting of cells, structural proteins, and signaling molecules, is recognized as playing a central role in tumor initiation, progression, and metastasis (Fig. 3). The level of aggression is greatly influenced by this environment, providing multiple targets for anti-cancer therapy. Targeting the stroma poses several obstacles, however. The fibroblasts, ECs, and inflammatory cells are themselves not malignant, therefore successful therapy needs to aim at phenotypic changes unique to this population, while avoiding normal cells elsewhere. Additionally, delivery of agents to the stroma can be problematic because of insufficient and defective vascular

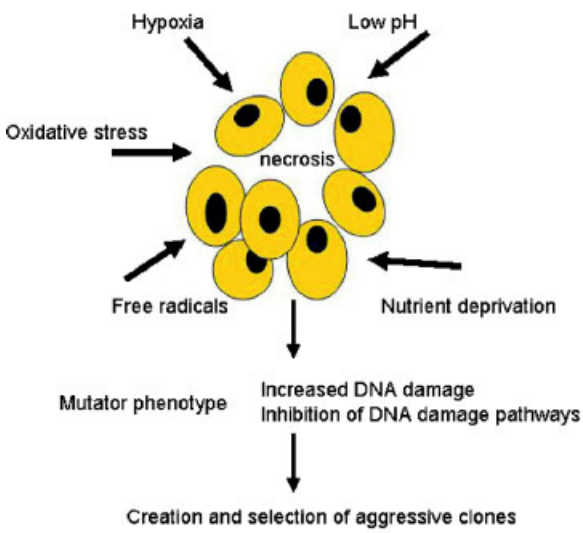

Fig. 3. The tumor stroma contributes to genetic instability of cancer cells. Hypoxia, inadequate delivery of nutrients, decreased pH and free radical formation created within the stroma as a result of insufficient blood supply, and factors released by inflammatory cells and activated fibroblasts drive DNA mutations and the suppression of DNA repair machinery. The mutator phenotype of cancer cells allows the creation and selection of aggressive clones with greater metastatic potential. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

structures, hypoxia, and pH alterations. These challenges should not dampen our enthusiasm for targeting the stroma, but successful approaches will require identifying appropriate targets and designing efficient delivery methods.

Examples of effective anti-stromal therapy include targeting cancer-associated inflammation through the use of non-steroidal anti-inflammatory drugs (NSAIDs). NSAID use is associated with a decrease in the incidence of colorectal cancer [Sandler et al., 2003] and the precursor lesion, adenomas [Baron et al., 2003]. Prevention of other types of cancer has been inferred but not yet confirmed by large trials [Schreinemachers and Everson, 1994; Castelao et al., 2000; Thun et al., 2002], adding support to targeting inflammation for cancer prevention.

The strong association between MMP expression and cancer spurred the development of biologically active MMP inhibitors, and subsequent clinical trials were designed to test the efficacy in a range of tumor types. Results from these trials have been disappointing but not entirely unexpected when one considers all the diverse functions of the various MMPs and realizes that only non-selective MMP inhibitor drugs entered trials. Also, MMP inhibition was employed at late stages of disease, where as preclinical data suggest the most efficacious time for therapy may be early in disease progression. As the net outcome of global MMP inhibition is dependent upon the interplay of multiple factors including the tissue where the tumor arises as well as the stage during which the drugs are given, the current push is for the development of highly selective MMP-inhibitors targeting single family members, and choosing the study population based on known alterations within specific cancer microenvironments.

Present efforts targeting molecules unique to the tumor microenvironment will provide strategies for modifying the tumor environment, while avoiding interrupting normal tissue homeostasis. The success of VEGF blockade [Presta et al., 1997] has encouraged blocking additional unique pathways, such as HIF-1 and HIF-1 target genes, in the hope that modifying the stroma alone or used in combination with conventions like radiation and chemotherapy to increase efficacy. It is through understanding the complex interactions of the stroma with the tumor that these therapies can be devised, and clinical trials developed which will allow the

agents to be tested in appropriate clinical situations.

ACKNOWLEDGMENTS

This study was supported by grants from NIH/NCI grant (1 RO1 CA119061 to G.H.), Funderberg Gastric Cancer award to J.H. and the Our Danny Cancer Fund Fellowship award to H.L.

REFERENCES

Aractingi SJ, Kanitakis S, Euvrard C, Le Danff I, Peguillet K, Khosrotehrani K, Latz O, Carosella ED. 2005. Skin carcinoma arising from donor cells in a kidney transplant recipient. Cancer Res 65:1755–1760.

Barilla ML, Crsons SE. 2000. Fibronectin fragments and their role in inflammatory arthritis. Semin Arthritis Rheum 29:252–265.

Baron JA, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, McKeown-Eyssen G, Summers RW, Rothstein R, Burke CA, Snover DC, Church TR, Allen JI, Beach M, Beck GJ, Bond JH, Byers T, Greenberg ER, Mandel JS, Marcon N, Mott LA, Pearson L, Saibil F, van Stolk RU. 2003. A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med 348(10):891–899.

Berger JA, Kipps TJ. 2006. CXCR4: A key receptor in the crosstalk between tumor cells and their microenvironment. Blood 107:1761–1767.

Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, Washington MK, Neilson EG, Moses HL. 2004. TGF-β signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science 303: 848–851.

Bindra RS, Glazer PM. 2005. Genetic instability and the tumor microenvironment: Towards the concept of microenvironment-induced mutagenesis. Mutat Res 569:75–85.

Boire A, Covic L, Agarwal A, Jacques S, Sherifi S, Kuliopulos S. 2005. Par1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. Cell 120:303–313.

Carmeliet P. 2005. VEGF as a key mediator of angiogenesis in cancer. Oncology 69:4–10.

Castelao JE, Yuan JM, Gago-Dominguez M, Yu MC, Ross RK. 2000. Non-steroidal anti-inflammatory drugs and bladder cancer prevention. Br J Cancer 82:1364–1369.

Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME. 2004. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 10:858–864.

Clayton A, Evans RA, Pettit E, Hallett M, Williams JD, Steadman R. 1998. Cellular activation through the ligation of intercellular adhesion molecule-1. J Cell Sci 111:443–453.

Comerford KM, Wallace TJ, Karhausen J, Louid NA, Montalto MC, Colgan SP. 2002. Hypoxia inducible factor-1 dependent regulation of the mutlidrug resistance (MDR1) gene. Cancer Res 62:3387–3394.

Coussens LM, Tinkle CL, Hanahan D, Werb Z. 2000. MMP-9 supplied by bone marrow derived cells contributes to skin carcinogenesis. Cell 103:481–490.

Coussens LM, Fingleton B, Matrisian LM. 2002. Matrix metalloproteinase inhibitors and cancer: Trials and tribulations. Science 295:2387–2392.

Crivellato F, Ribatti D. 2005. Involvement of mast cells in angiogenesis and chronic inflammation. Curr Drug Targets Inflamm Allergy 4:9–11.

Direkze NC, Hodivala-Dilke K, Jeffery R, Hunt T, Poulsom R, Oukrif D, Alison MR, Wright NA. 2004. Bone marrow contribution to tumor associated myofibroblasts and fibroblasts. Cancer Res 64:8492–8495.

Dvorak HF. 2005. Angiogenesis: Update. J Thromb Haemost 3:1835–1842.

Feldser D, Agani F, Iyer NV, Pak B, Ferreira G, Semenza GL. 1999. Reciprocal positive regulation of hypoxia inducible factor 1 alpha and insulin like growth factor 2. Cancer Res 59:3915–3918.

Genis L, Galvez BG, Gonzalo P, Arroyo AG. 2006. MT1-MMP: Universal or particular player in angiogenesis? Cancer Metastasis Rev 25:77–86.

Guo LJ, Luo XH, Xie H, Zhou HD, Yuan LQ, Wang M, Liao EY. 2006. Tissue inhibitor of matrix metalloproteinase-1 suppresses apoptosis of mouse bone marrow stromal cell line MBA-1. Calcif Tissue Int 78:285–292.

Hattori K, Dias S, Heissig B, Hackett NR, Lyden D, Tateno M, Hicklin DJ, Zhu Z, Witte L, Crystal RG, Moore MA, Rafii S. 2001. Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. J Exp Med 193:1005–1014.

Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Goldenring J, Fox JG, Wang TC. 2004. Gastric cancer originating from bone marrow-derived cells. Science 306:1568–1571.

Houghton AM, Grisolano JL, Baumann ML, Kobayashi DK, Hautamaki RD, Nehring LC, Cornelius LA, Shapiro SD. 2006. Macrophage elastase (matrix metalloproteinase-12) suppresses growth of lung metastases. Cancer Res 66:6149–6155.

Iwaguro H, Yamaguchi J, Kalka C, Murasawa S, Masuda H, Hayashi S, Silver M, Li T, Isner JM, Asahara T. 2002. Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. Circulation 105:732–738.

Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilsonet EG. 2002. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest 110:341–350.

Jodele S, Chantrain CF, Blavier L, Lutzko C, Crooks GM, Shimada H, Coussens LM, Declerck YA. 2005. The contribution of bone marrow derived cells to the tumor vasculature in neuroblastoma is matrix metalloproteinase-9 dependent. Cancer Res 65:3200–3208.

Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, l Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S, Lyden D. 2005. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 438:820–827.

Kopelovich L. 1982. Genetic predisposition to cancer in man: In vitro studies. Int Rev Cytol 77:63–88.

Kuperwasser C, Chavarria T, Wu M, Magrane G, Gray JW, Carey L, Richardson A, Weinberg RA. 2004. Reconstruction of functionally normal and malignant human breast

tissues in mice. Proc Natl Acad Sci USA 101:4966–4971.

Kurose K, Gilley K, Matsumoto S, Watson PH, Zhou XP, Enget C. 2002. Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. Nat Genet 32:355–357.

Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shaffie S. 1980. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature 284:67–68.

Nishi H, Nakada T, Kyo S, Inoue M, Shay JW, Isaka K. 2004. Hypoxia inducible factor 1 mediates upregulation of telomerase (hTERT). Mol Cell Biol 24:6076–6083.

Okuyama H, Krishnamachary B, Zhou YF, Nagasawa H, Bosch-Marce M, Semenza GL. 2006. Expression of vascular endothelial growth factor receptor 1 in bone marrow derived mesenchymal cells is dependent on hypoxia inducible factor 1. J Biol Chem 281:15554–15563.

Overall CM, Dean RA. 2006. Degradomics: Systems biology of the protease web. Pleiotropic roles of MMPs in cancer. Cancer Metastasis Rev 25:69–75.

Pei D. 2005. Matrix metalloproteinases target protease-activated receptors on the tumor cell surface. Cancer Cells 7:207–208.

Peters BA, Diaz LA, Polyak K, Meszler L, Romans K, Guinan EC. 2005. Contribution of bone marrow-derived endothelial cells to human tumor vasculature. Nat Med 11:261–262.

Petersen OW, Nielsen HL, Gudjonsson T, Villadsen R, Rank F, Niebuhr E, Bissell MJ, Rønnov-Jessen L. 2003. Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma. Am J Pathol 162:391–402.

Presta LG, Chen H, O'Connor SJ, Chisholm V, Meng YG, Krummen L, Winkler M, Ferrara N. 1997. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res 57:4593–4599.

Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, Werb Z, Bissell MJ. 2005. Rac1b and reactive oxygen species mediate MMP3-induced EMT and genomic instability. Nature 436:123–127.

Ribatti D, Vacca A, Nico B, Ria R, Dammacco F. 2002. Cross talk between hematopoiesis and angiogenesis signaling pathways. Curr Mol Med 2:537–547.

Ribatti D, Vacca A, Dammacco F. 2003. New non-angiogenesis dependent pathways for tumor growth. Eur J Cancer 39:1835–1841.

Ronnov-Jessen L, Petersen OW. 1993. Induction of α-smooth muscle actin by transforming growth factor-β1 in quiescent human breast gland fibroblasts. Implications for myofibroblast generation in breast neoplasia. Lab Invest 68:696–707.

Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC, Bernad A. 2005. Spontaneous human adult stem cell transformation. Cancer Res 65:3035–3039.

Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, Petrelli N, Pipas JM, Karp DD, Loprinzi CL, Steinbach G, Schilsky R. 2003. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med 348:883–890.

Sangeletti S, Stoppacciaro A, Guiducci C, Torrisi MR, Colombo MP. 2003. Leukocyte rather than tumor-produced SPARC determines stroma and collagen type IV deposition in mammary carcinoma. J Exp Med 198: 1475–1485.

Schor SL. 1986. Occurrence of a fetal fibroblast phenotype in familial breast cancer. Int J Cancer 37:831–836.

Schreinemachers DM, Everson RB. 1994. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. Epidemiology 5:138–146.

Serafini P, De Santo C, Marigo I. 2004. Derangement of immune responses by meyloid suppressor cells. Cancer Immunol Immunother 53:64–72.

Serakinci NP, Guldberg JS, Burns B, Abdallah H, Schrodder H, Jensen T, Kassem M. 2004. Adult human mesenchymal stem cell as a target for neoplastic transformation. Oncogene 23:5095–5098.

Siegel PM, Massague J. 2003. Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer. Nature 3: 807–820.

Silzle T, Randolph GJ. 2004. The fibroblast: Sentinel cell and local immune modulator in tumor tissue. Int J Cancer 108:173–180.

Sternlicht MD, Werb Z. 2001. How matric metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 17: 463–516.

Stringer SE. 2006. The role of heparan sulphate proteoglycans in angiogenesis. Biochem Soc Trans 34:451–453.

Stuelten CH, DaCosta Byfield S, Arany PR, Karpova TS, Stetlerr-Stevenson WG, Roberts AB, Anita B. 2005. Breast cancer cells induce stromal fibroblasts to express MMP-9 via secretion of TNF-alpha and TGF-beta. J Cell Sci 118:2143–2153.

Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T. 1999. Ischemia and cytokine induced mobilization of bone marrow derived endothelial progenitor cell for neovascularization. Nat Med 5:434–438.

Takura N. 2006. Role of hematopoietic lineage cells as accessory components in blood vessel formation. Cancer Sci 97:568–574.

Thun MJ, Henley SJ, Patrono C. 2002. Nonsteroidal anti-inflammatory drugs as anticancer agents: Mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst 94:252–266.

Tuhkanen H. 2004. Genetic alterations in the peritumoral stromal cells of malignant and borderline epithelial ovarian tumors as indicated by allelic imbalance on chromosome 3p. Int J Cancer 109:247–252.

Yamaguchi J, Kusano KF, Masou O, Kawamoto A, Silver M, Murasawa S, Bosch-Marce M, Masuda H, Losordo DW, Isner JM, Asahara T. 2003. Stromal cell-derived factor 1 effects an ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. Circulation 107:1316–1322.

Yang L, DeBusk LM, Fukuda K. 2004. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor bearing host directly promotes tumor angiogenesis. Cancer Cell 6:409–421.

Zeisberg M, Strultz F, Muller GA. 2000. Role of fibroblast activation in inducing interstitial fibrosis. J Nephrol 13: S111–S120.