Therapeutic targeting of the tumor microenvironment

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Introduction

Cancer is not a solo performance, but rather an ensemble production. Tumor cells play the leading villains, with a diverse supporting cast of normal cells that can be recruited to aid their malignant progression. The supporting players in the tumor microenvironment include stromal fibroblasts, infiltrating immune cells, the blood and lymphatic vascular networks, and the extracellular matrix (Figure 1). Normal cells can contribute both positive and negative signals to the tumor. They can be co-opted or modified by the cancer cells to produce a variety of growth factors, chemokines, and matrix-degrading enzymes that enhance the proliferation and invasion of the tumor. In addition, these conscripted normal cells may provide a support system for tumor cells to fall back on following traditional cytotoxic therapies. Furthermore, environmental conditions within the tumor, caused by changes in the stroma, such as increased interstitial fluid pressure and changes in vascular flow, reduce the effective delivery of anticancer drugs. Multitargeted approaches, in which tumor cells and co-opted cells in the microenvironment are simultaneously inhibited, may offer a more efficient way to treat cancer by circumventing these problems. One advantage of therapies targeting the microenvironment is that these nontumor cells are presumably genetically stable, which is in contrast to tumor cells that are known to be genetically unstable and thus can accumulate adaptive mutations and rapidly acquire drug resistance. However, a limitation of perturbing the cells in the tumor microenvironment is that a delicate balance exists between their tumor-inhibitory and tumor-promoting functions. As such, we need to identify and target the key molecular differences between these cells under normal tissue homeostasis versus when they have been co-opted or altered by the tumor microenvironment. Recent experiments have suggested various approaches to target different cell types in the tumor microenvironment, and will be the emphasis of this review.

The tumor stroma: An active player in cancer

All tissues require an extracellular network to provide structural support and facilitate the continuous cell-cell communication that maintains tissue homeostasis. An important regulator of normal tissue behavior is the extracellular matrix (ECM), which surrounds cells and is composed of many types of macromole-

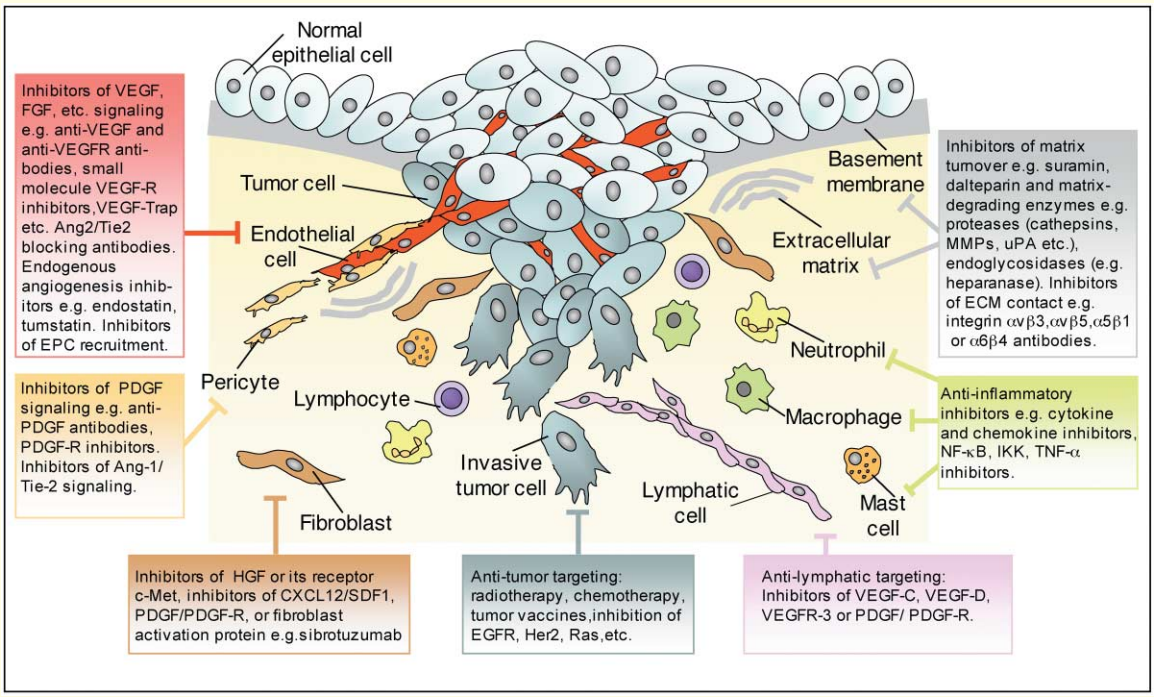

Figure 1. Therapeutic strategies that have been designed or suggested to target different cells in the tumor microenvironment

These strategies include targeting the tumor cells themselves, with traditional therapies such as chemotherapy and radiotherapy (which will also kill normal cells) and targeted vaccines, or molecular approaches to inhibit oncogenes such as Ras, EGFR, and Her2. Among the targets to inhibit in normal cells are members of the VEGF/VEGFR family (vascular or lymphatic endothelial cells), PDGF/PDGFR family (pericytes and potentially fibroblasts and lymphatics), matrix-degrading enzymes (made by inflammatory cells in large part, with BM/ECM components as substrates), and inhibitors of chronic inflammation.

Table 1. Drugs targeting the tumor microenvironment

Name	Cellular target	Mechanism of action	Status
Avastin (Bevacizumab)	Endothelial cells (ECs)	Humanized monoclonal antibody against VEGF-A	FDA-approved
Neovastat (AE941)	ECs	Natural antiangiogenic compound isolated from shark cartilage that inhibits VEGF signaling and MMP activity and specifically induces EC apoptosis	Phase III
PTK787 (vatalanib)	ECs	Small molecule receptor tyrosine kinase (RTK) inhibitor, inhibits VEGF-R2, but also VEGFR-1, VEGFR-3, PDGFR-β	Phase II/III
Interferon-alpha	ECs	Inhibits angiogenesis in part by downregulating bFGF expression	Phase II/III
Combretastatin A4	ECs	Specifically interferes with endothelial microtubule assembly, resulting in rapid vascular dysfunction in tumors	Phase II
LY317615	ECs	Inhibitor of protein kinase Cβ	Phase II
Atrasentan	ECs	Small molecule selective inhibitor of endothelin A receptor	Phase II
ZD6474	ECs	Small molecule RTK inhibitor, inhibits VEGF-R2 and EGFR, and to a lesser extent VEGFR-1 and R-3	Phase I/II
VEGF-Trap	ECs	Soluble VEGF receptor: composite fusion protein of VEGFR-1 and VEGFR-2 with Fc fragment of IgG	Phase I/II
2-ME	ECs	2-Methoxyestradiol (2-ME) is a small molecule inhibitor that blocks angiogenesis in part by HIF-1α downregulation	Phase I
Thalidomide	ECs, other cell types	Glutamic acid derivative that inhibits angiogenesis, in part by inhibition of NF-κB, TNF-α, IL-6, and VEGF; also increases apoptosis and stimulates immune response	Phase III
CC-5013	ECs, other cell types	Synthetic analog of thalidomide, with more potent inhibition and less toxic side effects	Phase II/III
Gleevec (imatinib)	Pericytes, stromal fibroblasts	RTK inhibitor: in addition to inhibiting Bcr-Abl kinase, also inhibits PDGF-R and c-Kit kinases	FDA-approved
SU11248	Pericytes, ECs	Small molecule RTK inhibitor, inhibits VEGF-R family members (VEGFR-1, -2, -3), PDGFR-β, and CSF-1R	Phase II
Vitaxin	ECM	Humanized monoclonal anti-αvβ3 antibody	Phase II
Volociximab	ECM	Humanized monoclonal anti-α5β1 antibody	Phase II
Cilengitide (EMD121974)	ECM	Cyclic peptide inhibitor of αvβ3 and αvβ5	Phase I/II
Suramin	Inhibits ECM turnover	Polysulfonated napthylurea; inhibits matrix remodeling and blocks FGF, PDGF, IGF-1, TGF-α, and TGF-β function	Phase I/II
Sibrotuzumab	Carcinoma-associated fibroblasts	Humanized monoclonal antibody against fibroblast activation protein	Phase I
PI-88	Inflammatory cells, ECs	Heparan sulfate mimetic; inhibits heparanase activity and function of heparin-binding growth factors	Phase I/II

Table listing agents that target different cells in the tumor microenvironment that are currently in clinical trials for cancer or have been approved by the FDA. Abbreviations: EC, endothelial cell; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; EGFR, epidermal growth factor receptor; HIF-1, hypoxia-inducible factor; NF-κB, nuclear factor of κB; TNF, tumor necrosis factor; IL-6, interleukin 6; CSF-1R, colony-stimulating factor receptor; ECM, extracellular matrix; IGF, insulin-like growth factor; TGF, transforming growth factor. See Supplemental Data at http://www.cancercell.org/cgi/content/full/7/6/513/DC1/ for table and references in full.

cules, including collagen, laminin, fibronectin, and heparan sulfate proteoglycans (HSPGs). These proteins are linked in an intricate, three-dimensional matrix (Kalluri, 2003). One specialized ECM, the basement membrane (BM), separates the epithelium from the stroma, and underlies endothelial cells, pericytes, and other cell types. Maintaining organ homeostasis can prevent neoplastic transformation in normal tissues by ensuring firm cell-cell contacts, mediated by tight-junction proteins and cell adhesion molecules, such as β1 integrins and E-cadherin (Weaver et al., 1997; Wrobel et al., 2004; reviewed in Bissell and Radisky, 2001). In tumors, these protective constraints can be overridden by remodeling of the BM/ECM.

BM/ECM remodeling is mediated in an orchestrated manner by several families of matrix-degrading enzymes, including proteases of the cysteine, serine, and matrix metalloprotease (MMP) classes, as well as endoglycosidases such as heparanase (reviewed in Carmeliet, 2003; Vlodavsky et al., 2002). Controlled BM/ECM degradation is necessary for angiogenesis and invasion of tumor cells, into both the surrounding normal tissue and the blood and lymphatic systems. The BM/ECM is also a rich source of sequestered heparin binding progrowth and proangiogenic factors, which are made available following increased production of matrix-degrading enzymes (reviewed in Pupa et al., 2002). In many cancers, matrix-degrading enzymes are provided by infiltrating innate immune cells (Coussens et al., 2000; Coussens and Werb, 2002; Pollard, 2004), and the inhibition of certain enzymes, such as cysteine cathepsins and heparanase, offers the potential to block multiple nodes in the tumor microenvironment (Joyce et al., 2004; Joyce et al., 2005) (Table 1).

However, caution should be exercised in considering broad-spectrum inhibitors of matrix-degrading enzymes for clinical use, as ECM degradation is a delicate balance. Some matrix-degrading enzymes such as MMPs can also release antiangio-

genic proteins such as endostatin, angiostatin, and tumstatin (Hamano et al., 2003; Kalluri, 2003), which inhibit tumorigenesis. This inhibitory role of certain MMPs may explain, at least in part, the failure of the broad-spectrum MMP inhibitors (MMP-I) in the clinic. This example highlights the importance of certain criteria in considering antistromal therapies, which include the appropriate patient selection, the requisite demonstration that drugs specifically inhibit their target in these patients, and the selection of the appropriate endpoints for what is likely to be cytostatic rather than cytotoxic therapy (see Coussens et al., 2002 for a discussion of the MMP inhibitor trials).

Additional therapeutic targets in the extracellular milieu include the integrins, a family of heterodimeric receptors that connect cells to ECM proteins and transduce intracellular signals. Several integrins, including $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha 5\beta 1$, and $\alpha 6\beta 4$, are upregulated in cancer, and are expressed on endothelial cells, tumor cells, or both (Guo and Giancotti, 2004; Hood and Cheresh, 2002). Based on the known functions of these integrins, blocking these proteins would be expected to interfere with cell survival, angiogenesis, cell adhesion, and motility, thus impacting tumor viability. Indeed, disruption of individual integrins using antibodies or small molecule inhibitors produced encouraging results in animal models, and some of these drugs are now in clinical trials (reviewed in Jin and Varner, 2004) (Table 1). However, contradicting results have been observed between pharmacologic versus genetic ablation of αv integrins, with αv knockout mice showing enhanced pathological angiogenesis, whereas antiangiogenic results were obtained with the inhibitors (reviewed in Hynes, 2002). Thus, it will be of particular interest to see how the $\alpha v\beta 5$ and $\alpha v\beta 3$ antagonists perform in the ongoing phase II trials (Table 1). Another compelling reason to target integrins is that some of them have been shown to be involved in cell adhesion-mediated drug resistance (CAM-DR), which has been observed in a variety of cancer cell types, including small cell lung cancer, myeloma, and glioma cells, among others (Damiano et al., 1999; Sethi et al., 1999; reviewed in Shain and Dalton, 2001). $\beta 1$-integrin blocking antibodies have been shown to abrogate CAM-DR (Sethi et al., 1999). Thus, ECM remodeling during tumorigenesis could contribute to CAM-DR by both altering the integrin repertoire and affecting the local ECM composition (Morin, 2003). Based on current results, $\beta 1$ integrin appears to be a promising therapeutic target.

Fibroblasts are another essential component of the tissue stroma, and play an important role in preventing the progression of transformed epithelial cells. This has been demonstrated by coculture experiments in which normal fibroblasts prevented transformation of initiated prostatic epithelial cells (Olumi et al., 1999), and could even promote the reversion of neoplastic epithelial cells (Hayashi and Cunha, 1991). Recent elegant genetic experiments in mice suggest that transforming growth factor β (TGF-β) is one of the fibroblast-supplied factors involved in suppression of epithelial transformation, in part by controlling c-Myc and c-Met signaling in the adjacent tumor cells via a paracrine mechanism involving hepatocyte growth factor (HGF) (Bhowmick et al., 2004a). Simply rendering fibroblasts unresponsive to TGF-β by genetic inactivation of the TGF-βRII receptor resulted in transformation of the otherwise genetically normal epithelial cells in the prostate and forestomach, a striking demonstration of the powerful influence of stromal fibroblasts on adjacent epithelial transformation.

Conversely, many studies have shown that fibroblasts resident in established tumors differ considerably from those in normal tissues, and are "activated," highly proliferative, and display typical markers of smooth muscle differentiation (Bhowmick et al., 2004b). These cells are termed myofibroblasts or carcinoma-associated fibroblasts (CAFs), and have been shown to enhance malignant epithelial transformation (Hayward et al., 2001; Olumi et al., 1999; Orimo et al., 2005; reviewed in Bhowmick et al., 2004b). Thus, stromal fibroblasts can have a bipolar role in cancer, depending on their differentiation state and the stage of tumor development. Molecules enriched in activated fibroblasts, such as the fibroblast activation protein (FAP) (Garin-Chesa et al., 1990; Park et al., 1999), CXCL12/ stromal derived factor-1 (Allinen et al., 2004; Orimo et al., 2005), HGF (Bhowmick et al., 2004a), and cathepsin K (Allinen et al., 2004), could provide promising selective targets in the tumor stroma, with encouraging evidence to date in preclinical models and a phase I clinical trial for an anti-FAP antibody, sibrotuzumab (Cheng et al., 2002; Scott et al., 2003) (Table 1).

Targeting the tumor vasculature: Endothelial cells, pericytes, and endothelial progenitor cells

It is now well established that in order for a tumor to grow beyond a certain size, it needs to recruit its own blood supply to deliver oxygen and nutrients (Hanahan and Folkman, 1996). This size limitation is governed by the diffusion limit for oxygen from the nearest blood vessel, which is approximately 100–200 μm (Folkman et al., 2000). The tumor vasculature is derived by angiogenesis, new blood vessel growth from pre-existing vessels, and vasculogenesis, the recruitment of circulating endothelial progenitor cells (Carmeliet, 2003; Rafii et al., 2002). The concept of angiogenesis as a target for cancer therapy, initially proposed by Folkman (Folkman, 1971), was met with skepticism for decades, but is now widely accepted and being applied to the armament of cancer therapeutics. A plethora of antiangiogenic agents inhibiting either angiogenic growth factors or their receptors have been developed (see Table 1 for examples) and tested in preclinical experiments (Papetti and Herman, 2002).

Recently, tumor vascular targeting has been expanded to include pericytes, which provide both survival signals and structural support to endothelial cells, contributing to a mature, functional vasculature. The process of vascular maturation involves interactions between endothelial cells and pericytes, employing several growth factor signaling pathways; and PDGF-B/PDGFRβ, VEGF-A/VEGFR2, TGF-$\beta 1$, and the Angiopoietin/Tie-2 system (Carmeliet, 2003). When pericytes are not present, or cannot produce VEGF in mouse models, the endothelium is now vulnerable to VEGF blockade (Benjamin et al., 1999). One way to reduce pericyte coverage is to block the signaling pathways involved in recruiting pericytes to endothelial cells. PDGFR inhibitors offer a means to do this and have been tested as single agents, but with limited efficacy (reviewed in Pietras et al., 2003). However, combinations of PDGFR antagonists with a VEGFR2 inhibitor have been shown to greatly perburb pericyte-endothelial cell interactions and resulted in tumor regression in a mouse cancer model (Bergers et al., 2003). It is important to note that the pericyte-endothelial connections in the normal tissues examined did not appear to be affected by this treatment (Bergers et al., 2003), suggesting there are differences between these cell types in tumors and normal tissues which can be therapeutically exploited. Modifications of this therapy have proven even more effective. For example, when an adapted chemotherapeutic regimen is added, in which tumors are first "debulked" using high-dose

chemotherapy, and then treated with continuous low-dose (metronomic) chemotherapy in combination with RTK inhibitors (Pietras and Hanahan, 2005), tumor regression and survival are markedly improved.

Another way to potentially block a tumor's blood supply is to prevent endothelial progenitor cells (EPCs) from either homing to the tumor site or eliciting their vasculogenic program once there. There is considerable controversy as to the exact contribution of EPCs to tumor endothelium, with estimates varying from 0 to >90% in mouse cancer models (De Palma et al., 2003; Li et al., 2004: Lyden et al., 2001; Rafii et al., 2002; Rajantie et al., 2004), contrasting with a <5% median contribution in the human cancers that have been analyzed (Hilbe et al., 2004; Peters et al., 2005; Yu et al., 2004). While simultaneous inhibition of VEGFR1 and VEGFR2 resulted in tumor regression in mouse xenograft models, and was suggested to be a consequence of a reduction in the recruitment of EPCs (Lyden et al., 2001; Rafii et al., 2002), it remains to be seen whether EPC reduction will ever be a viable antiangiogenic option in the clinic. However, the quantitation of EPCs or viable circulating endothelial cells (CEC) in the peripheral blood may at least offer a potential surrogate marker for monitoring the correct administration and subsequent efficacy of antiangiogenic therapies (Shaked et al., 2005; Willett et al., 2004; reviewed in Kerbel and Folkman, 2002).

Given the various successes of antiangiogenic drugs in mouse cancer models, it was somewhat surprising that the initial results of these agents tested singly in the clinic were not particularly encouraging (reviewed in Garber, 2002). One possible explanation for the discrepancy between preclinical and clinical results is that subcutaneous xenograft models are often used in preclinical testing of anticancer agents, including antiangiogenic drugs. The particular concern with using these models to test antistromal therapies is that the tumor microenvironment of cancer cells growing subcutaneously in a nude mouse may be very different from the microenvironment of endogenous tumors. This may result in substantial molecular changes, such as differences in the profile of vascular-specific proteins (Joyce et al., 2003). In this regard, orthotopic transplantation models or genetically engineered mice (GEM) may more accurately recapitulate the tumor microenvironment of each organ-specific cancer.

More recent data from the clinical trials of the VEGF-specific antibody, bevacizumab (Avastin) (Table 1), showed that in patients with metastatic colorectal cancer, there was a significant survival benefit when combined with chemotherapy (Ferrara et al., 2004; Hurwitz et al., 2004), leading to the FDA approval of bevacizumab and renewing confidence in antiangiogenic therapies. Recently reported interim analyses from phase III trials in non-small cell lung cancer and metastatic breast cancer show similar survival improvements when Avastin is combined with standard chemotherapy, as the first-line therapy (see http://cancer.gov/newscenter/pressreleases/AvastinBreast and http://www.gene.com/gene/news/press-releases/display.do?method=detail&id=8207). This suggests that combinatorial approaches targeting both tumor cells and supporting cells will likely prove most effective in treating human cancers. Intriguingly, VEGF receptors have been shown to be expressed in some human cancers, including colon cancer (Ryden et al., 2003; Fan et al., 2005), thus raising the possibility that anti-VEGF therapies may simultaneously target endothelial and tumor cells in this subset of cancers.

Improving drug delivery to the tumor

The success of many anticancer therapies, whether they target the tumor or host cells, depends upon effective drug delivery. One of the reasons that many chemotherapeutic regimens may ultimately fail is that in addition to the development of multidrug resistance, microenvironmental influences, such as the previously mentioned CAM-DR, and increases in interstitial fluid pressure (IFP) can affect the bioavailability and efficacy of anticancer agents (reviewed in Morin, 2003). In order for an anticancer agent to be therapeutically effective, it should be uniformly distributed throughout the tumor circulation, cross the vessel wall, and pass through the extracellular matrix. Tumors create multiple obstacles to drug transport, and as the microenvironment can be heterogenous within a tumor, the requirements for effective drug delivery may vary considerably. One of these barriers is an increase in IFP within tumors (particularly at the tumor core) that is significantly higher than in normal tissues (reviewed in Heldin et al., 2004). Elevated IFP is caused in part by changes in ECM composition, fibroblast-mediated contraction of the interstitial space, and a nonoperational lymphatic system within the tumor. Another barrier results from the chaotic nature of the tumor vasculature, as tumor vessels are dilated, leaky, and tortuous (Carmeliet and Jain, 2000), which further contributes to decreased drug delivery.

Strategies to overcome these environmental barriers, by reducing IFP or "normalizing" the vasculature for example, have been championed by Jain, Heldin, and others (Heldin et al., 2004; Jain, 2001; Jain, 2005). In fact, these two phenomena are closely linked: vessel normalization, in which inefficient and immature blood vessels are selectively eliminated, results in decreased IFP and increased uptake of drugs in the tumor. The administration of VEGFR2-blocking antibodies, PDGF antagonists, and TGFβ inhibitors, among others, effectively reduces IFP, resulting in enhanced drug uptake (Lammerts et al., 2002; Pietras et al., 2002; Tong et al., 2004), as exemplified by the combinatorial efficacy of chemotherapy and PDGF inhibitors in reducing tumor growth in preclinical models (Pietras et al., 2002). An exciting recent development has been the demonstration in the clinic that even a single dose of the VEGF-specific antibody, bevacizumab, results in vascular normalization and decreased IFP (Willett et al., 2004). However, getting the timing right will be crucial with these agents. The prediction is that normalization of the tumor vasculature would result in a temporary increase in drug and oxygen uptake, before the tumor vessels (and thus the delivery system) are ultimately destroyed. In this regard, establishing the correct treatment "window" for each inhibitor, or for drugs in combination, will be essential. The proof of this concept was elegantly demonstrated using VEGFR2 blockade followed by radiation therapy in an orthotopic glioma model. The effects of radiation synergized with the antiangiogenic, normalization therapy, but only in a very narrow treatment window (Winkler et al., 2004).

Inflammation and cancer

The association between chronic inflammation and cancer has been noted by epidemiologists for many years, and has recently come to the fore of cancer research, as evidence accumulates of the causal link between the two. Among the human cancers associated with chronic inflammation are colorectal, gastric, bladder, liver, lung, pancreatic, and cervical cancers, in which the inflammatory stimulus can range from chronic pancreatitis to *Heliobacter pylori* infection (Balkwill et al., 2005;

Coussens and Werb, 2002). While infiltrates of adaptive immune cells, particularly CD4+ and CD8+ T cells, in tumors are typically associated with a more favorable prognosis, in cancers associated with chronic inflammation, the infiltrating cells are of the innate immune class, and invariably correlate with a poor prognosis (Pollard, 2004; Vakkila and Lotze, 2004). The innate immune infiltrate predominantly consists of cells of the myeloid lineage (macrophages, granulocytes, mast cells, neutrophils, etc.), which produce chemokines, angiogenic growth factors, and matrix-degrading enzymes, contributing to a rich environment for tumor growth and invasion (Coussens and Werb, 2002; Pollard, 2004). This class of inflammatory cells can also enhance the genomic instability of the tumor through the production of reactive oxygen and nitrogen species, which can form peroxynitrate, a DNA-damaging agent (Maeda and Akaike, 1998; reviewed in Hussain et al., 2003).

These observations would suggest that reducing chronic inflammation would lower the risk of developing cancer, and indeed epidemiological studies have demonstrated that people taking nonsteroidal anti-inflammatory drugs (NSAIDs) have a clear reduction in their risk of developing colorectal cancer (Thun et al., 1991), and possibly other tumors. As a result, there were high expectations for the next-generation NSAIDs, the selective COX-2 inhibitors, in the prevention and treatment of cancers associated with chronic inflammation. However, the current controversy surrounding the association of long-term high-dose COX-2 inhibitor use with an elevated risk of cardiovascular events (Bresalier et al., 2005) indicates that alternative drugs or molecular targets will need to be identified. Additional proinflammatory factors that are potential targets for cancer prevention and treatment include IκB kinase (IKK), the upstream kinase that activates NF-κB (Karin et al., 2004; Lam et al., 2005), TNF-α (Palladino et al., 2003), interleukins IL-1, IL-6, and IL-8, and certain chemokines and their receptors (Balkwill, 2004), inhibitors of many of which are currently being tested in clinical trials for inflammatory conditions.

An alternative strategy could be to specifically target the protumorigenic factors supplied by innate immune cells during chronic inflammation, rather than systemic inhibition of the inflammatory response per se. Of course, this requires identifying the proteins that are either selectively expressed or highly upregulated in tumor-infiltrating immune cells. Tumor-infiltrating immune cells undergo a processive maturation or "education" within the tumor microenvironment (reviewed by Pollard, 2004), resulting in molecular and phenotypic changes that could be exploited therapeutically. Some molecules that are upregulated, though not exclusively expressed by these cells, include urokinase plasminogen activator receptor (uPAR) (Hildenbrand et al., 1999), CSF-1 (Lin et al., 2001), MMP-9 (Coussens et al., 2000; Giraudo et al., 2004), cathepsins (Joyce et al., 2004), and heparanase (Joyce et al., 2005); pharmacologic or genetic ablation of these molecules has been shown to significantly perturb tumor progression in animal models. Future identification of genes that are truly specific to tumor-infiltrating innate immune cells and functionally contribute to tumorigenesis should further enhance the antitumor selectivity of this approach.

Finally, in addition to switching innate immune cells toward a protumorigenic function, changes in the tumor microenvironment can actively contribute to immune tolerance, preventing rejection of the tumor by the immune system. One way this can occur is by changes in the balance of cytokines (increased VEGF, TGFβ, IL-10, IL-6, COX-2, etc., and reduced IL-4, IL-12, IFN-α, IFN-γ, and GM-CSF) within the tumor, which actively suppresses dendritic cell (DC) maturation. This significantly dampens the antigen presenting function of DCs, which then contributes to immune tolerance (recently reviewed in Zou, 2005). Various strategies have been adopted to break immune tolerance, including systemic administration of effector cytokines that enhance the immune response (e.g., IL-2, IL-12, IFN-γ). While there is a therapeutic benefit from these treatments in some patients with melanoma, renal cell carcinoma, and prostate cancer, there are significant side effects associated with prolonged use (see Dranoff, 2004 for review). Thus, cytokine administration directly into the tumor (Forni et al., 1988) or strategies to restore the cytokine imbalance in a more localized manner may be more effective and less toxic.

The metastatic microenvironment

In any discussion of the tumor microenvironment, we should also consider the environmental conditions in which metastatic tumors develop. Metastatic cells need an appropriate microenvironment in which they can survive and proliferate (Fidler, 2003). While experimental systems have shown that tumor cells arrive at secondary sites at relatively high rates, they only thrive in certain, stereotypical sites (Chambers et al., 2002). For example, prostate cancer cells predominantly metastasize to bone, whereas colorectal cancer cells preferentially metastasize to the liver. This suggests that tumor cells from a given tissue of origin may be more prone than others to possess the capabilities that are necessary to invade and prosper in a particular distant organ. Thus, defining the molecular microenvironment in the organs to which tumor cells successfully metastasize is of vital importance in selecting targets for interfering with either the homing or the survival of metastatic cells.

An important advance in this direction came from the gene expression analysis of chemokines and their receptors in breast cancer (Muller et al., 2001). The authors found that two chemokine receptors (CXCR4 and CCR7) were highly expressed on metastatic breast cancer cells. Their respective ligands (CXCL12 and CCL21) were preferentially expressed in the lung and regional lymph nodes, two sites that breast cancer frequently metastasizes to. When the interaction between one of these pairs (CXCL12/CXCR4) was blocked in vivo using neutralizing antibodies, there was a significant reduction in breast cancer metastases to both the lung and lymph nodes (Muller et al., 2001). Identification of similar chemokine ligand-receptor pairs for other primary cancers and the organs to which they preferentially metastasize (reviewed in Zlotnik, 2004) could be a promising avenue for neutralization strategies.

Inhibitors of chemokines and their receptors are in preclinical development (reviewed in Balkwill, 2004), and offer one means to interfere with the homing of tumor cells to secondary organs. Another therapeutic strategy is to block the dissemination of tumor cells at the source, by inhibiting the development of blood and lymphatic circulatory systems within the tumor. Angiogenesis inhibitors are expected to block metastatic spread and growth. This hypothesis is supported by recent experiments in which potent VEGF inhibition (using the VEGF-Trap, Table 1) in a breast xenograft model with established lung metastases resulted in regression of both the primary and secondary tumors (Huang et al., 2003). Similarly, interfering with tumor lymphangiogenesis using a soluble VEGFR3 inhibitor (He et al., 2002) or a VEGF-C antibody (Stacker et al., 2001) resulted in decreased lymph node metastases (see Achen et al., 2005 for

review). Recent findings have implicated PDGF-BB and its receptors in tumor lymphangiogenesis (Cao et al., 2004), thus suggesting additional therapeutic applications for PDGFR inhibitors (Table 1).

Challenges and perspectives

Whereas cancer cells employ an enormous variety of genetic changes to elicit tumorigenesis, changes in the cancer microenvironment may be common to many tumor types, raising the hope that therapeutic targeting of these events could be generally applicable. Identifying the most important molecular players in the microenvironment of each tumor type is the first step toward this goal. Expression and proteomic profiling of the individual cell types constituting the cancer microenvironment (Allinen et al., 2004; Oh et al., 2004; St Croix et al., 2000) represent important advances. Determining how the molecular profile of the microenvironment changes as tumors progress will be critical for identifying targets to select "normal" cells in the tumor microenvironment that have been altered during tumor progression. Recent studies of endothelial cells (Benezra et al., 2001; Hoffman et al., 2003; Joyce et al., 2003) and stromal fibroblasts (Park et al., 1999; Bhowmick et al., 2004a, 2004b) have identified molecular differences between nontumor cells in the microenvironment of preneoplastic lesions compared to tumors. These proteins themselves could be therapeutically targeted or used as "zip codes" to specifically deliver cytotoxic agents to these converted "normal" cells. Thus, if we can truly selectively target only the cells have been modified by the tumor microenvironment, it should allow their unmodified precursors in normal tissues to remain untouched. Another fundamental question to answer will be how much the cancer microenvironment varies from one tumor type to another. While the individual molecules may vary from organ to organ, there should be common lessons that we can learn about the fundamental mechanisms used by tumor cells to co-opt normal cells.

A potential benefit of many of the targets that have been discussed in this review is that they are implicated in more than one pathway or cell type in the tumor microenvironment (Figure 1). Thus, targeting certain chemokines and their receptors may reduce chronic inflammation in the primary tumor, while simultaneously preventing the development of secondary tumors. Similarly, blocking PDGF and its receptors could interfere with both tumor angiogenesis and tumor lymphangiogenesis. The challenge now is to specifically inhibit the protumorigenic roles of these normal cells in cancer, while maintaining their homeostatic functions in normal tissues.

The good news is that we have a vastly improved set of tools to meet this challenge. Both organotypic 3D culture systems, as pioneered by Bissell and adopted by Brugge and others (Muthuswamy et al., 2001; Petersen et al., 1992; Schmeichel and Bissell, 2003), and the recent development of a "humanized" mammary gland xenograft model (Kuperwasser et al., 2004), allow human epithelial cells and stromal cells to be studied in vitro and in vivo, and are important advances in identifying molecular changes mediated by tumor-host interactions. Along with tissue-specific genetic knockouts, siRNA-mediated knockdown, and selective inhibitors to ablate the key players, these approaches ought to facilitate advances in our understanding of the tumor microenvironment in the next few years, which should translate into improved therapies in the clinic. The recent success of antiangiogenic therapy should continue to serve as an example for all agents targeting the tumor microenvironment. Rational approaches in which cytotoxic agents are administered with cytostatic antistromal agents hold considerable promise. For example, we could envisage a three-step combinatorial approach in which the tumor microenvironment is first normalized by antiangiogenic or antistromal therapy, followed by treatment with cytotoxic therapies to shrink or even eradicate the tumor, then a maintenance regimen, such as low-dose chemotherapy or other antistromal drugs, could be administered to keep any remaining cancer cells in check. In conclusion, an important step in this direction is the recognition that to effectively eliminate renegade cancer cells, we should also consider targeting the cast of normal cells that have been co-opted into supporting them.

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References

Achen, M.G., McColl, B.K., and Stacker, S.A. (2005). Focus on lymphangiogenesis in tumor metastasis. Cancer Cell *7*, 121–127.

Allinen, M., Beroukhim, R., Cai, L., Brennan, C., Lahti-Domenici, J., Huang, H., Porter, D., Hu, M., Chin, L., Richardson, A., et al. (2004). Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell *6*, 17–32.

Balkwill, F. (2004). Cancer and the chemokine network. Nat. Rev. Cancer *4*, 540–550.

Balkwill, F., Charles, K.A., and Mantovani, A. (2005). Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell *7*, 211–217.

Benezra, R., Rafii, S., and Lyden, D. (2001). The Id proteins and angiogenesis. Oncogene *20*, 8334–8341.

Benjamin, L.E., Golijanin, D., Itin, A., Pode, D., and Keshet, E. (1999). Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. J. Clin. Invest. *103*, 159–165.

Bergers, G., Song, S., Meyer-Morse, N., Bergsland, E., and Hanahan, D. (2003). Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J. Clin. Invest. *111*, 1287–1295.

Bhowmick, N.A., Chytil, A., Plieth, D., Gorska, A.E., Dumont, N., Shappell, S., Washington, M.K., Neilson, E.G., and Moses, H.L. (2004a). TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science *303*, 848–851.

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

Bissell, M., and Radisky, D. (2001). Putting tumors in context. Nat. Rev. Cancer *1*, 46–54.

Bresalier, R.S., Sandler, R.S., Quan, H., Bolognese, J.A., Oxenius, B., Horgan, K., Lines, C., Riddell, R., Morton, D., Lanas, A., et al. (2005). Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N. Engl. J. Med. *352*, 1092–1102.

Cao, R., Bjorndahl, M.A., Religa, P., Clasper, S., Garvin, S., Galter, D., Meister, B., Ikomi, F., Tritsaris, P., Dissing, S., et al. (2004). PDGF-BB induces intratumoral lymphangiogenesis and promotes lymphatic metastasis. Cancer Cell *6*, 333–345.

Carmeliet, P. (2003). Angiogenesis in health and disease. Nat. Med. *9*, 653–660.

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

Chambers, A.F., Groom, A.C., and MacDonald, I.C. (2002). Dissemination and growth of cancer cells in metastatic sites. Nat. Rev. Cancer *2*, 563–572.

Cheng, J.D., Dunbrack, R.L., Jr., Valianou, M., Rogatko, A., Alpaugh, R.K., and Weiner, L.M. (2002). Promotion of tumor growth by murin fibroblast activation

protein, a serine protease, in an animal model. Cancer Res. *62*, 4767–4772.

Coussens, L.M., and Werb, Z. (2002). Inflammation and cancer. Nature *420*, 860–867.

Coussens, L.M., Tinkle, C.L., Hanahan, D., and Werb, Z. (2000). MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell *103*, 481–490.

Coussens, L.M., Fingleton, B., and Matrisian, L.M. (2002). Matrix metalloproteinase inhibitors and cancer: Trials and tribulations. Science *295*, 2387–2392.

Damiano, J.S., Cress, A.E., Hazlehurst, L.A., Shtil, A.A., and Dalton, W.S. (1999). Cell adhesion mediated drug resistance (CAM-DR): Role of integrins and resistance to apoptosis in human myeloma cell lines. Blood *93*, 1658–1667.

De Palma, M., Venneri, M.A., Roca, C., and Naldini, L. (2003). Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. Nat. Med. *9*, 789–795.

Dranoff, G. (2004). Cytokines in cancer pathogenesis and cancer therapy. Nat. Rev. Cancer *4*, 11–22.

Fan, F., Wey, J.S., McCarty, M.F., Belcheva, A., Liu, W., Bauer, T.W., Somcio, R.J., Wu, Y., Hooper, A., Hicklin, D.J., and Ellis, L.M. (2005). Expression and function of vascular endothelial growth factor receptor-1 on human colorectal cancer cells. Oncogene *24*, 2647–2653.

Ferrara, N., Hillan, K.J., Gerber, H.P., and Novotny, W. (2004). Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat. Rev. Drug Discov. *3*, 391–400.

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

Folkman, J. (1971). Tumor angiogenesis: Therapeutic implications. N. Engl. J. Med. *285*, 1182–1186.

Folkman, J., Hahnfeldt, P., and Hlatky, L. (2000). Cancer: Looking outside the genome. Nat. Rev. Mol. Cell Biol. *1*, 76–79.

Forni, G., Fujiwara, H., Martino, F., Hamaoka, T., Jemma, C., Caretto, P., and Giovarelli, M. (1988). Helper strategy in tumor immunology: Expansion of helper lymphocytes and utilization of helper lymphokines for experimental and clinical immunotherapy. Cancer Metastasis Rev. *7*, 289–309.

Garber, K. (2002). Angiogenesis inhibitors suffer new setback. Nat. Biotechnol. *20*, 1067–1068.

Garin-Chesa, P., Old, L.J., and Rettig, W.J. (1990). Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. Proc. Natl. Acad. Sci. USA *87*, 7235–7239.

Giraudo, E., Inoue, M., and Hanahan, D. (2004). An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. J. Clin. Invest. *114*, 623–633.

Guo, W., and Giancotti, F.G. (2004). Integrin signalling during tumour progression. Nat. Rev. Mol. Cell Biol. *5*, 816–826.

Hamano, Y., Zeisberg, M., Sugimoto, H., Lively, J.C., Maeshima, Y., Yang, C., Hynes, R.O., Werb, Z., Sudhakar, A., and Kalluri, R. (2003). Physiological levels of tumstatin, a fragment of collagen IV alpha3 chain, are generated by MMP-9 proteolysis and suppress angiogenesis via alphaV beta3 integrin. Cancer Cell *3*, 589–601.

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

Hayashi, N., and Cunha, G.R. (1991). Mesenchyme-induced changes in the neoplastic characteristics of the Dunning prostatic adenocarcinoma. Cancer Res. *51*, 4924–4930.

Hayward, S.W., Wang, Y., Cao, M., Hom, Y.K., Zhang, B., Grossfeld, G.D., Sudilovsky, D., and Cunha, G.R. (2001). Malignant transformation in a nontumorigenic human prostatic epithelial cell line. Cancer Res. *61*, 8135–8142.

He, Y., Kozaki, K., Karpanen, T., Koshikawa, K., Yla-Herttuala, S., Takahashi, T., and Alitalo, K. (2002). Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. J. Natl. Cancer Inst. *94*, 819–825.

Heldin, C.H., Rubin, K., Pietras, K., and Ostman, A. (2004). High interstitial fluid pressure - an obstacle in cancer therapy. Nat. Rev. Cancer *4*, 806–813.

Hilbe, W., Dirnhofer, S., Oberwasserlechner, F., Schmid, T., Gunsilius, E., Hilbe, G., Woll, E., and Kahler, C.M. (2004). CD133 positive endothelial progenitor cells contribute to the tumor vasculature in non-small cell lung cancer. J. Clin. Pathol. *57*, 965–969.

Hildenbrand, R., Wolf, G., Bohme, B., Bleyl, U., and Steinborn, A. (1999). Urokinase plasminogen activator receptor (CD87) expression of tumor-associated macrophages in ductal carcinoma in situ, breast cancer, and resident macrophages of normal breast tissue. J. Leukoc. Biol. *66*, 40–49.

Hoffman, J.A., Giraudo, E., Singh, M., Zhang, L., Inoue, M., Porkka, K., Hanahan, D., and Ruoslahti, E. (2003). Progressive vascular changes in a transgenic mouse model of squamous cell carcinoma. Cancer Cell *4*, 383–391.

Hood, J.D., and Cheresh, D.A. (2002). Role of integrins in cell invasion and migration. Nat. Rev. Cancer *2*, 91–100.

Huang, J., Frischer, J.S., Serur, A., Kadenhe, A., Yokoi, A., McCrudden, K.W., New, T., O'Toole, K., Zabski, S., Rudge, J.S., et al. (2003). Regression of established tumors and metastases by potent vascular endothelial growth factor blockade. Proc. Natl. Acad. Sci. USA *100*, 7785–7790.

Hurwitz, H., Fehrenbacher, L., Novotny, W., Cartwright, T., Hainsworth, J., Heim, W., Berlin, J., Baron, A., Griffing, S., Holmgren, E., et al. (2004). Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N. Engl. J. Med. *350*, 2335–2342.

Hussain, S.P., Hofseth, L.J., and Harris, C.C. (2003). Radical causes of cancer. Nat Rev Cancer *3*, 276–285.Hynes, R.O. (2002). A reevaluation of integrins as regulators of angiogenesis. Nat. Med. *8*, 918–921.

Hynes, R.O. (2002). A reevaluation of integrins as regulators of angiogenesis. Nat. Med. *8*, 918–921.

Jain, R.K. (2001). Normalizing tumor vasculature with anti-angiogenic therapy: A new paradigm for combination therapy. Nat. Med. *7*, 987–989.

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

Jin, H., and Varner, J. (2004). Integrins: Roles in cancer development and as treatment targets. Br. J. Cancer *90*, 561–565.

Joyce, J.A., Laakkonen, P., Bernasconi, M., Bergers, G., Ruoslahti, E., and Hanahan, D. (2003). Stage-specific vascular markers revealed by phage display in a mouse model of pancreatic islet tumorigenesis. Cancer Cell *4*, 393–403.

Joyce, J.A., Baruch, A., Chehade, K., Meyer-Morse, N., Giraudo, E., Tsai, F.Y., Greenbaum, D.C., Hager, J.H., Bogyo, M., and Hanahan, D. (2004). Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. Cancer Cell *5*, 443–453.

Joyce, J.A., Freeman, C., Meyer-Morse, N., Parish, C.F., and Hanahan, D. (2005). A functional heparan sulfate mimetic implicates both heparanase and heparan sulfate in tumor angiogenesis and invasion in a mouse model of multistage cancer. Oncogene, in press.

Kalluri, R. (2003). Basement membranes: Structure, assembly and role in tumour angiogenesis. Nat. Rev. Cancer *3*, 422–433.

Karin, M., Yamamoto, Y., and Wang, Q.M. (2004). The IKK NF-kappa B system: A treasure trove for drug development. Nat. Rev. Drug Discov. *3*, 17–26.

Kerbel, R., and Folkman, J. (2002). Clinical translation of angiogenesis inhibitors. Nat. Rev. Cancer *2*, 727–739.

Kuperwasser, C., Chavarria, T., Wu, M., Magrane, G., Gray, J.W., Carey, L., Richardson, A., and Weinberg, R.A. (2004). Reconstruction of functionally normal and malignant human breast tissues in mice. Proc. Natl. Acad. Sci. USA *101*, 4966–4971.

Lam, L.T., Davis, R.E., Pierce, J., Hepperle, M., Xu, Y., Hottelet, M., Nong, Y., Wen, D., Adams, J., Dang, L., and Staudt, L.M. (2005). Small molecule inhibitors of IkappaB kinase are selectively toxic for subgroups of diffuse large B-cell lymphoma defined by gene expression profiling. Clin. Cancer Res. *11*, 28–40.

Lammerts, E., Roswall, P., Sundberg, C., Gotwals, P.J., Koteliansky, V.E., Reed, R.K., Heldin, N.E., and Rubin, K. (2002). Interference with TGF-beta1 and -beta3 in tumor stroma lowers tumor interstitial fluid pressure independently of growth in experimental carcinoma. Int. J. Cancer *102*, 453–462.

Li, H., Gerald, W.L., and Benezra, R. (2004). Utilization of bone marrow-

derived endothelial cell precursors in spontaneous prostate tumors varies with tumor grade. Cancer Res. *64*, 6137–6143.

Lin, E.Y., Nguyen, A.V., Russell, R.G., and Pollard, J.W. (2001). Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J. Exp. Med. *193*, 727–740.

Lyden, D., Hattori, K., Dias, S., Costa, C., Blaikie, P., Butros, L., Chadburn, A., Heissig, B., Marks, W., Witte, L., et al. (2001). Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat. Med. *7*, 1194–1201.

Maeda, H., and Akaike, T. (1998). Nitric oxide and oxygen radicals in infection, inflammation, and cancer. Biochemistry (Mosc.) *63*, 854–865.

Morin, P.J. (2003). Drug resistance and the microenvironment: Nature and nurture. Drug Resist. Updat. *6*, 169–172.

Muller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M.E., McClanahan, T., Murphy, E., Yuan, W., Wagner, S.N., et al. (2001). Involvement of chemokine receptors in breast cancer metastasis. Nature *410*, 50–56.

Muthuswamy, S.K., Li, D., Lelievre, S., Bissell, M.J., and Brugge, J.S. (2001). ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini. Nat. Cell Biol. *3*, 785–792.

Oh, P., Li, Y., Yu, J., Durr, E., Krasinska, K.M., Carver, L.A., Testa, J.E., and Schnitzer, J.E. (2004). Subtractive proteomic mapping of the endothelial surface in lung and solid tumours for tissue-specific therapy. Nature *429*, 629–635.

Olumi, A.F., Grossfeld, G.D., Hayward, S.W., Carroll, P.R., Tlsty, T.D., and Cunha, G.R. (1999). Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res. *59*, 5002–5011.

Orimo, A., Gupta, P.B., Sgroi, D.C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., Carey, V.J., Richardson, A.L., and Weinberg, R.A. (2005). Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell *121*, 335–348.

Palladino, M.A., Bahjat, F.R., Theodorakis, E.A., and Moldawer, L.L. (2003). Anti-TNF-alpha therapies: The next generation. Nat. Rev. Drug Discov. *2*, 736–746.

Papetti, M., and Herman, I.M. (2002). Mechanisms of normal and tumor-derived angiogenesis. Am. J. Physiol. Cell Physiol. *282*, C947–C970.

Park, J.E., Lenter, M.C., Zimmermann, R.N., Garin-Chesa, P., Old, L.J., and Rettig, W.J. (1999). Fibroblast activation protein, a dual specificity serine protease expressed in reactive human tumor stromal fibroblasts. J. Biol. Chem. *274*, 36505–36512.

Peters, B.A., Diaz, L.A., Polyak, K., Meszler, L., Romans, K., Guinan, E.C., Antin, J.H., Myerson, D., Hamilton, S.R., Vogelstein, B., et al. (2005). Contribution of bone marrow-derived endothelial cells to human tumor vasculature. Nat. Med. *11*, 261–262.

Petersen, O.W., Ronnov-Jessen, L., Howlett, A.R., and Bissell, M.J. (1992). Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. Proc. Natl. Acad. Sci. USA *89*, 9064–9068.

Pietras, K., and Hanahan, D. (2005). A multitargeted, metronomic, and maximum-tolerated dose "chemo-switch" regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. J. Clin. Oncol. *23*, 939–952.

Pietras, K., Rubin, K., Sjoblom, T., Buchdunger, E., Sjoquist, M., Heldin, C.H., and Ostman, A. (2002). Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. Cancer Res. *62*, 5476–5484.

Pietras, K., Sjoblom, T., Rubin, K., Heldin, C.H., and Ostman, A. (2003). PDGF receptors as cancer drug targets. Cancer Cell *3*, 439–443.

Pollard, J.W. (2004). Tumour-educated macrophages promote tumour progression and metastasis. Nat. Rev. Cancer *4*, 71–78.

Pupa, S.M., Menard, S., Forti, S., and Tagliabue, E. (2002). New insights into the role of extracellular matrix during tumor onset and progression. J. Cell. Physiol. *192*, 259–267.

Rafii, S., Lyden, D., Benezra, R., Hattori, K., and Heissig, B. (2002). Vascular and haematopoietic stem cells: Novel targets for anti-angiogenesis therapy? Nat. Rev. Cancer *2*, 826–835.

Rajantie, I., Ilmonen, M., Alminaite, A., Ozerdem, U., Alitalo, K., and Salven, P. (2004). Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. Blood *104*, 2084–2086.

Ryden, L., Linderholm, B., Nielsen, N.H., Emdin, S., Jonsson, P.E., and Landberg, G. (2003). Tumor specific VEGF-A and VEGFR2/KDR protein are co-expressed in breast cancer. Breast Cancer Res. Treat. *82*, 147–154.

Schmeichel, K.L., and Bissell, M.J. (2003). Modeling tissue-specific signaling and organ function in three dimensions. J. Cell Sci. *116*, 2377–2388.

Scott, A.M., Wiseman, G., Welt, S., Adjei, A., Lee, F.T., Hopkins, W., Divgi, C.R., Hanson, L.H., Mitchell, P., Gansen, D.N., et al. (2003). A Phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. Clin. Cancer Res. *9*, 1639–1647.

Sethi, T., Rintoul, R.C., Moore, S.M., MacKinnon, A.C., Salter, D., Choo, C., Chilvers, E.R., Dransfield, I., Donnelly, S.C., Strieter, R., and Haslett, C. (1999). Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: A mechanism for small cell lung cancer growth and drug resistance in vivo. Nat. Med. *7*, 186–191.

Shain, K.H., and Dalton, W.S. (2001). Cell adhesion is a key determinant in de novo multidrug resistance (MDR): New targets for the prevention of acquired MDR. Mol. Cancer Ther. *1*, 69–78.

Shaked, Y., Bertolini, F., Man, S., Rogers, M.S., Cervi, D., Foutz, T., Rawn, K., Voskas, D., Dumont, D.J., Ben-David, Y., et al. (2005). Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis; Implications for cellular surrogate marker analysis of antiangiogenesis. Cancer Cell *7*, 101–111.

Stacker, S.A., Caesar, C., Baldwin, M.E., Thornton, G.E., Williams, R.A., Prevo, R., Jackson, D.G., Nishikawa, S.-I., Kubo, H., and Achen, M.G. (2001). VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat. Med. *7*, 186–191.

St Croix, B., Rago, C., Velculescu, V., Traverso, G., Romans, K.E., Montgomery, E., Lal, A., Riggins, G.J., Lengauer, C., Vogelstein, B., and Kinzler, K.W. (2000). Genes expressed in human tumor endothelium. Science *289*, 1197–1202.

Thun, M.J., Namboodiri, M.M., and Heath, C.W., Jr. (1991). Aspirin use and reduced risk of fatal colon cancer. N. Engl. J. Med. *325*, 1593–1596.

Tong, R.T., Boucher, Y., Kozin, S.V., Winkler, F., Hicklin, D.J., and Jain, R.K. (2004). Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res. *64*, 3731–3736.

Vakkila, J., and Lotze, M.T. (2004). Inflammation and necrosis promote tumour growth. Nat. Rev. Immunol. *4*, 641–648.

Vlodavsky, I., Goldshmidt, O., Zcharia, E., Atzmon, R., Rangini-Guatta, Z., Elkin, M., Peretz, T., and Friedmann, Y. (2002). Mammalian heparanase: Involvement in cancer metastasis, angiogenesis and normal development. Semin. Cancer Biol. *12*, 121–129.

Weaver, V.M., Petersen, O.W., Wang, F., Larabell, C.A., Briand, P., Damsky, C., and Bissell, M.J. (1997). Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. J. Cell Biol. *137*, 231–245.

Willett, C.G., Boucher, Y., di Tomaso, E., Duda, D.G., Munn, L.L., Tong, R.T., Chung, D.C., Sahani, D.V., Kalva, S.P., Kozin, S.V., et al. (2004). Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat. Med. *10*, 145–147.

Winkler, F., Kozin, S.V., Tong, R.T., Chae, S.S., Booth, M.F., Garkavtsev, I., Xu, L., Hicklin, D.J., Fukumura, D., di Tomaso, E., et al. (2004). Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: Role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell *6*, 553–563.

Wrobel, C.N., Debnath, J., Lin, E., Beausoleil, S., Roussel, M.F., and Brugge, J.S. (2004). Autocrine CSF-1R activation promotes Src-dependent disruption of mammary epithelial architecture. J. Cell Biol. *165*, 263–273.

Yu, Y., Flint, A.F., Mulliken, J.B., Wu, J.K., and Bischoff, J. (2004). Endothelial progenitor cells in infantile hemangioma. Blood *103*, 1373–1375.

Zlotnik, A. (2004). Chemokines in neoplastic progression. Semin. Cancer Biol. *14*, 181–185.

Zou, W. (2005). Immunosuppressive networks in the tumor environment and their therapeutic relevance. Nat. Rev. Cancer *5*, 263–274.