Chapter 2

Role of Microenvironment on Gene Expression, Angiogenesis and Microvascular Function in Tumors

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Abstract: Solid tumors are organ-like entities. In addition to neoplastic cells, they consist of non-transformed host stromal cells such as endothelial cells, fibroblasts and inflammatory cells. All of these cells are embedded in a characteristic extracellular matrix and are surrounded by specific molecular and metabolic microenvironments. Blood and lymphatic vessels, which are important for maintaining the homeostasis of living organisms, are compromised in solid tumors, causing various physiological barriers to the delivery of therapeutic agents to tumors in sufficient quantity and under optimal conditions. There is a growing body of evidence that stromal cells are not quiescent bystanders; instead, they significantly influence the pathophysiology of tumors. Both stromal cells and tumor cells participate in the formation of this milieu, and the microenvironment, which includes the expression of positive and negative regulators of angiogenesis, influences the biology of these cells. Any of these factors – tumor cells, stromal cells, and the local microenvironment of particular organs – may vary during treatment and may influence the efficiency of various treatment modalities. Therefore, stromal cells and the tumor microenvironment offer novel targets for tumor detection and treatment. A better understanding of host-tumor interaction and formation, as well as of the function of blood and lymphatic vessels in tumors in different microenvironments, is warranted in order to facilitate the development of such strategies.

Key words: Microenvironment, angiogenesis, stromal cells, extracellular matrix, fibroblasts, lymphocytes macrophages, hypoxia, intravital microscopy, VEGF, blood vessels, colon carcinoma, AKT, PI3K, acidosis, urokinase plasminogen activator, hypoxia inducing factor, gene expression

1. INTRODUCTION

Tumors consist not only of cancer cells but also of host stromal cells – non-malignant cells which include endothelial cells, peri-vascular cells, fibroblasts, macrophages, lymphocytes, dendritic cells and mast cells. These cells, embedded within a protein-rich extracellular matrix, face a hostile metabolic microenvironment characterized by hypoxia and acidosis (Figure 1). Each of these cells is capable of producing positive and negative regulators of angiogenesis in response to microenvironmental cues (1, 2). These local interactions vary with tumor type and site of growth (host organ), and may change during the course of tumor growth and treatment. In this chapter, we will discuss the characteristics of tumor vasculature and the resulting microenvironment, as well as the roles of the metabolic microenvironment, the host stromal cells, and the host organ microenvironment in the regulation of tumor angiogenesis and physiological functions.

G. G. Meadows (ed.), Integration/Interaction of Oncologic Growth, 23-36.

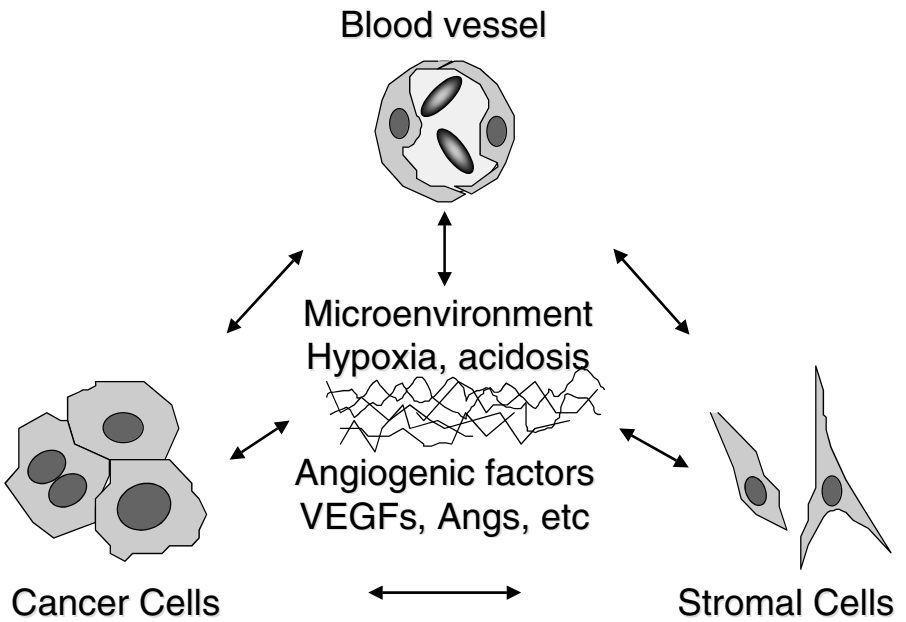

Figure 1. Composition of solid tumor.

2. INTRAVITAL MICROSCOPY FOR THE DISSECTION OF TUMOR PATHOPHYSIOLOGY

In vivo tumor models and their monitoring systems must be used in order to understand the inner workings of solid tumors (i.e. angiogenesis and the functions of newly formed blood and lymphatic vessels). Intravital microscopy provides molecular, cellular, structural, and functional insights *in vivo* with high spatial and temporal resolutions, and is thus ideal for the investigation of tumor pathophysiology (3). Intravital microscopy requires four essential components: a) appropriate animal models that allow optical access to tissues of interest, b) molecular probes (usually fluorescent) that can be imaged, c) microscopes and detection systems, and d) computer-assisted image processing and analysis systems (Figure 2). Our laboratory has established and modified many animal tumor models for intravital microscopy, including transparent window models such as the mouse dorsal skin chamber (4) and cranial window (5), and orthotopic tumor models such as liver (6), gall bladder (7), pancreas (8), mammary gland (9) and lung tumors (10). Molecular probes include the green fluorescent protein (GFP) driven by a promoter of interest (11), optical probes that are activated by specific enzymes (12), or microenvironments (for example, pO_2 and pH-activated probes) (13) and endogenous and exogenous tracers that label specific target cells (14, 15) or molecules (for example, second harmonic generation by fibrillar collagen) (16). Conventional fluorescent microscopes (15, 17), confocal laser scanning microscopes (11), and, more recently, multiphoton laser-scanning microscopes (10, 18) have been used for intravital studies. The development and application of multiphoton laser-scanning microscopy has provided significant advances because it allows deep tissue penetration, high signal-to-noise ratio, and minimal photo-damage.

Structural analyses reveal quantitative information regarding tumor growth and angiogenesis (vessel density, diameter, volume), as well as information about the size of pores in the vessel walls and in the extracellular matrix (3, 10). In addition, functional analyses can evaluate hemo- and lympho-dynamics (blood and lymph flow, vasomotor action), leukocyte-endothelial interaction,

vascular permeability, cell migration (of tumor cells, lymphocytes, macrophages, fibroblasts, etc), and interstitial transport (3, 10). The combination of these tumor models with intravital microscopy techniques has allowed us to discern the regulation of tumor angiogenesis and microcirculation as well as the effects of various treatments on this regulation.

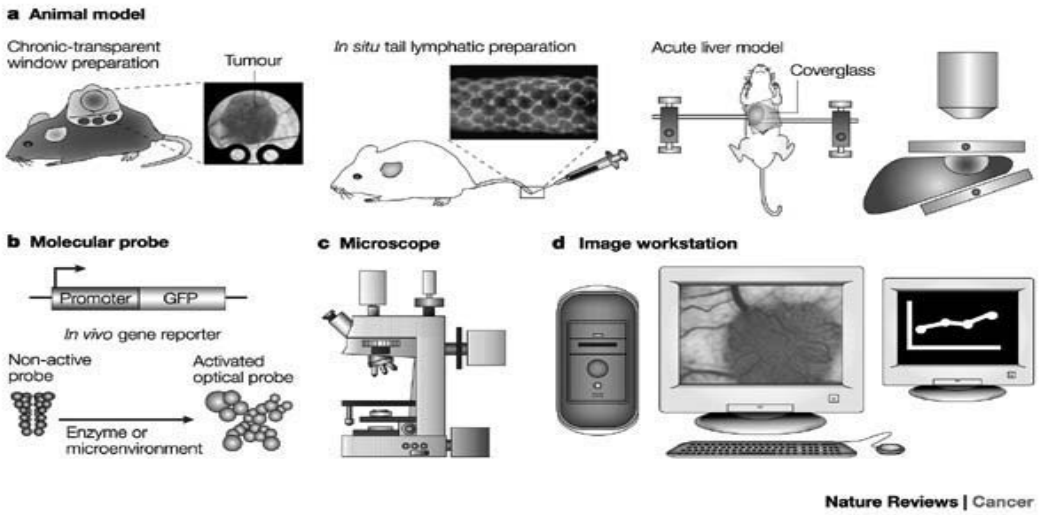

Figure 2. Four requirements for intravital microscopy. Reproduced from Reference (3).

3. CHARACTERISTICS OF TUMOR VASCULATURE AND THE RESULTING MICROENVIRONMENT

Vascular function is an important determinant of the local microenvironment. There are two vascular systems in the body: blood vessels and lymphatic vessels. In tumors, both types of vessels are morphologically and functionally abnormal (3). The normal microcirculation has a well-organized architecture consisting of arterioles, capillaries, and venules. Arterioles, the upstream element, have circumferential pericyte coverage and higher vessel tone. From arterioles, blood flows into capillaries, which represent the major portion of the microvasculature and have the smallest diameter and the thinnest vessel wall. Capillaries maintain tissue homeostasis by mediating the exchange of nutrients, gas, and waste. From the capillaries, the blood is collected into venules, the downstream portion of the microcirculation. Venules have intermediate wall thickness and non-circumferential pericyte coverage. In contrast to these highly organized, functional networks from normal tissue, the tumor vascular network is immature and mesh-like, similar to the primarily vascular plexus in early stage embryos. Tumor vessels are dilated, tortuous, and have an irregular surface and a heterogeneous spatial distribution. Recent studies show the presence of pericyte-like cells in tumor vessels. However, their morphology and association with vessels are abnormal (19), which results in poorly regulated vascular function. In normal vessels, arterioles have a higher flow velocity and nutrient/oxygen level, while venules have lower velocity and oxygen level. In each vessel category, flow velocity correlates well with vessel size. In tumor vessels, blood flow is very slow, is sometimes static, and may even change direction over time. Moreover, there is no correlation between tumor vessel diameter and flow rate or oxygen level (5, 13). Some tumor vessels contain almost no oxygen or other nutrients despite relatively good perfusion. This abnormal and non-

homogenous blood flow creates a physiological barrier to the delivery of therapeutic agents to tumors (20) and can also lead to the hypoxia and acidosis which are often seen in tumors (13). Such a severe metabolic microenvironment reduces the efficacy of anti-tumor therapies. In addition, the abnormality of tumor vessels also results in high vascular permeability, and leaked plasma proteins such as fibronectin form an optimal provisional matrix for angiogenesis. Finally, leukocyte adhesion in tumor vessels is generally low, hiding tumors from immune surveillance. All of these vascular, interstitial, and cellular barriers have to be overcome for efficient delivery of anti-tumor therapies.

By transporting both immune cells and interstitial fluid out of tissue, the normal lymphatic network plays an important role in immune function and in the maintenance of tissue interstitial fluid balance. Tumor cells grow in a confined space and, thus, create a mechanical stress (solid stress), which compresses the intratumor blood and lymph vessels (21). Consequently, there are no functional lymphatic vessels inside solid tumors (22). High permeability of intratumor blood vessels and impaired lymphatic drainage cause significant elevation of interstitial fluid pressure and oncotic pressure in solid tumors. As a result, the pressure gradient between blood vessel and tumor tissue is lost (23). High interstitial fluid pressure and the loss of pressure gradients constitute additional physiological barriers to the delivery of therapeutic agents to tumors. Nevertheless, in the peripheral region of the tumor, lymph-angiogenesis, lymphatic hypertrophy, and lymphatic dilatation are often found (22, 24, 25). Dysfunction of the lymphatic valves allows retrograde flow in these lymphatic vessels (25). Tumor cells can invade these peripheral lymphatic vessels and form metastases within the lymphatic system. Hence, a better understanding of the formation and function of blood and lymphatic vessels in tumors is necessary in order to develop new strategies to overcome these barriers to tumor treatment.

4. VEGF, THE MAIN REGULATOR OF NEW BLOOD VESSEL FORMATION

Because tumor cells depend on a nutrient supply from blood vessels, neovascularization (angiogenesis) is required for tumors to grow beyond 1-2 mm in diameter (26). Newly formed blood vessels are important not only for the growth of primary tumors, but also for the metastatic spread of cancers (27). A variety of positive and negative regulators govern vasculogenesis, angiogenesis, and subsequent vessel maturation (1, 2, 28). More than 20 angiogenic stimulators and inhibitors have been discovered in the past two decades, including vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), and angiopoietin (Ang) (1, 2, 28, 29). These factors not only mediate tumor vessel formation but also affect the function of these vessels. VEGF is one of the most potent angiogenic factors and is the target of the first FDA approved anti-angiogenic agent, Avastin (approved for colorectal cancer in 2004) (30). VEGF expression levels in tumors, and the concentrations of this protein in the bodily fluids (serum, urine, CSF) of cancer patients, show significant correlation with the extent of angiogenesis and/or metastasis and have therefore been suggested as diagnostic and prognostic markers (29, 31). VEGF is a mitogen that acts primarily on endothelial cells (29). VEGF signaling is critical for the development of a nascent vascular network via physiological vasculogenesis as well as pathological angiogenesis (2, 29). VEGF induces nitric oxide-mediated relaxation of the arterial vessels (32), signaled through PI3K and Akt, and has a role as a survival factor in maintaining the vasculature (29, 33). VEGF is also believed to contribute to an angiogenic phenotype by increasing the permeability of existing vessels (31). This leads to the extravasation of fibrin, plasmin, and clotting factors, resulting in a fibrin-rich stroma that supports the migration of endothelial and peri-endothelial cells and the formation of new vasculature (31). Local variation in the concentrations of VEGF in the tumor may explain the heterogeneous angiogenesis and vascular dysfunction in tumor vessels as well the non-uniform response of these vessels to various therapies. The tumor microenvironment, in turn,

regulates the expression of VEGF and thereby regulates the formation and function of blood vessels in tumors.

5. REGULATION OF VEGF AND ANGIOGENESIS BY THE METABOLIC MICROENVIRONMENT

Hypoxia and acidosis are hallmarks of the metabolic environment in solid tumors (34-37). Both oxygen tension (pO_2) and pH are important determinants of tumor growth, metabolism, and response to a variety of therapies such as radiation therapy, chemotherapy, hyperthermia, and photodynamic therapy (34-39). Hypoxia upregulates various angiogenic growth factors, including VEGF, Ang2, PDGF, Placenta growth factor (PlGF), transforming growth factor (TGF), interleukin (IL)-8, and hepatocyte growth factor (35, 40). Hypoxia inducible factor (HIF) is a transcriptional factor that binds to the hypoxia responsive element (HRE) in the promoter of hypoxia-responsive genes such as VEGF, PDGF and TGF (35, 40). HIF-1 also mediates hypoxia-induced apoptosis via p53 and other mechanisms (35, 41). Tumor cells have developed many mechanisms to evade HIF-1-mediated cell death under hypoxic conditions (35). A few other factors, such as IL-8 and PlGF, are activated by HIF-independent mechanisms (35, 42). Several lines of evidence have shown that microenvironmental hypoxia upregulates VEGF, both in tumors *in vivo* and in multicellular tumor spheroids *in vitro* (43, 44). Hypoxia may play an important role in the angiogenic switch (45) which is required for tumor growth and expansion. However, an immunohiostochemical study revealed a lack of spatial correlation between the staining of redox marker and VEGF in squamous cell carcinomas (46). There is a wide heterogeneity in intratumor and intertumor pO_2 distributions (13, 47). To understand the extent of hypoxia which is required to upregulate VEGF *in vivo*, pO_2 distributions should be measured simultaneously with VEGF profiles *in vivo*.

Low extracellular pH, another characteristic of solid tumors, causes stress-induced alteration of gene expression, including the upregulation of VEGF in macrophages and in tumor cells *in vitro* (48, 49). Furthermore, low pH synergistically enhances the hypoxia-induced upregulation of VEGF in cancer cells *in vitro* (49). Despite its importance, the effect of the low and heterogeneous interstitial pH on hypoxia-induced VEGF production *in vivo* remained unknown for many years due to the lack of appropriate techniques and animal models. However, two non-invasive optical techniques have now been developed: fluorescence ratio imaging microscopy for pH measurements (50) and phosphorescence quenching microscopy for pO_2 measurements (51). These two techniques provide high spatial resolution and are routinely used in combination to map temporal and spatial pH and pO_2 profiles at the same tumor locations (13). Recently, we also developed a GFP reporter system (*$VEGF^p$-GFP*) that monitors VEGF promoter activity *in vivo* (11). The combination of these techniques allows the coordinated study of pH, pO_2, and VEGF expression *in vivo* (52).

We first determined the effect of hypoxia on *$VEGF^p$-GFP* transfected cells *in vitro,* as a means of confirming the system. Both the endogenous VEGF gene and exogenous construct-derived GFP were comparably upregulated by hypoxia (52). Then, *$VEGF^p$-GFP* U87 tumors were implanted in SCID mouse cranial windows (5) and grown into well-vascularized tumors over a period of 7-8 days. GFP fluorescence in U87 tumor cells was visualized by fluorescence microscopy (10). To translate GFP fluorescence intensity into instantaneous GFP concentration, we generated calibration curves using known quantities of recombinant EGFP and the same intravital microscopy set-up. Tissue pO_2 and pH were determined by phosphorescence quenching microscopy and ratio imaging microscopy, respectively (13). We analyzed the relationship between pO_2, pH, and VEGF expression in *$VEGF^p$-GFP* U87 tumors by dividing the measurements into either hypoxic (pO_2 < 30 mmHg) or oxygenated (pO_2 > 30 mmHg) groups and either low pH (pH < 6.8) or neutral (6.8 < pH < 7.4) groups. This allowed for the comparison of GFP expression under each of these conditions using linear regression. Figures 3A and 3D show that, under hypoxic conditions or neutral pH conditions, pO_2, but not pH, is correlated

to GFP expression. Conversely, Figures 3B and 3C show that, under low pH or oxygenated conditions, pH and not pO_2 is related to GFP expression. These results indicated, for the first time, that VEGF transcription in brain tumors is independently regulated by the tissue pO_2 and pH (52).

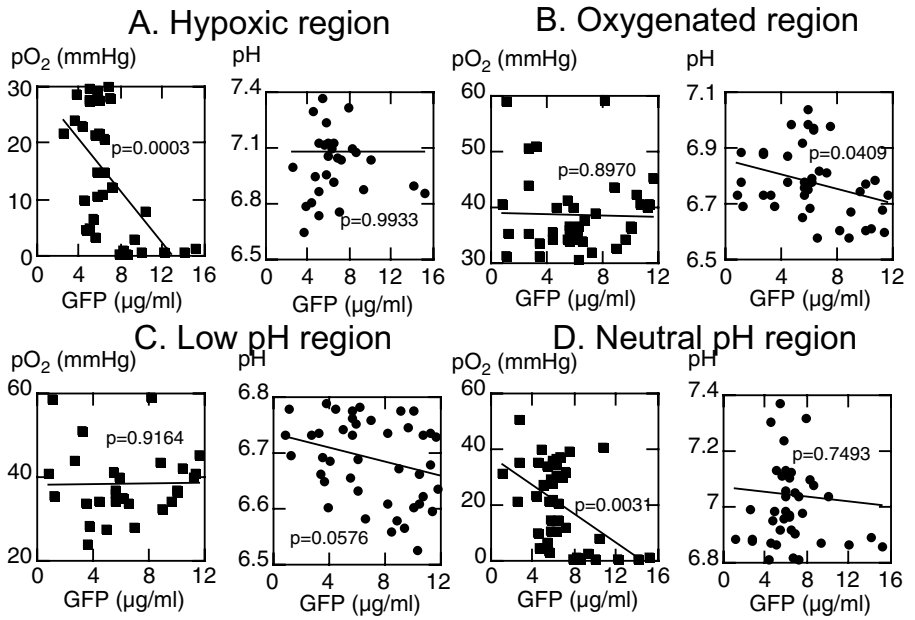

Figure 3. Relationships between VEGF promoter activity, tissue pO2, and extracellular pH. Reproduced from (52).

Our findings prompted us to study the signaling pathway in order to mediate acidic-pH-induced VEGF upregulation. Serial deletion of the VEGF promoter showed that the promoter region contains the activating protein (AP)-1 binding site but not HRE or AP-2, and also indicated that stimulatory protein-1 binding sites were crucial for acidic-pH-induced VEGF upregulation. This is clearly a different signaling pathway from that which regulates hypoxia-induced VEGF expression, which is mediated by HIF-1 complex binding to HRE. Acidic pH also stabilized VEGF mRNA. Furthermore, the acidic-pH-activated *Ras* oncogene and downstream signaling requires extracellular signal-related kinase1/2 but not p38 or Jun amino-terminal kinase (53). These data confirmed that two major metabolic environments in solid tumors regulate VEGF expression in a complimentary manner via distinct signaling pathways.

6. INVOLVEMENT OF HOST STROMAL CELLS IN TUMOR ANGIOGENESIS

Traditionally, cancer researchers have focused their studies on genetically transformed neoplastic cells. However, it is becoming increasingly apparent that the development and pathophysiology of a tumor cannot be explained simply by the genes carried by the tumor cells (54). The cell population in a tumor includes numerous non-neoplastic bystanders (stromal cells) such as endothelial cells, peri-vascular cells, fibroblasts, and inflammatory cells (Figure 1). We are beginning to understand that stromal cells profoundly influence many steps of tumor progression, such as tumor cell proliferation, invasion, angiogenesis, metastasis, and even malignant transformation (11, 55-60). Crosstalk between the diverse cell types within a tumor, via both soluble factors and direct cell-to-cell contact,

plays an important role in the induction, selection, and expansion of the neoplastic cells. Successful tumor cells are those that have acquired the ability to co-opt their normal neighbors by inducing them to release abundant fluxes of growth-stimulating signals (54, 58, 60).

Although the importance of angiogenesis in tumor development has been appreciated for some time, the involvement of host stromal cells in tumor angiogenesis was overlooked due to a lack of methodology for determining the specific contributions of stromal cells. To this end, we have engineered transgenic mice bearing the VEGF promoter-GFP reporter construct (*VEGF^p-GFP* mice). *VEGF^p-GFP* mice showed green cellular fluorescence around the healing margins and throughout the granulation tissue of superficial ulcerative wounds (11). Tumor implantation in the *VEGF^p-GFP* mice led to an accumulation of green fluorescence resulting from the tumor induction of stromal VEGF promoter activity (15). Initially, surface-weighed confocal laser scanning microscopy of both wounds and tumors revealed that GFP-positive cells are mainly spindle shaped fibroblast-like cells (11). Subsequently, we used multiphoton laser-scanning microscopy to determine gene expression and function at depths of over 400 microns in the tumor tissue (Figure 4). We then found that VEGF-expressing stromal cells co-localize with the vasculature and even surround tumor blood vessels deep inside the tumor (18). These findings suggest that activated fibroblasts are involved in angiogenesis, the fortification of blood vessels, and the function of these vessels. In fact, co-implantation of fibroblasts enhanced the tumorigenicity of breast cancer cells *in vivo* (61) and fibroblastic expression of three components of the urokinase-type plasminogen activator system (uPA, uPA receptor, PA inhibitor-1), which are also known to be angiogenic factors, showed a positive correlation with clinical parameters of breast cancers such as tumor size and grade (62). In addition to fibroblasts, some inflammatory cells recruited to tumors may also promote (rather than eliminate) angiogenesis and tumor cell growth (59).

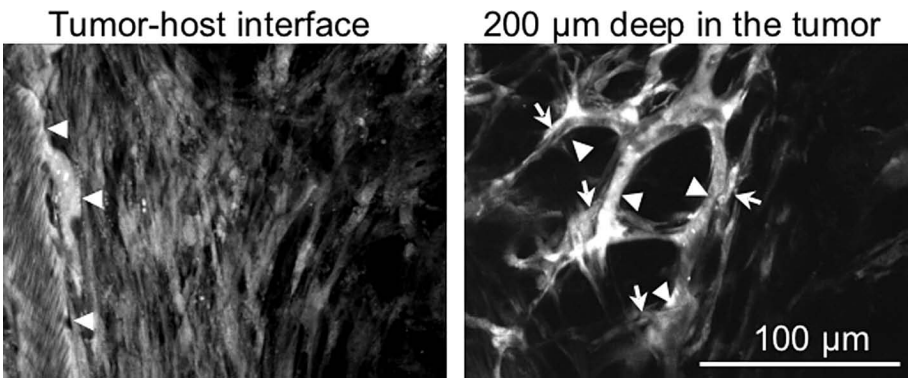

Figure 4. Imaging VEGF promoter activity in vivo. MCaIV murine breast tumor was grown in the dorsal skin chamber in VEGFp-GFP mice. The tumor vasculature was highlighted by injection of tetramethylrhodamine-labeled BSA (arrow head). Fluorescence of GFP and rhodamine was visualized by multiphoton laser-scanning microscopy (18). Deep inside the tumor (~200 μm) GFP positive cells (arrow) were often associated with angiogenic vessels (arrow). Adapted from Reference (18).

The next question would be the extent of stromal cell contribution in tumor angiogenesis. To answer this question, we determined angiogenesis and tissue VEGF protein level in various tumors derived from genetically engineered embryonic stem cells (ES cells). We compared ES cells with mutations that can influence VEGF expression, including mutations in HIF-1, in HRE in the VEGF promoter, and in VEGF itself (63). We found that angiogenesis in these tumors correlated well with their VEGF levels,

i.e., *VEGF*$^{-/-}$ ≈ *HRE*$^{-/-}$ < *HIF-1*$^{-/-}$ < wild type (WT). VEGF protein levels in *VEGF*$^{-/-}$ ES cell-derived tumors, which have VEGF only from host stromal cells, were approximately half of those in WT ES cell-derived tumors, which contain both tumor cell-derived and stromal cell-derived VEGF. This suggests that host stromal cells make a significant contribution (~50%) to the production of VEGF in these tumors (63). The ratio of tumor/host-derived VEGF may vary depending on tumor type, stage, and organ site. For an example, late stage orthotopic pancreatic tumors expressed significantly higher tumor cell-derived VEGF compared to early stage or ectopically-grown tumors (8).

The contribution of host stromal cells to tumor angiogenesis was also altered by tumor treatments. The blockade of human epidermal growth factor receptor (HER)-2 signaling by a neutralizing antibody (Herceptin) downregulates VEGF, TGFα, Ang1, and PAI-1, and also induces thrombospondin-1, producing significant inhibition of angiogenesis and tumor growth (Figure 5) (64). Based on these findings, we proposed that Herceptin mimics an anti-angiogenic cocktail (64). It is noteworthy that although Herceptin significantly inhibited VEGF expression in tumor cells, the overall VEGF expression in tumor tissue did not change, due to compensation by the host stromal cells. Increased host stromal contribution to VEGF-promoter activity was observed following various anti-angiogenesis/anti-vascular treatments such as hormone withdrawal (65) and photodynamic therapy (66). These findings suggest that host stromal cells may compensate for the loss of critical growth factors during anti-tumor treatment, and will thus provide a survival window for repopulation with treatment-resistant tumor cells.

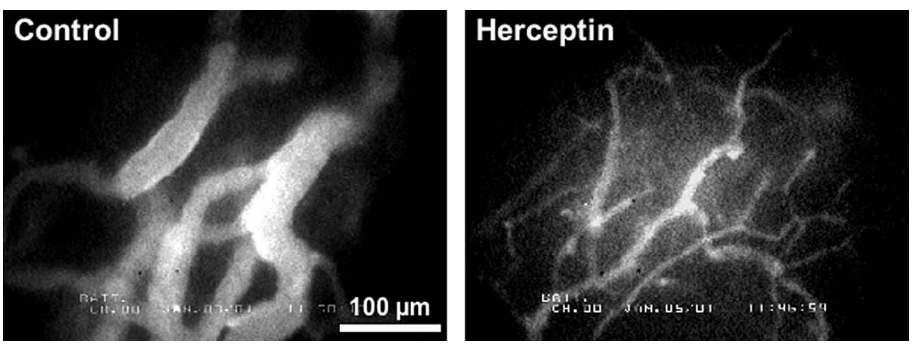

Figure 5. Effect of Herceptin on tumor vessels. Vasculature of control (left) and Herceptin (right) treated MDA-MB-361HK tumor grown in the cranial windows of SCID mice on day-15. The blood vessels are contrast enhanced by i.v. injection of FITC-dextran. Reproduced from Reference (64).

In addition to primary tumor angiogenesis and growth, host stromal cells may also contribute to the spread of disease. Stromal cells and cancer cells exchange enzymes and cytokines that modify the local extracellular matrix and stimulate migration (56, 58). Macrophages have been shown to induce tumor cell intravasation (59). Fibroblasts promote tumor cell invasion by upregulation of matrix metalloproteinase-9 production (67), and proliferative activity of fibroblasts closely correlates with metastasis of breast carcinoma (68). The fact that stromal cells can survive and proliferate in distant organs (69) is particularly important. Heterotypic multicellular tumor fragments have been found in blood and lymphatic vessels (57, 70). Tumor cell aggregates form more metastatic tumors than single tumor cells (70-72). Co-injection of non-neoplastic cells such as fibroblasts or embryo cells significantly increase metastasis in experimental tumor models (71, 73). Taken together these findings support the provocative idea that primary tumor-derived stromal cells may form the provisional stroma for the initial survival and growth of metastasic tumors. Stromal cells should subsequently be considered as an additional target

for both anti-tumor treatments and prevention of metastasis.

7. REGULATION OF ANGIOGENESIS AND VESSEL FUNCTIONS BY ORGAN MICROENVIRONMENT

Gene expression, angiogenesis, and microcirculatory functions differ significantly between sites of implantation when the same tumor cells are grown in different host organs (3, 74). These observations imply that growth factor expression and function are determined by a complicated interaction between tumor cells, host stromal cells, and the organ microenvironment where the tumor is growing. This hypothesis is supported by the facts that various human tumors have particular organ preferences for their metastases (75) and that tumor growth, angiogenesis, metastatic potential, and response to treatment differs among various host organs (76-78). These preferences/differences seem to be due to the organ-specific microenvironment. Organ-specific upregulation of bFGF (79), IL-8 (80), VEGF (6, 8), and various metastasis-related genes (81) in tumors has been reported (Table 1). Human renal cell carcinoma (HRCC) xenografts grown in the kidneys of immunodeficient mice were highly vascularized and metastatic, and they expressed levels of bFGF mRNA which were 10- to 20-fold higher than those from the same tumor grown subcutaneously (79). We found that LS174T tumors (human colon adenocarcinoma) grown in the liver expressed lower levels of VEGF mRNA and had a lower vessel density than those in subcutaneous tissue (6). Similarly, the levels of IL-8 mRNA were always high in A375 tumors (human melanoma) re-established in the skin and were low in the tumors re-established in the liver (80).

Table 1. Role of host organ microenvironment on angiogenesis and vascular function.

Comparison	Key conclusions	Reference
Cranium *vs.* skin	Faster angiogenesis in a collagen gel in the cranial window.	(82)
	Smaller pore cut off size in the cranial tumors.	(83)
	Lower vascular permeability in baseline and after VEGF superfusion in pial vessels.	(84)
	Higher interstitial diffusion in the cranial tumors due to less collagen (fibroblast) involvement.	(85)
	Tumor blood barrier formation in HGL21 human glioma only when grown in the cranial window.	(86)
Liver *vs.* skin	Higher IL-8 expression in skin tumors and induction of IL-8 by co-culture with keratinocytes.	(80)
	Lower VEGF expression and angiogenesis but higher vascular permeability in the liver tumor.	(6)
Colon *vs.* skin	Higher EGFR, FGF, collagenase, mdr-1 gene expression, and metastatic potential in colon tumors.	(81)
Kidney *vs.* skin	Higher bFGF expression, angiogenesis, and metastatic potential in kidney tumors.	(79)
Gall bladder *vs.* skin	Higher production of anti-angiogenesis factor (TGFβ1) in the gall bladder tumor.	(7)
Pancreas *vs.* abdominal wall	Higher VEGF protein level, angiogenesis, and tumor growth in tumors grown in the pancreas.	(8)
Cranium vs. mammary fat pad	Higher VEGF/receptor expression and permeability but lower angiogenesis in the mammary tumor.	(9)

The expression of endogenous anti-angiogenic factors is also regulated by organ specific host-tumor interaction. Human gall bladder primary tumors inhibit angiogenesis and growth of secondary tumors at a distant site. However, this result was only observed when the primary tumor was grown in the gall bladder (orthotopic), rather than the subcutaneous space (ectopic) (7). TGFβ1 mediates this process. Murine fibrosarcoma FsaII can also inhibit angiogenesis in secondary tumors *in vivo*. Surgical removal of the primary tumor cancelled this effect. On the other hand, irradiation of the primary tumor induced a more pronounced inhibitory effect on angiogenesis in the distant site (87). Plasma endostatin levels correlate well with these endogenous antiangiogenic effects caused by tumor burden and/or treatments. Knowledge of organ-dependent profiles of gene expression and protein level, in stromal cells and tumor cells from different organ microenvironments, will provide new insight into tumor biology and should allow us to understand why a given tumor behaves differently in different organs.

Knowledge of gene expression alone, without corresponding functional analysis, provides an incomplete understanding at best of the putative role of the gene and/or its product *in vivo*. For example, it is widely accepted that VEGF is responsible for high permeability in tumors, but the organ microenvironment also plays a role: HGL21, a human glioblastoma, has high vascular permeability (similar to that in the majority of other tumors) when it is grown in subcutaneous tissue, but these tumors are no longer as permeable when grown in the cranium (88), despite similar expression levels of VEGF and its receptors in HGL21 tumors grown in both sites. Furthermore, the vascular pore cut-off size (the maximum functional pore size for trans-vascular transport of macromolecules through the vessel wall) in various tumors decreased when the tumors were grown in the cranial window as compared to the dorsal skin chamber (89). Similarly, with the presence of a blood-brain-barrier, a significantly higher amount of VEGF was required to induce vascular hyperpermeability in normal vessels in the cranial window than in the dorsal skin chamber (84). On the other hand, the cranial environment is more angiogenic and forms new vessels faster than the subcutaneous tissue does in response to a given angiogenic factor (82). These differences are presumably due to differences in the phenotype of vascular endothelial cells, which is defined by their origin, by cell-cell and cell-matrix interactions, and by the surrounding microenvironment. These findings indicate that VEGF level alone may not be a sufficient predictor of angiogenesis or vascular permeability in the tumors of different organs. Indeed, the vascular permeability of LS174T human colon cancer grown in the liver versus subcutaneous space was inversely correlated with the expression levels of VEGF at these sites, while angiogenesis was parallel to VEGF levels (6). Conversely, higher VEGF expression and permeability but lower angiogenesis were observed in ZR75 human breast cancers grown in the mammary fat pad (primary site) compared to those grown in the cranial window (metastatic site) (9). These findings underscore the need for functional studies in conjunction with gene expression studies.

In conclusion, host-tumor interaction influences the biology of both tumor cells and host stromal cells, including their expression of positive and negative regulators of angiogenesis. This interaction depends on the cross-talk between tumor cells and stromal cells. In addition, the local microenvironment of different organs may vary during treatment, which in turn will influence the efficiency of various treatment modalities. Unlike neighboring malignant cells, stromal cells are genetically stable, yet they play important roles in multiple steps of tumor progression. Thus, targeting stromal cells may be a superior strategy for tumor detection and treatment. The cellular, molecular, and metabolic environment of solid tumors activates stromal cells and tumor cells, inducing angiogenesis, tumor cell proliferation, invasion, and metastasis and reducing the therapeutic efficacy of conventional anti-tumor treatments. However, these characteristic tumor environments may offer novel targets for tumor detection and treatment. A better understanding of host-tumor interaction during tumor growth, response to treatments, regression, and regrowth would facilitate the development of innovative tumor treatment strategies.

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